PART I

19

Co-funded by the
Erasmus+ Programme
of the European Union

Healthy and Denervated Muscles Physiology

CK4Stim

Co-funded by the Erasmus+ Programme of the European Union

Healthy Muscle Physiology

EVA ILIE

Introduction

Muscle physiology is a fascinating field of study that plays a critical role in human health and performance. Healthy muscle physiology is essential for a wide range of activities, from simple movements such as walking and lifting objects to high-intensity exercise and athletic performance. In this chapter, we will explore the fundamental principles of healthy muscle physiology, including muscle contraction, energy metabolism, and muscle functionality. By the end of this chapter, you will have a deeper understanding of how healthy muscle physiology works.

Looking at the human body from an engineering perspective, it can be viewed as a mechanism for transmitting loads. Within this framework, skeletal muscle serves as the primary biological system for generating force and powering movement. Around a century ago, the action of muscles in the human body was initially understood through anatomical considerations of their origin and insertion points. However, further exploration was undertaken by Duchenne in his 1867 book, "Physiologie des Mouvements" in which he described the response of several human muscles to Faradic stimulation.[1] Since the pioneering work of individuals such as Duchenne, Ranvier, and Hill, our understanding of the structure and function of skeletal muscle has advanced significantly.[2,3] Today, virtually every aspect of muscle biology has been explored in detail, leaving few areas left unexamined.

The human body is home to over 600 individual skeletal muscles, with approximately 300 muscles located on each side of the body (Figure 1.1). Each of these muscles is specialized for specific functions and possesses unique properties that are determined during early developmental stages. For example, the anatomical structure of each muscle, including its shape, spatial organization, and orientation of component fascicles, as well as its connections with specific tendons, bones, and aponeuroses, is determined irreversibly during development. Together, these properties contribute to the remarkable precision and versatility of human movement.[4]

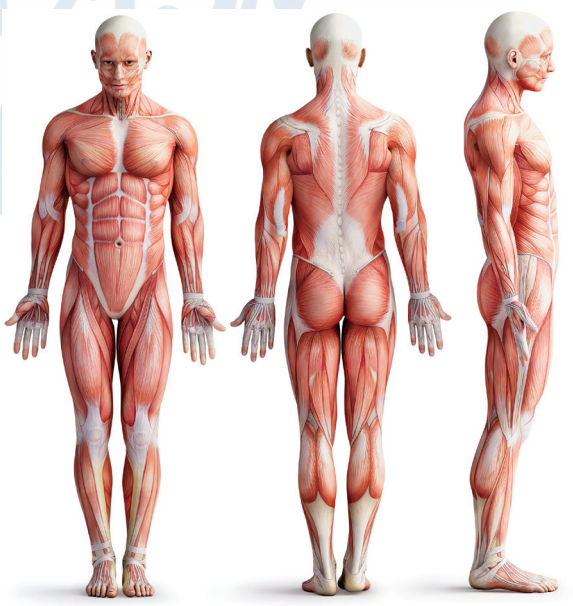

Figure 1.1 Human body skeletal muscles.

As the "final common pathway" in Sherrington's model of motor control, muscle represents the ultimate effector of a complex array of neuronal processes.[5] Accordingly, it plays a critical role in the expression of movement, serving as the ultimate site of force generation and control within the body. The basic functional unit of skeletal muscle is the motor unit, consisting of a group of muscle fibers innervated by a single motor neuron. For a successful performance of the human body, each motor unit must be capable of fulfilling any reasonable task that is required of it. In this way, the muscle units work together to produce the complex and varied movements necessary for daily living.

Skeletal Muscle Ontogeny

Skeletal muscles have their origin in the paraxial mesoderm, which arises during gastrulation and embryonic axis elongation. This tissue forms the presomitic mesoderm at the posterior end of the embryo, which consists of an immature posterior and a committed anterior region that eventually segments to form the somites. Within the somites, skeletal myogenesis is initiated through the specification of premyogenic progenitors and skeletal myoblasts. Differentiation and proliferation phases lead to the formation of multinucleated myofibers via the fusion of mononucleated myocytes. This process involves a series of molecular and cellular events that regulate the progression through different developmental stages in the embryo.[6]

Muscle Tissue as A Source of Endocrine Signaling Molecules

The human body's locomotor system heavily relies on the skeletal muscle, which is one of the largest organs. This essential component helps to maintain posture, generate force and power during intentional movements, and assists with involuntary actions such us breathing and reflexes.[7]

Muscles were traditionally viewed as solely being responsible for movement and locomotion. However, recent research has revealed that muscles are also involved in a wide range of metabolic processes and functions, including glucose homeostasis, lipid metabolism, and energy expenditure. Additionally, it has been discovered that the muscle tissue secretes a variety of molecules, including myokines, which act as signaling molecules in the body and have a wide range of physiological effects beyond muscle contraction. This has led to the recognition of skeletal muscle as an endocrine organ, capable of influencing a variety of physiological processes throughout the body.[8,9] The first myokine to be discovered was Interleukin-6 (IL-6), which was found to be released into the bloodstream by contracting muscles, even in the absence of inflammation.[10] IL-6 is known to play a role in the regulation of glucose and lipid metabolism during exercise. Specifically, it has been shown to promote glucose uptake and fatty acid oxidation in skeletal muscle, which is mediated in part by the activation of Adenosine Monophosphate-activated Protein Kinase (AMPK) and enhances the production of hepatic glucose. This suggests that IL-6 is involved in the metabolic adaptation of skeletal muscle to exercise, promoting the use of stored energy sources for fuel.[11] In addition to IL-6, other cytokines such as IL-7, IL-15, and Leukaemia Inhibitory Factor have been identified as being secreted by skeletal muscles and capable of exerting metabolic effects.[12]

New research has revealed that AMPK signaling in skeletal muscle plays an important role in the biogenesis of microRNAs by regulating the expression of DICER, a critical enzyme involved in this process.[13]

The brain-derived neurotrophic factor (BDNF) is a myokine that has been shown to increase in response to exercise in both rodents and humans. It is the most abundant neurotrophin in the brain and plays a critical role in neuronal survival, differentiation, and plasticity. In addition to its neurotrophic functions, BDNF has been found to have metabolic effects in skeletal muscle, including promoting glucose uptake and enhancing mitochondrial function. The release of BDNF from contracting muscle may contribute to the beneficial effects of exercise on brain function and cognitive health.[14]

Recent studies have shown that muscle-specific deletion of BDNF in mice leads to the development of metabolic myopathy and insulin resistance, highlighting its essential autocrine and paracrine roles as a metabolic regulator.[15] Furthermore, evidence suggests that BDNF released from muscle may also act in an endocrine manner by signaling to vascular cells and perivascular adipose depots.[16]

The myokine irisin, produced by the Fibronectin Type 3 Domain Containing Protein 5 (FNDC5) precursor protein, has been shown to induce BDNF expression in the brain and stimulate thermogenic gene expression in beige adipocytes, promoting their conversion to brown adipocytes in response to exercise in both mice and humans.[17,18] Irisin is also involved in the regulation of bone mineral density and synaptic plasticity in the brain, suggesting that the skeletal muscle plays a significant role in regulating body metabolism and physiology.[19]

Microstructure of Skeletal Muscle

Skeletal muscle is responsible for voluntary movement and is under conscious control. It is composed of muscle fibers that can contract and generate force, but also require a large amount of energy to do so, which can lead to fatigue. Examples of skeletal muscles include the Quadriceps Femoris in the thigh and the Biceps Brachii in the forearm. Skeletal muscle is a complex tissue with a highly organized structure and function. It consists of several bundles of muscle fibers (myofibers), each containing several myofibrils, which are the basic cellular units called sarcomeres. The fascicles are bundles of myofibers, which in turn form the muscle tissue, encapsulated by the Extracellular Matrix and supported by the cytoskeletal networks.

Skeletal muscle is highly vascularized and innervated, and contains components of the metabolic and regulatory machinery necessary for efficient energy production and cellular homeostasis. The precise coordination between each of these components is essential for maintaining muscular health and associated motor activity. Any perturbations, whether genetic or environmental, can result in loss of muscle health and function, typically characterized by muscle fiber loss, reduced motor output, and in some cases, death.[20]

The muscle as a biomechanical device requires the coordination between several factors (or components) both intrinsic (e.g. genetic) and extrinsic (e.g. environmental stressors, circulatory factors) essential for normal muscle function. Over the decades, reviews in skeletal muscle research have focused extensively on specific aspects of muscle structure or function. However, a holistic understanding of the integrated nature of these components is essential for a complete understanding of muscle health and function. The structural and functional aspects of skeletal muscle can be divided into three fundamental units that drive muscle contraction: (a) the neuromuscular junction (NMJ), which serves as a junction between nerve and muscle; (b) the machinery involved in excitation-contraction coupling, which is the process of transducing electrical impulses from the nerve to the muscle, required to initiate mechanical contraction; and (c) the sarcomere, the contractile apparatus responsible for force generation. These three units work together in a highly coordinated fashion to generate the mechanical force required for muscle movement. Dysfunction or impairment in any of these units can lead to muscle weakness or loss of function.[21]

Skeletal muscle fibers are unique in their large size and multinucleated structure, with each fiber containing numerous nuclei that are located peripherally adjacent to the plasma membrane. In fact, the number of nuclei in a single muscle fiber can range from hundreds to thousands depending on the size and function of the muscle. This peripheral distribution of nuclei is thought to be important for efficient protein synthesis and repair, as the nuclei can quickly respond to stimuli and produce the necessary proteins. While it is uncommon for nuclei to migrate to the center of a muscle fiber, this can occur in certain circumstances such as during development, regeneration, or disease. Myonuclei are specialized nuclei that are unique to skeletal muscle fibers. Unlike other cell types, myonuclei

are post-mitotic, meaning that they do not divide or replicate. They have a flattened and elongated shape, with their long-axis typically running parallel to the longitudinal axis of the muscle fiber. Interestingly, myonuclei are not randomly distributed within the muscle fiber. Rather, they appear to repel each other during positioning, resulting in an evenly distributed configuration throughout the muscle fiber. This organization is thought to be important for efficient muscle function and force generation. Under certain conditions such as muscle damage or growth, additional myonuclei may be added to the muscle fiber through the activation and fusion of satellite cells, a population of muscle stem cells.[22]

The concept of myonuclear domains suggests that each myonucleus is responsible for regulating gene expression within a specific portion of the muscle fiber, known as its myonuclear domain.[21] This concept is based on the idea that a myonucleus can only efficiently regulate gene expression within a limited volume of cytoplasm, as diffusion of mRNA and proteins beyond a certain distance becomes inefficient. Thus, the number of myonuclei in a muscle fiber is thought to directly impact the size and metabolic capacity of the fiber, with larger fibers having a greater number of myonuclei to regulate gene expression within their larger myonuclear domains.[22]

The myonuclear domain theory suggests that each nucleus in a muscle fiber is responsible for controlling the transcription and translation of proteins in a limited volume of the cytoplasm surrounding it. This is because the diffusion of transcription factors and mRNA molecules is limited, and thus the transcriptional capacity of each nucleus is limited to a specific volume of the muscle fiber, also known as the myonuclear domain. Therefore, in order to maintain proper protein synthesis across the entire muscle fiber, the number of myonuclei must increase proportionally to the fiber size. The concept of the myonuclear domain is therefore important for understanding muscle physiology and adaptation to exercise.[23]

The concept of the myonuclear domain theory has been widely accepted by many researchers, but it remains a topic of intense debate within the field. According to this theory, there is a linear relationship between the total number of myonuclei and the size or volume of the muscle fiber. However, some researchers have questioned the validity of this theory and have proposed alternative models for the regulation of protein synthesis in muscle fibers. Despite the ongoing debate, the myonuclear domain theory remains an important concept in the field of skeletal muscle physiology.[24-26]

Dynamic and plastic nature of skeletal muscle allows it to adapt to changes in external and internal environments, such as changes in physical activity levels, nutrient availability, and hormonal balance. Regular exercise, for instance, can stimulate muscle protein synthesis and growth, leading to increased muscle mass and strength. On the other hand, prolonged periods of inactivity or nutrient deficiency can lead to muscle atrophy, which is characterized by a loss of muscle mass and function. Understanding the factors that regulate muscle protein turnover and adaptation is crucial for maintaining muscle health and function, particularly in populations at risk of muscle wasting, such as older adults or individuals with chronic diseases.[27]

Skeletal muscle is a highly versatile tissue that contributes significantly to multiple bodily functions. Mechanically, skeletal muscle is responsible for generating force and power, maintaining posture, and producing movement, which can impact daily activities, social interactions, occupational settings, and overall health. Metabolically, skeletal muscle plays a key role in basal energy metabolism, storing important substrates such as amino acids and carbohydrates, producing heat to maintain core temperature, and consuming the majority of oxygen and fuel used during physical activity and exercise. Additionally, skeletal muscle serves as a reservoir of amino acids needed by other tissues for the synthesis of organ-specific proteins.[28]

Several techniques are used to quantify muscle mass and determine the body composition, including dual-energy X-ray absorptiometry, bioelectrical impedance analysis, magnetic resonance imaging, and computed tomography. These

techniques have been utilized in various research and clinical settings to evaluate changes in muscle mass and function in response to interventions such as exercise, nutrition, and disease.[7]

Muscular System Structure

The organization of skeletal muscle is distinguished by a highly structured and extensively documented pattern of muscle fibers (also known as myofibers or muscle cells) and their corresponding supportive connective tissues. Muscle size can vary greatly among individuals and can also be influenced by factors such as genetics, age, and physical activity. The arrangement of muscle fibers within a muscle is also highly organized, with fibers oriented in parallel to the line of force generated by the muscle. This arrangement allows for efficient force transmission and contributes to the overall strength and function of the muscle. Additionally, the connective tissue surrounding and interweaving between muscle fibers provides structural support and helps to transmit force throughout the muscle.[29]

Additionally, muscle fibers are highly specialized and can be categorized into different types based on their metabolic and contractile properties. These properties are determined by the expression of different isoforms of contractile and metabolic proteins within the fiber. Thus, the overall architecture and composition of skeletal muscle are critical to its function and are influenced by a variety of factors including genetics, physical activity, nutrition, and disease.[30] Non-uniformity of contractile properties within a single muscle fiber may be caused by a variety of factors, such as differences in the expression of contractile proteins, differences in the types of motor units innervating different regions of the fiber, or differences in the mechanical loading experienced by different regions of the fiber. These differences can result in variations in contractile force, speed of contraction, and fatigue resistance within a single fiber. Understanding the factors that contribute to these variations is an area of active research in muscle physiology.[31]

Satellite cells are known to be quiescent (inactive) in healthy muscles, but become activated in response to mechanical stress, injury, or disease. Upon activation, satellite cells undergo proliferation, followed by differentiation and fusion with existing muscle fibers or formation of new fibers, thus contributing to muscle repair and growth. Dysfunction or depletion of satellite cells has been implicated in various muscle diseases and age-related decline in muscle function.[32]

When activated by myogenic factors, satellite cells undergo a series of steps that ultimately lead to the formation of new muscle fibers. These steps include proliferation, migration, and differentiation. Once activated, satellite cells proliferate to form a population of undifferentiated myoblasts. Some of these myoblasts migrate to the site of injury or damage, where they fuse with existing muscle fibers to repair or replace damaged muscle tissue. Other myoblasts continue to proliferate and differentiate into new muscle fibers, which contribute to muscle growth and hypertrophy.[33]

The internal myofilament structure is responsible for the contractile properties of skeletal muscle. The basic unit of this structure is the sarcomere, which is composed of interdigitating thick (myosin) and thin (actin) filaments. The interaction between these two types of filaments, facilitated by a number of regulatory proteins, results in the generation of force and movement. The sarcomere is organized into repeating units along the length of the muscle fiber, giving rise to the striated appearance of skeletal muscle when viewed under a microscope.[34]

Muscle Fiber Phenotype Diversity

The capillary supply networks that support the metabolic demands of skeletal muscle vary depending on the fiber type. Oxidative, slow-twitch fibers have a higher density of capillaries than glycolytic and fast-twitch fibers due to their greater reliance on oxygen for energy production. This difference in capillary density contributes to the differences in metabolic and endurance capacity between muscle fibers.[35]

The classification of muscle fibers based on different criteria has led to the identification of various fiber types. In humans, there are typically three main

fiber types: type I (slow, oxidative, fatigue-resistant), type IIa (fast, oxidative, intermediate metabolic features), and type IIx (or IIb) (faster, glycolytic, fatigable). Type I fibers are often referred to as slow-twitch oxidative fibers and are characterized by their high oxidative capacity and resistance to fatigue. Type II fibers, on the other hand, are typically faster and more powerful, but have lower oxidative capacity and are more prone to fatigue. Type IIa fibers have an intermediate profile between type I and type IIx fibers, while type IIx fibers have the highest glycolytic capacity and are the fastest and most fatigable of all fiber types. The specific distribution of fiber types within a muscle is determined by various factors, including genetics, age, sex, and training status.[36,37]

Additionally, within these three major fiber types, there is a continuum of properties, resulting in a range of intermediate fiber types. These fiber types are not fixed and can be altered by various factors such as exercise, aging, and disease. For example, endurance training can increase the proportion of slow oxidative fibers, whereas resistance training can increase the proportion of fast glycolytic fibers. Understanding the properties and plasticity of different fiber types is important for optimizing exercise and rehabilitation programs and for understanding the pathophysiology of various neuromuscular disorders.[4]

It should also be noted that some sources may refer to Type IIx fibers as Type IIb fibers. This nomenclature difference does not reflect a difference in the properties of the fibers themselves, but rather a difference in historical classification schemes. Additionally, some sources may recognize a fourth fiber type, Type IIb, which is even more glycolytic and fatigable than Type IIx. However, this classification is not as widely used as the three fiber types mentioned earlier.[4]

Muscle Fiber Cellular Components

In addition to the myofibrils, a muscle fiber contains other important cellular components such as the T-tubule system, the sarcoplasmic reticulum, and mitochondria. The T-tubule system is a network of invaginations of the sarcolemma that extends deep into the muscle fiber. Its main function is to transmit the action potential generated at the NMJ into the muscle fiber to initiate muscle contraction.[38] The T-tubule system also ensures that the action potential reaches all parts of the muscle fiber uniformly. The sarcoplasmic reticulum is a specialized endoplasmic reticulum that is responsible for the storage and release of calcium ions, which are essential for muscle contraction. Finally, the mitochondria in muscle fibers produce adenosine triphosphate (ATP), which is the main source of energy for muscle contraction. The amount and organization of these components may differ between fiber types, reflecting their different metabolic and contractile properties.[39] When the muscle fiber is stimulated, an action potential propagates along the T-tubules, which triggers the release of calcium ions from the terminal cisternae into the sarcoplasm. The calcium ions then bind to the regulatory proteins troponin and tropomyosin, which allows for the interaction between actin and myosin filaments and the generation of force. When the stimulus ends, the sarcoplasmic reticulum reuptakes the calcium ions, causing the muscle fiber to relax. This process of calcium release and reuptake is essential for muscle contraction and relaxation. The amount and distribution of sarcoplasmic reticulum and T-tubules vary depending on the fiber type, with fast-twitch fibers having a larger and more extensive network of these structures than slow-twitch fibers.[39] The mitochondrial network in muscle fibers is crucial for generating ATP, which is necessary for muscle contraction. Mitochondria are able to perform oxidative phosphorylation, a process in which they use oxygen and nutrients to create ATP. This process is much more efficient than the anaerobic glycolysis used by fast-twitch fibers, and allows for sustained muscle activity. Mitochondria are particularly abundant in slow-twitch fibers, which have a high oxidative capacity and are able to sustain the contraction for long periods of time. In fast-twitch fibers, the mitochondrial network is less extensive, and glycolysis is the primary source of ATP during high-intensity exercise.[40] Mitochondria are essential for the production of energy in muscle fibers, and their distribution within the cell can vary de-

pending on the fiber type and metabolic demands. In some muscle fibers, mitochondria are located closer to the sarcolemma, which allows for more efficient transport of oxygen from the capillary supply to the mitochondria. This is especially important during aerobic exercise when the demand for oxygen increases. In other fibers, mitochondria are located in the inter-myofibrillar space, which allows for more efficient energy transfer during high-intensity, short-duration activities. The number and distribution of mitochondria can also be influenced by training, with endurance training leading to an increase in mitochondrial content and density in slow-twitch fibers.[40]

Transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) is a master regulator of mitochondrial biogenesis and function, as well as other metabolic processes in muscle cells. It is activated by various signaling pathways, including AMPK and calcium/calmodulin-dependent protein kinase, which are activated during exercise. PGC-1α stimulates the expression of nuclear respiratory factor 1 and mitochondrial transcription factor A, which in turn promote mitochondrial deoxyribose nucleic acid (DNA) replication and transcription, leading to an increase in mitochondrial biogenesis and function. Additionally, endurance exercise training increases the capillary density and the expression of transporters and enzymes involved in oxidative metabolism, leading to improved oxygen delivery and usage in muscle fibers.[41] The fragmentation of the sarcoplasmic reticulum in aging muscle can lead to impaired calcium release and muscle activation. This can contribute to age-related muscle weakness and loss of function. Additionally, aging is associated with a decline in mitochondrial function and number, which can lead to a decrease in energy production and contribute to muscle weakness and fatigue. Exercise training, particularly endurance or aerobic exercise, has positive effects on both mitochondrial function and the sarcoplasmic reticulum, which can improve muscle function and overall health in aging individuals.[42]

Abnormalities in the mitochondrial network have been reported in a number of neuromuscular diseases, including amyotrophic lateral sclerosis, mitochondrial myopathies, and Duchenne muscular dystrophy. These abnormalities can include alterations in mitochondrial size and distribution, as well as changes in mitochondrial function and metabolism. In addition, chronic conditions such as metabolic syndrome and obesity are also associated with mitochondrial dysfunction, which can contribute to muscle weakness and atrophy.[42] Understanding the changes in cellular elements in muscle fibers in response to various stimuli or pathologies is important for developing effective strategies for preventing and treating muscle dysfunction.

Muscle Proteins

Single muscle fibers are composed mostly of protein, specifically contractile proteins (such as actin and myosin), regulatory proteins (such as troponin and tropomyosin), and cytoskeletal proteins (such as titin and nebulin), which make up about 80% of the fiber's weight. The remaining 8% is composed of sarcoplasm, which includes various organelles such as mitochondria, the sarcoplasmic reticulum, and the T-tubule system, as well as other substances such as glycogen and myoglobin.[43] The myofibrils within muscle fibers are organized into distinct regions called sarcomeres, which are the basic contractile units of skeletal muscle. Sarcomeres are composed of two main types of myofilaments: actin and myosin. Actin filaments are thin and form a lattice-like structure, whereas myosin filaments are thick and contain globular heads that interact with actin to generate force and movement. In addition to actin and myosin, there are many other proteins present in the sarcomere and sarcoplasm that contribute to the structure and function of skeletal muscle, including proteins involved in the cytoskeleton, excitation-contraction coupling, energy release, and force and power generation. Modern Proteome analysis techniques have allowed for the identification and characterization of many of these muscle proteins, leading to a better understanding of muscle physiology and pathophysiology.[44] The troponin complex is responsible for binding calcium ions and initiating

a conformational change that results in the movement of tropomyosin away from the myosin-binding sites on the actin filament. This movement exposes the binding sites and allows the myosin heads to interact with the actin, leading to the formation of cross-bridges and the subsequent sliding of the myofilaments past each other. Tropomyosin also helps to stabilize the actin filament and maintain the position of the myosin-binding sites when there is no calcium present. In this way, the troponin complex and tropomyosin act as key regulators of muscle contraction, ensuring that force is generated only when calcium is present and the proper signaling pathways are activated.[44]

Titin and nebulin are also included in the category of proteins that affect the physiology and mechanical properties of the muscle.[45] Titin is the largest known protein and plays a crucial role in maintaining the structural integrity of the sarcomere, as well as in determining the passive stiffness of muscle fibers. It acts as a molecular spring that provides an elastic restoring force when muscle fibers are stretched. Nebulin, on the other hand, is a giant protein that runs along the length of the thin filament and regulates its length. It also plays a role in anchoring the thin filament to the Z-disc, which is a protein structure that separates adjacent sarcomeres.[45]

Muscle Fiber Activation Process

In response to this influx of calcium, a calcium release channel on the sarcoplasmic reticulum, known as the ryanodine receptor, is activated and releases a large amount of calcium into the cytoplasm. This calcium binds to the troponin complex on the actin filament, causing a conformational change that moves tropomyosin out of the way and exposes the myosin-binding site on the actin filament. Myosin then binds to actin, forming a cross-bridge. ATP is hydrolyzed to provide the energy needed for myosin to change its conformation and pull the actin filament toward the center of the sarcomere, shortening the muscle fiber and generating force. As the nerve stimulus ends, calcium is pumped back into the sarcoplasmic reticulum, causing the myofilaments to detach and the muscle fiber to relax. This process can be repeated rapidly to produce the repetitive contractions needed for muscle movement.[46] As the subsequent power stroke that results from the movement of the myosin head, is responsible for the sliding of the actin and myosin filaments past each other, resulting in muscle contraction. This process continues as long as ATP is available and calcium levels remain high enough to sustain the interaction between actin and myosin filaments. When the nerve impulse ceases, calcium is pumped back into the sarcoplasmic reticulum by the calcium ATPase pump and the actin and myosin filaments return to their relaxed state.[46]

In addition to the proteins and molecular events involved in the excitation-contraction coupling process, recent research has identified several genes that play a role in modulating this process. For example, myotubularin-related protein 14 has been shown to be involved in regulating the amount of calcium released from the sarcoplasmic reticulum during muscle contraction, while mitsugumin 29 has been implicated in maintaining the structural integrity of the sarcoplasmic reticulum. Kruppel-like factor 15, on the other hand, appears to be involved in regulating muscle fiber type and energy metabolism. Together, these genes and their protein products play important roles in the regulation of muscle function and physiology.[47]

Muscle Energy Pathways

The relationship between the capillary network and the mitochondrial network within muscle fibers is critical for sustained aerobic exercise. The mitochondria produce ATP through oxidative phosphorylation, which requires oxygen, and they are especially abundant in slow-twitch muscle fibers. These fibers are also rich in myoglobin, which facilitates the transport of oxygen from the capillaries to the mitochondria. In contrast, fast-twitch muscle fibers are poor in mitochondria and myoglobin, but rich in glycogen, which is the substrate for anaerobic glycolysis. The balance of fast-twitch and slow-twitch muscle fibers in a muscle determines its contractile properties and metabolic capacity. Physical training can induce adaptations that increase the proportion of slow-twitch fibers in a muscle, which

can improve endurance performance.[48] Carbohydrates and fats are the two primary sources of fuel for the muscle cell to produce ATP. During exercise, the body relies on a mixture of both carbohydrates and fats, with the relative contribution of each fuel source depending on factors such as exercise intensity and duration, as well as the individual's diet and training status. For example, during high-intensity exercise, the body primarily relies on carbohydrates for fuel, while during low-intensity exercise, fat is the primary fuel source.[49]

Muscle Force Generation Types

Muscle actions can also be classified based on their functional role in movement. For example, agonist muscles are those that contract to produce a desired movement, while antagonist muscles work in opposition to the agonist to control the movement or slow it down. Synergist muscles work with the agonist to enhance the movement, while stabilizer muscles work to stabilize the joint or body segment during movement. Additionally, some movements involve a combination of muscle actions, such as isometric contractions to stabilize a joint while a dynamic concentric or eccentric action occurs in another joint or body segment. Eccentric muscle actions cause muscle damage if not executed properly. However, exercise training, especially when incorporating eccentric actions, can help reduce this damage and the resulting inflammatory response.[50] For patients undergoing rehabilitation for cardio-pulmonary diseases, eccentric actions can be beneficial as they require less muscle activation, energy consumption, and oxygen consumption compared to concentric actions at a given level of force.[50]

Muscle Performance

The sliding filament theory of muscle contraction proposes that muscle fibers generate force by the sliding of the thin actin filaments over the thick myosin filaments. When an action potential reaches the muscle fiber, calcium ions are released from the sarcoplasmic reticulum, which binds to the regulatory protein troponin on the actin filament. This causes tropomyosin to move away from the active sites on the actin filament, allowing myosin heads to bind to the actin filament and form cross-bridges. The myosin heads then undergo a conformational change, pulling the actin filament towards the center of the sarcomere, which shortens the muscle fiber and generates force. The sliding filament theory has been modified over the years to account for additional complexities, but it remains the widely accepted mechanism for muscle contraction.[51] The force generated by the actin-myosin cross-bridges is transmitted along the length of the muscle fiber and also laterally to neighboring fibers. This force transmission is important for producing movement and generating tension in the muscle. The force is transmitted to the Z-disk of the sarcomeres in series, and ultimately reaches the myotendinous junction, tendons, and joints to produce movement. This process involves a complex interplay of various proteins and structures within the muscle fiber and is regulated by nervous and hormonal signals.[51]

The specific force of a muscle is defined as the force generated by the muscle per unit of cross-sectional area. It is a measure of the muscle's ability to generate force relative to its size and is considered an important indicator of muscle quality. Generally, muscles with a higher specific force are considered to be of higher quality, as they are able to generate more force per unit of muscle mass.[51]

The rate of ATP consumption during muscle contraction depends on various factors such as the number of myosin heads, the fraction of myosin heads that form cross-bridges, and the rate constant for cross-bridge detachment. The rate of ATP consumption is also influenced by the expression of myosin heavy chain isoforms, which determines the metabolic properties of muscle fibers. In human muscles, type I fibers have the lowest ATP consumption rate, followed by type IIa fibers, while type IIx fibers have the highest rate of ATP consumption.[52]

Neuromuscular Junction

The NMJ plays a crucial role in the control of muscle contraction and maintenance of muscle function (Figure 1.2). Dysfunction of the NMJ has been implicated in a variety of neuromuscular disor-

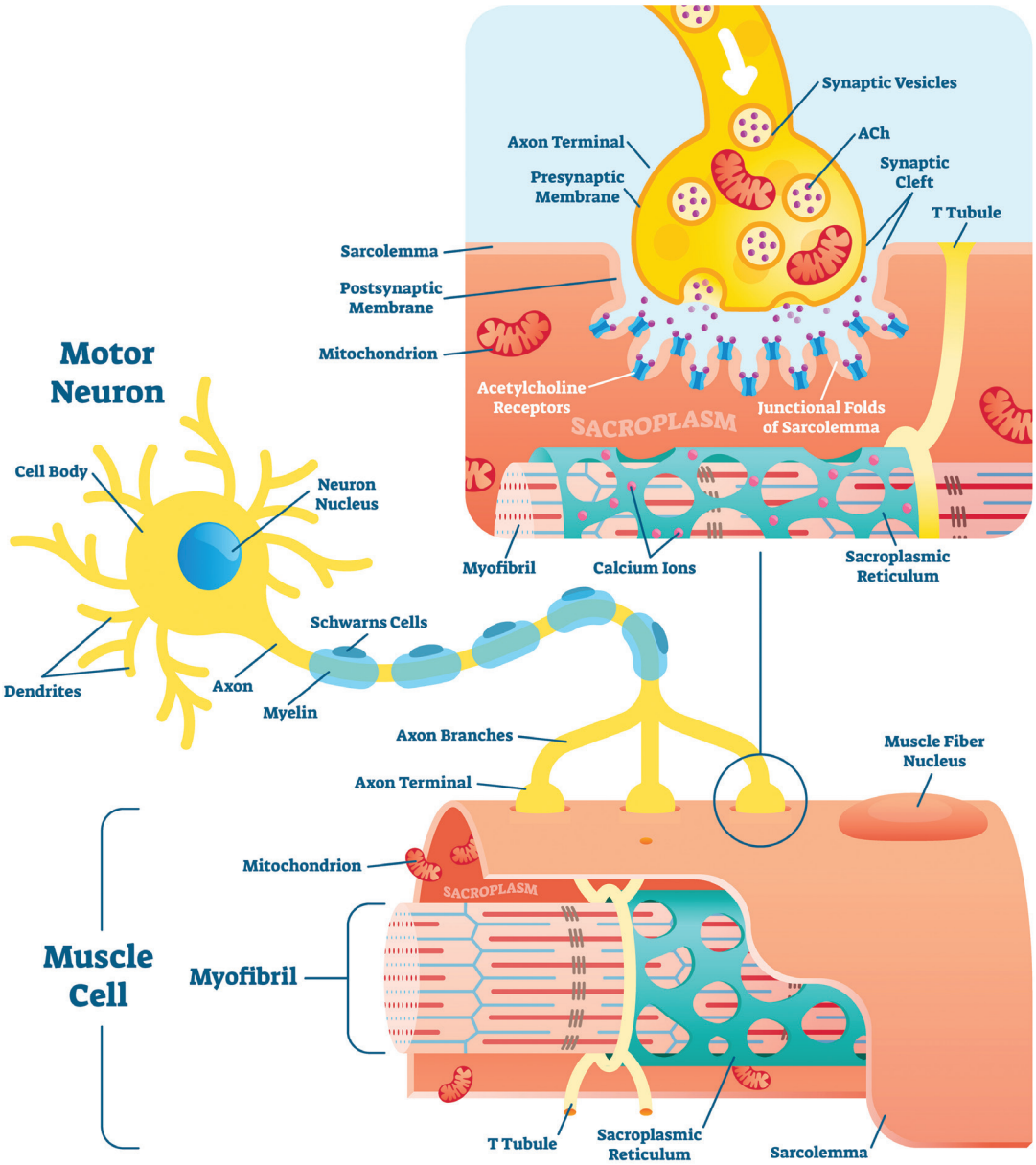

Figure 1.2 Neuromuscular junction.

ders, including myasthenia gravis, Lambert-Eaton myasthenic syndrome, and amyotrophic lateral sclerosis. In these disorders, the communication between the motor neuron and muscle fiber at the NMJ is disrupted, leading to muscle weakness and fatigue.

The study of NMJ structure and function is therefore an important area of research for understanding neuromuscular diseases and developing effective treatments.[53]

Presynaptic Region

At the NMJ, the Schwann cell surrounds the majority of the nerve terminal, excluding the part facing the postsynaptic membrane. The nerve terminal is packed with synaptic vesicles (SVs), which store, release and uptake the neurotransmitter acetylcholine (ACh).[54] These SVs fuse to the presynaptic membrane at "active zones", leading to the initiation of neuromuscular transmission. The Schwann cell plays an important role in maintaining the

structure and function of the NMJ by providing trophic support to the nerve terminal and regulating the release of ACh.[54]

Synaptic Space and Synaptic Basal Lamina

The synaptic space is the narrow gap between the pre- and post-synaptic membranes, where the neurotransmitter molecules are released from the presynaptic neuron and bind to the receptors on the postsynaptic membrane, triggering a response in the muscle fiber. The synaptic basal lamina is a specialized extracellular matrix that lines the synaptic space, providing structural support for the NMJ and anchoring the pre- and post-synaptic membranes.[55] The basal lamina is composed of several proteins, including laminin, collagen, and perlecan, which play important roles in the formation, maintenance, and regeneration of the NMJ. Disruption of the synaptic basal lamina can lead to NMJ dysfunction and muscle weakness, as seen in certain neuromuscular disorders.[55]

Postsynaptic Region

The postsynaptic region at the NMJ contains junctional folds that increase the surface area of the postsynaptic membrane, leading to a larger volume of synaptic space. The junctional sarcoplasm, which fills the synaptic space, contains various cellular structures such as mitochondria, Golgi apparatus, and intermediate filaments, that provide metabolic and structural support to the postsynaptic region. The crests of the junctional folds are densely packed with nicotinic acetylcholine receptors (nAChRs), which are ion channels composed of subunits alpha, beta, gamma, delta, and epsilon. These subunits are linked together via a protein called rapsyn.[56,57]

The depolarization potential created by the activation of nAChRs by ACh reaching the postsynaptic membrane allows for the permeability of Na+ and K+ ions and to a lesser extent Cl- and Ca2+ ions, while the voltage-gated sodium channels (VGSCs) concentrated in the troughs of junctional folds generate an action potential which is transmitted through the fiber via the T-tubules. Ankyrin-G and ß-spectrin are necessary for maintaining VGSC densities in the postsynaptic folds, which are essential for impulse propagation.[58]

References

1. Duchenne GB, Kaplan EB. (1949), Physiology of motion. Philadelphia: JB Lippincott Co.
2. Ranvier L. (1873), Propriétés et structures differentes des muscles rouges et des muscles blancs chez lapins et chez les rares. Comptes Rendus Hebdomadaires des Séances de I'Académie des Sciences.
3. Hill AV, Baltimore MD. (1965), Trails and trials in physiology. The Williams & Wilkins Co.
4. Blaauw B, Schiaffino S, Reggiani C. Mechanisms modulating skeletal muscle phenotype. Compr Physiol. 2013;3(4):1645-87. doi:10.1002/cphy.c130009.
5. Sherrington CS. Ferrier Lecture: Some functional problems attaching to convergence. Proc R Soc Lond [Biol]. 1929;105(737):332-62. doi:10.1098/rspb.1929.0047
6. Chal J, Pourquié O. Making muscle: Skeletal myogenesis in vivo and in vitro. Development. 2017;144(12):2104-22. doi:10.1242/dev.151035.
7. Frontera WR, Ochala J. Skeletal muscle: A brief review of structure and function. Calcif Tissue Int. 2015;96:183-95. doi:10.1007/s00223-014- 9915-y.
8. Pedersen BK. Muscle as a secretory organ. Compr Physiol. 2013;3(3):1337-62. doi:10.1002/cphy.c120033.
9. Severinsen MCK, Pedersen BK. Muscle–organ crosstalk: The emerging roles of myokines. Endocr Rev. 2020;41:594-609. doi:10.1210/endrev/bnaa016.
10. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. Diabetes. 2004; 53:1643-8. doi:10.2337/diabetes.53.7.1643.
11. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, et al. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes. 2006;55(10):2688-97. doi:10.2337/db05-1404.
12. Haugen F, Norheim F, Lian H, Wensaas AJ, Dueland S, Berg O, et al. IL-7 is expressed and secreted by human skeletal muscle cells. Am J Physiol Cell Physiol. 2010;298(4):C807-16. doi:10.1152/ajpcell.00094.2009.
13. Brandao BB, Madsen S, Rabiee A, Oliverio M, Ruiz GP, Ferrucci DL, et al. Dynamic changes in DICER levels in adipose tissue control metabolic adaptations to exercise. Proc Natl Acad Sci USA. 2020;117(38):23932-41. doi:10.1073/pnas.2011243117.
14. Matthews VB, Astrom MB, Chan MHS, Bruce CR, Krabbe KS, Prelovsek O, et al. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia. 2009;52(7):1409-18. doi:10.1007/s00125-009-1364-1.
15. Yang X, Brobst D, Chan WS, Tse MCL, Herlea-Pana O, Ahuja P, et al. Muscle-generated BDNF is a sexually dimorphic myokine that controls metabolic flexibility. Sci Signal. 2019;12(594):eaau1468. doi:10.1126/scisignal.aau1468.
16. Zierold S, Buschmann K, Gachkar S, Bochenek ML, Velmeden D, Hobohm L, et al. Brain-Derived Neurotrophic Factor expression and signaling in different perivascular adipose tissue depots of patients with coronary artery disease. J Am Heart Assoc. 2021;10(6):e018322. doi:10.1161/JAHA.120.018322

17. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell Metab. 2013;18(5):649-59. doi:10.1016/j.cmet.2013.09.008.
18. Kim H, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, et al. Irisin mediates effects on bone and fat via αv integrin receptors. Cell. 2018;175(7):1756-68.e17. doi:10.1016/j.cell.2018.10.025
19. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med. 2019;25(1):165-75. doi:10.1038/s41591-018-0275-4
20. Lieber, R. L. (2009). Skeletal muscle structure, function, and plasticity. Baltimore, MD: Lippincott Williams & Wilkins.
21. Mukund K, Subramaniam S. Skeletal muscle: A review of molecular structure and function, in health and disease. Wiley Interdiscip Rev Syst Biol Med. 2020;12(1):e1462. doi:10.1002/wsbm.1462
22. Bruusgaard JC, Liestøl K, Gundersen K. Distribution of myonuclei and microtubules in live muscle fibers of young, middle-aged, and old mice. J Appl Physiol (1985). 2006;100(6):2024-30. doi:10.1152/japplphysiol.00913.2005
23. Hall ZW, Ralston E. Nuclear domains in muscle cells. Cell. 1989;59(5):771-2. doi:10.1016/0092-8674(89)90597-7
24. Pavlath GK, Rich K, Webster SG, Blau HM. Localization of muscle gene products in nuclear domains. Nature. 1989;337(6207):570-3. doi:10.1038/337570a0
25. Gundersen K, Bruusgaard JC, Egner IM, Eftestøl E, Bengtsen M. Muscle memory: Virtues of your youth?. J Physiol. 2018;596(18):4289-90. doi:10.1113/JP276354
26. Snijders T, Smeets JS, van Kranenburg J, Kies AK, van Loon LJ, Verdijk LB. Changes in myonuclear domain size do not precede muscle hypertrophy during prolonged resistance-type exercise training. Acta Physiol (Oxf). 2016;216(2):231-9. doi:10.1111/apha.12609
27. Schwartz LM. Skeletal muscles do not undergo apoptosis during either atrophy or programmed cell death-revisiting the myonuclear domain hypothesis. Front Physiol. 2019;9:1887. doi:10.3389/fphys.2018.01887.
28. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006;84(3):475-82. doi:10.1093/ajcn/84.3.475
29. Javan R, Horvath JJ, Case LE, Austin S, Corderi J, Dubrovsky A, et al. Generating color-coded anatomic muscle maps for correlation of quantitative magnetic resonance imaging analysis with clinical examination in neuromuscular disorders. Muscle Nerve. 2013;48(2):293-5. doi:10.1002/mus.23780
30. Hikida RS. Aging changes in satellite cells and their functions. Curr Aging Sci. 2011;4(3):279-97. doi:10.2174/1874609811104030279. PMID: 21529324.
31. Wilkins JT, Krivickas LS, Goldstein R, Suh D, Frontera WR. Contractile properties of adjacent segments of single human muscle fibers. Muscle Nerve. 2001;24(10):1319-26. doi:10.1002/mus.1150
32. Macaluso F, Myburgh KH. Current evidence that exercise can increase the number of adult stem cells. J Muscle Res Cell Motil. 2012;33(3-4):187-98. doi:10.1007/s10974-012-9302-0
33. Bareja A, Holt JA, Luo G, Chang C, Lin J, Hinken AC, et al. Human and mouse skeletal muscle stem cells: Convergent and divergent mechanisms of myogenesis. PLoS One. 2014;9(2):e90398. doi:10.1371/journal.pone.0090398
34. Thomas GD. Functional muscle ischemia in Duchenne and Becker muscular dystrophy. Front Physiol. 2013;4:381. 2013. doi:10.3389/fphys.2013.00381
35. Liu G, Mac Gabhann F, Popel AS. Effects of fiber type and size on the heterogeneity of oxygen distribution in exercising skeletal muscle. PLoS One. 2012;7(9):e44375. doi:10.1371/journal.pone.0044375
36. Lamboley CR, Murphy RM, McKenna MJ, Lamb GD. Sarcoplasmic reticulum Ca2+ uptake and leak properties, and SERCA isoform expression, in type I and type II fibres of human skeletal muscle. J Physiol. 2014;592(6):1381-95. doi:10.1113/jphysiol.2013.269373
37. Galpin AJ, Raue U, Jemiolo B, Trappe TA, Harber MP, Minchev K, et al. Human skeletal muscle fiber type specific protein content. Anal Biochem. 2012;425(2):175-82. doi:10.1016/j.ab.2012.03.018
38. Jayasinghe I, Launikonis BS. Three-dimensional reconstruction and analysis of the tubular system of vertebrate skeletal muscle. J Cell Sci. 2013;126(Pt 17):4048-58. doi:10.1242/jcs.131565
39. Kerr JP, Ward CW, Bloch RJ. Dysferlin at transverse tubules regulates Ca(2+) homeostasis in skeletal muscle. Front Physiol. 2014;5:89. doi:10.3389/fphys.2014.00089
40. Dahl R, Larsen S, Dohlmann TL, Qvortrup K, Helge JW, Dela F, et al. Three-dimensional reconstruction of the human skeletal muscle mitochondrial network as a tool to assess mitochondrial content and structural organization. Acta Physiol (Oxf). 2015;213(1):145-155. doi:10.1111/apha.12289
41. Yan Z, Lira VA, Greene NP. Exercise training-induced regulation of mitochondrial quality. Exerc Sport Sci Rev. 2012;40(3):159-64. doi:10.1097/JES.0b013e3182575599
42. Weisleder N, Brotto M, Komazaki S, Pan Z, Zhao X, Nosek T, et al. Muscle aging is associated with compromised Ca2+ spark signaling and segregated intracellular Ca2+ release. J Cell Biol. 2006;174(5):639-45. doi:10.1083/jcb.200604166
43. Hoppeler H, Lüthi P, Claassen H, Weibel ER, Howald H. The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. Pflugers Arch. 1973;344(3):217-32. doi:10.1007/BF00588462
44. Ottenheijm CA, Granzier H. Lifting the nebula: novel insights into skeletal muscle contractility. Physiology (Bethesda). 2010;25(5):304-10. doi:10.1152/physiol.00016.2010
45. Monroy JA, Powers KL, Gilmore LA, Uyeno TA, Lindstedt SL, Nishikawa KC. What is the role of titin in active muscle?. Exerc Sport Sci Rev. 2012;40(2):73-8. doi:10.1097/JES.0b013e31824580c6
46. Rebbeck RT, Karunasekara Y, Board PG, Beard NA, Casarotto MG, Dulhunty AF. Skeletal muscle excitation-contraction coupling: Who are the dancing partners?. Int J Biochem Cell Biol. 2014;48:28-38. doi:10.1016/j.biocel.2013.12.001
47. Manring H, Abreu E, Brotto L, Weisleder N, Brotto M. Novel excitation-contraction coupling related genes reveal aspects of muscle weakness beyond atrophy-new hopes for treatment of musculoskeletal diseases. Front Physiol. 2014;5:37. doi:10.3389/fphys.2014.00037
48. Weibel ER. The structural conditions for oxygen supply to muscle cells: The Krogh cylinder model. J Exp Biol. 2013;216(Pt 22):4135-7. doi:10.1242/jeb.076497
49. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol. 1993;265(3 Pt 1):E380-E391. doi:10.1152/ajpendo.1993.265.3.E380

50. Fulford J, Eston RG, Rowlands AV, Davies RC. Assessment of magnetic resonance techniques to measure muscle damage 24 h after eccentric exercise. Scand J Med Sci Sports. 2015;25(1):e28-e39. doi:10.1111/sms.12234.
51. Huxley H, Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature. 1954;173(4412):973-6. doi:10.1038/173973a0.
52. Larsson L, Moss RL. Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. J Physiol (Lond).1993; 472:595-614. doi:10.1113/jphysiol.1993.sp019964.
53. Hughes BW, Kusner LL, Kaminski HJ. Molecular architecture of the neuromuscular junction. Muscle Nerve. 2006;33(4):445-61. doi:10.1002/mus.20440.
54. Denker A, Rizzoli SO. Synaptic vesicle pools: An update. Front Synaptic Neurosci. 2010;2:135. doi:10.3389/fnsyn.2010.00135.
55. Rogers RS, Nishimune H. The role of laminins in the organization and function of neuromuscular junctions. Matrix Biol. 2017;57-58:86-105. doi:10.1016/j.matbio.2016.08.008.
56. Kramer IM. (2016), Cholinergic signaling and muscle contraction. In: Signal transduction. 3rd ed. Boston, MA: Academic Press. doi:10.1016/B978-0-12-394803-8.00004-8.
57. Zuber B, Unwin N. Structure and superorganization of acetylcholine receptor–rapsyn complexes. Proc Natl Acad Sci USA. 2013;110(26):10622-7. doi:10.1073/pnas.1301277110.
58. Rafuse VF, Polo-Parada L, Landmesser LT. Structural and functional alterations of neuromuscular junctions in NCAM-deficient mice. J Neurosci. 2000;20(17):6529-39. doi:10.1523/JNEUROSCI.20-17-06529.2000.

CK4STEM

Co-funded by the Erasmus+ Programme of the European Union

Pathophysiology of the Muscle

LIGIA RUSU

Overview About Muscle Atrophy

The muscle atrophy refers to the breaking down of muscle fibers and it is described as occurring in skeletal muscle. This process could affect skeletal, cardiac, and smooth muscles. The muscle atrophy has three mechanisms– physiologic, pathologic, and neurogenic. While some forms can be reversed, others cannot. Depend on the condition the evolution of muscle atrophy could be different and the symptoms, but usually is reduced muscle mass.[1,2]

The main signs of muscle atrophy are:

1. One arm or one leg is thinner than the other.
2. Weakness in one arm and or one leg.
3. Tingling in arms and legs.
4. Trouble in walking or balance
5. Difficulty of speaking.
6. Facial weakness.

Many of physical or pathological stimuli could generate a lot of muscle tissue changes fiber content, capillary distribution, and the components of intracellular connective tissue. The effect increases the pathologic mechanism that support development of atrophy or hypertrophy.[3]

One of the therapeutic interventions is Neuromuscular Electrical Stimulation (NMES) a kind of electrical stimulation that uses a device to send electrical stimulations to nerves and will cause muscle contraction. This kind of intervention does not require any muscle strength. This is a passive muscle contraction and is effective in treating muscle atrophy.[4]

What Causes Muscle Atrophy?

The cause of muscle atrophy depends on the type you have. Disuse (physiologic) atrophy is caused by not using the muscles enough. If you stop using your muscles, the body will not waste the energy it needs to take care of them. Instead, body will start to break the muscles down, which causes them to decrease in size and strength. Disuse atrophy may affect if you:

1. Lead a sedentary lifestyle.
2. Are malnourished.
3. Do not get enough exercise.
4. Sit at a desk job all day.
5. Are on best rest.
6. Have a genetic disorder such as muscular dystrophy or Charcot-Marie-Tooth disease.
7. Cannot move your limbs due to a stroke or other conditions such as dermatomyositis.
8. Have age-related atrophy (sarcopenia).

Neurogenic atrophy is caused by an injury or disease affecting nerves that connect to muscles. When the nerves are damaged, they cannot trigger the muscle contractions that are needed to stimulate muscle activity. When the muscles do not contract, the body thinks that those muscles are not needed anymore. Therefore, the body starts breaking them down, which causes them to decrease in size and strength. Diseases and other conditions that can affect these nerves include:

1. Amyotrophic Lateral Sclerosis.
2. Guillain-Barre Syndrome.
3. Carpal Tunnel Syndrome.
4. Poliomyelitis
5. Spinal Cord Injury.
6. Multiple Sclerosis.

How Long Does It Take Muscles to Atrophy?

The amount of time it takes to muscle atrophy depends on the age, fitness level, and cause of atrophy. If muscle atrophy is due to disuse (physiologic), the process can start within 2-3 weeks of not using muscles. Neurogenic muscle atrophy may develop sooner depending on health condition.

Diagnosis and Tests

How is muscle atrophy diagnosed?

To diagnose muscle atrophy, the healthcare provider will give a physical exam and ask about the symptoms. They'll look at to arms and legs and measure the muscle mass. In addition, the healthcare provider may order tests, including:[2]

- Blood test
- Muscle or nerve biopsy
- Electromyography
- Nerve conduction studies
- X-ray imaging
- Computed tomography (CT)scan
- Magnetic resonance imaging (MRI) scan

Management and Treatment

Can Muscle Atrophy Be Reversed?

Disuse (physiologic) atrophy can sometimes be reversed with exercise and a healthy diet. The healthcare provider may start a program that includes exercises in the pool. Working out in the water can reduce your muscle workload. Neurogenic atrophy typically cannot be reversed because of the physical damage in the nerves.[3]

What Treatments Are Used for Muscle Atrophy?

Treatment for muscle atrophy depends on the type. Disuse (physiologic) atrophy can be treated with regular exercise and better nutrition. The healthcare provider may recommend physical therapy or an exercise plan. Even if the patient cannot actively move certain joints in the body, the patient can still do exercises wearing a splint or brace. The healthcare provider may require to work with a dietitian on a healthy eating plan. They may suggest nutritional supplements as well.

Neurogenic atrophy can sometimes be treated with a special kind of physical therapy called electrical stimulation. The physical therapist place electrodes on the skin over the muscles. The electrodes send small electrical impulses to nerves and muscles. The electrical impulses try to artificially exercise or contract muscles. This can help the patient maintain muscle mass and strength. The physical therapist may also recommend ultrasound therapy. Ultrasound therapy uses sound waves to promote muscle healing.

If a contracture developed due to the muscle atrophy, the healthcare provider may perform surgery to correct it. A contracture occurs when muscle tissues become fibrous. This tissue makes it hard to stretch the muscle and prevents movement.

Muscle Composition

Muscle mass, quality, and composition are important factors that contribute to overall muscle function. However, these factors can be negatively impacted by conditions such as atrophy and sarcopenia. Atrophy refers to a decrease in muscle mass due to a reduction in muscle fiber size, while sarcopenia is characterized by a loss of both muscle mass and quality that typically occurs with aging.[3,4]

During atrophy and sarcopenia, there is often a decrease the size and number of muscle fibers, as well as a decrease in muscle protein synthesis and an increase in muscle protein breakdown. This leads to a decrease in muscle mass and an alteration in muscle composition, with a reduction in muscle fiber cross-sectional area and an increase in intramuscular fat and connective tissue. In addition to changes in muscle mass and composition, atrophy and sarcopenia also result in a decline in

muscle strength and function. This can lead to decreased mobility, falls, and an increased risk of disability and mortality.

To counteract these negative effects, interventions such as resistance training and adequate protein intake can be effective in maintaining and improving muscle mass, quality, and composition in older adults and individuals with muscle wasting conditions.

Age-related decline in muscle mass and strength is known as sarcopenia. Sarcopenia is a multifactorial process that involves a combination of factors such as decreased physical activity, hormonal changes, chronic inflammation, mitochondrial dysfunction, and altered protein metabolism. In addition to age-related sarcopenia, muscle mass and quality can also be affected by acute or chronic conditions such as disuse, immobilization, malnutrition, and certain diseases.[3,4]

Muscle quality is an important factor that determines muscle function and performance. It refers to the force-generating capacity of muscle adjusted for its size and is often assessed by specific force, which is the force per unit of muscle cross-sectional area. Specific force can be affected by changes in muscle fiber type composition, myosin heavy chain isoform expression, and contractile protein content.

Muscle composition also plays a crucial role in muscle function and performance. Skeletal muscle is composed of various tissues such as muscle fibers, connective tissues, blood vessels, and nerves. Changes in the composition of these tissues can lead to alterations in muscle function and performance. For instance, an increase in connective tissue content can reduce muscle elasticity and contractile force, while a decrease in capillary density can impair muscle metabolism and endurance.

Overall, maintaining muscle mass, quality, and composition is essential for optimal muscle function and performance, and preventing or delaying age-related sarcopenia and other muscle-related conditions. Regular exercise, adequate nutrition and appropriate medical care can help promote muscle health and function throughout the lifespan.

This alteration in muscle composition has been attributed to various factors, including changes in hormones, inflammation, and oxidative stress among others. Moreover, the quality of muscle fibers also changes with aging, with an increased proportion of type II fibers and a reduction in type I fibers, resulting in a decreased oxidative capacity of the muscle. These changes, together with a reduction in neuromuscular activation and motor unit recruitment, contribute to the age-related decline in muscle strength and power are seen in sarcopenia. Additionally, inactivity, malnutrition, and certain diseases or medical conditions can exacerbate the loss of muscle mass and function. Therefore, maintaining or increasing muscle mass, quality, and function is crucial for healthy aging and prevention of sarcopenia.[1]

The rate of muscle strength or power loss is much greater than the rate of skeletal muscle mass loss with advancing age. While muscle strength decreases at a rate of 2.5% to 4% per year, skeletal muscle mass only decreases at a rate of 0.5% to 1% per year. This suggests that other factors, such as changes in muscle quality and composition, may be responsible for the decline in muscle function associated with aging.[2]

Muscle Atrophy During Aging

Age-related transformation of human skeletal muscle fibers is a well-known phenomenon. Research by Lexell has shown that the limb muscles of aging individuals are 25-35% smaller and have more fat and connective tissue compared to those of younger individuals. The size of type II fibers is reduced in older individuals, while type I fibers are less affected by aging.[3]

In addition to the reduction in muscle fiber size and number, aging is also associated with a decline in overall muscle cross-sectional area. This decline is primarily due to a reduction in the number and size of type II muscle fibers, which are responsible for generating power and speed during muscle contractions. Type I muscle fibers, which are used for endurance activities, are less affected by aging. However, even the type I fibers show some decline in size and number with aging.[5]

With aging, there is a natural decrease in skeletal muscle cross-sectional area, ranging between 21% and 40% compared to healthy young individuals.[6] This reduction is associated with poorer physical performance.[7] Additionally, muscle disuse can exacerbate this decline in the elderly, resulting in more pronounced changes.

Sarcopenia is a term used to describe the loss of muscle mass and strength that occurs with aging. It is a natural part of the aging process and can lead to functional decline and increased risk of falls and fractures in older adults. Cachexia, on the other hand, refers to the loss of muscle mass and weight that occurs in the context of an underlying illness, such as cancer or heart failure. While sarcopenia and cachexia share some similarities, they are distinct conditions with different causes and clinical implications.

The loss of muscle fibers is more pronounced in the fast-twitch (type II) fibers compared to the slow-twitch (type I) fibers. This is significant as type II fibers are responsible for generating the most force and power. Additionally, the loss of muscle fibers can also result in a reduction of muscle protein synthesis and an increase in muscle protein breakdown, leading to muscle wasting. This decline in muscle mass and function can have significant impacts on the overall health and quality of life in aging individuals, increasing the risk of falls, fractures, and loss of independence. Therefore, interventions such as exercise and proper nutrition are important to maintain muscle mass and function in aging individuals.[8]

The accumulation of Mitokondriyal DNA mutations in aging muscles is thought to be a result of oxidative damage from free radicals generated during energy production in the mitochondria. The Cytochrome C Oxidase (COX) deficient muscle fibers have reduced or absent activity of the COX enzyme, which is involved in the electron transport chain and Adenosine triphosphate production in the mitochondria. This can lead to a decrease in energy production and contribute to muscle weakness and atrophy. Ragged-red fibers are muscle fibers with abnormal accumulations of mitochondria, and they are often seen in mitochondrial myopathies. In aging muscles, ragged-red fibers are less common and are usually only found in small areas.[9]

Measurements of Muscle Atrophy

Ultrasonography (US) is a non-invasive imaging technique that uses high-frequency sound waves to produce images of internal structures.[10] It is a useful tool for assessing muscle structure characteristics, such as muscle thickness, fascicle length, and pennation angle. However, US images can be affected by artefacts that may interfere with automatic segmentation software, leading to inaccurate measurements. Therefore, careful attention must be paid to image acquisition and interpretation to ensure accurate results. Other imaging techniques, such as CT and MRI, may also be used to assess muscle structure, but they are more expensive and may expose the patient to ionizing radiation.[11]

MRI is considered the gold standard for soft-tissue segmentation and evaluation of muscle structure characteristics. MRI can provide high-resolution images with excellent tissue contrast and can detect changes in muscle composition and quality. It can also be used to assess muscle size, muscle fiber orientation, and muscle fat infiltration. The multipoint Dixon fat mapping MRI technique is a promising technique that can be used to accurately quantify fatty degeneration in various musculoskeletal disorders, including lumbar disc pathology.[12]

Cross-sectional area (CSA) of a muscle can be measured using different imaging techniques, including CT, MRI, and US. The CSA measurement by means of MRI has been found to be highly correlated with the clinical measure of muscle strength. This correlation can be explained by the fact that muscle strength is largely determined by muscle size, and CSA is a good indicator of muscle size. Therefore, changes in muscle CSA can be used to track changes in muscle strength over time.[13]

Physical Exercises for Muscle Atrophy

Therapeutic interventions for muscle atrophy aim to increase muscle protein synthesis and reduce

protein degradation. Some potential approaches include resistance exercise, dietary protein supplementation, pharmacological agents, and gene therapy. Resistance exercises have been shown to increase muscle protein synthesis and improve muscle strength in both young and older individuals. Dietary protein supplementation has also been shown to increase muscle protein synthesis, especially when consumed after exercise. Pharmacological agents such as anabolic steroids and growth hormone have been used to promote muscle growth, but they also have potential side effects. Gene therapy is a promising approach that involves the delivery of genes encoding for growth factors or other proteins that can promote muscle growth and regeneration[14]. Exercise has been shown to have a positive effect on muscle mass and function, especially in older adults. Resistance training, in particular, has been found to be effective in stimulating muscle protein synthesis and promoting muscle growth. In addition to resistance training, aerobic exercise has been shown to improve cardiovascular health and overall fitness. The recommended amount of exercise for adults is at least 150 minutes of moderate-intensity aerobic activity or 75 minutes of vigorous-intensity aerobic activity per week, along with muscle-strengthening activities at least 2 days per week.[15]

Exercise can stimulate muscle protein synthesis and increase muscle mass, which makes it an effective strategy for treating and preventing muscle atrophy. Resistance training, such as weight lifting, has been shown to be particularly effective at stimulating muscle growth and increasing muscle strength. It is important to note, however, that the specific exercise regimen needed to promote muscle growth and prevent atrophy may vary depending on the individual's age, health status, and other factors. It is recommended to consult with a healthcare professional or a certified exercise specialist to determine the most appropriate exercise program for each individual.[16]

Exercise can have a positive effect on mitochondrial function and biogenesis in aging skeletal muscle. Mitochondria are responsible for producing energy in cells, and their dysfunction has been linked to several age-related diseases, including sarcopenia. Exercise training can improve mitochondrial function and biogenesis by increasing the expression of certain genes involved in mitochondrial biogenesis, such as peroxisome proliferator-activated receptor γ coactivator-1α and nuclear respiratory factors. Moreover, exercise can also increase the volume of mitochondria in skeletal muscle, which can improve the metabolic capacity of the muscle and increase its endurance.[17]

Reverse Atrophy in Lower Motor Nerve Denervation

Skeletal muscle atrophy can result from a variety of factors, including neural and skeletal muscle injuries, prolonged bed rest, space flight, normal aging, and various diseases. It can lead to a significant loss of muscle size and strength, resulting in reduced physical function and increased morbidity and mortality. Therefore, developing effective treatments and prevention strategies for skeletal muscle atrophy is important for maintaining overall health and quality of life.[18]

Spinal cord injury can lead to muscle wasting due to the interruption of the communication between the brain and muscles below the level of the injury. The severity of the muscle wasting depends on the level and extent of the injury. In cases of complete and permanent damage to the lower motor neurons, the muscles innervated by those neurons can undergo severe wasting and become weakened or paralyzed. This can also result in decreased range of motion, impaired balance and coordination, and decreased quality of life. Rehabilitation programs that include exercise and physical therapy can help to mitigate the effects of muscle wasting and improve muscle strength and function in individuals with spinal cord injury.[19]

The preservation of the connection between the muscle and the nerve is crucial in spinal cord injury as denervation can lead to severe muscle atrophy and degeneration. In cases of complete peripheral nerve lesion, the denervated muscle loses its excitability with electrical stimulators and undergoes disorganization at the ultrastructural lev-

el within a few months. Over time, severe atrophy with nuclear clumping and fibro-fatty degeneration can occur, typically within 3 to 6 years. Therefore, it is essential to preserve the muscle-nerve connection and prevent denervation to maintain muscle function and prevent muscle wasting in individuals with spinal cord injury.[20]

Spasticity

Spasticity definition proposed by Lance is a "motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes with exaggerated tendon jerk, resulting from hyper-excitability of the stretch reflex". In this moment this definition omits several clinical manifestations of spasticity, like clonus and flexor withdrawal spasms seen with spasticity arising from spinal cord injury.[21,22]

Spasticity is a finding in the upper motor neuron syndrome, defined as injury to the motor neurons in the brain or their connecting pathways leading to the lower motor neurons of the spinal cord. Development of these pathways generate many clinical signs and symptoms. The main signs are: clonus, flexor spasms, and hyperactive tendon reflexes from excessive or inappropriate muscle activity. The development of spasticity is based on stretch reflex disorders. Stretch reflex refers to proprioceptive reflexes, which are tonic and included sustained stretch as in clonus and short stretch as in deep tendon reflexes.[13]

Pathophysiology of the Spasticity

Spasticity results from the loss of descending inhibition of spinal cord reflex arcs as well as loss of cortical inhibition on the postural centers. By this way, the upper motor neuron both inhibitory and excitatory pathways are affected, and the anterior horn cells and the corticospinal tract are disinhibited. The results are development of increasing the spinal cord reflexes secondary to a loss of cerebral inhibition. The anatomic structures involved in development of spasticity are presented in Figure 2.1.[23]

The spasticity physiopathology has two mechanisms; one refers to change in the function of spinal neurons and second are the cerebral mechanism encompassing supraspinal and suprasegmental mechanisms.[21] We can speak about hypersensitivity of the reflex arc due to changes in the spinal cord but also a decrease of inhibition to the supraspinal central nervous system and generate abnormal impulses.

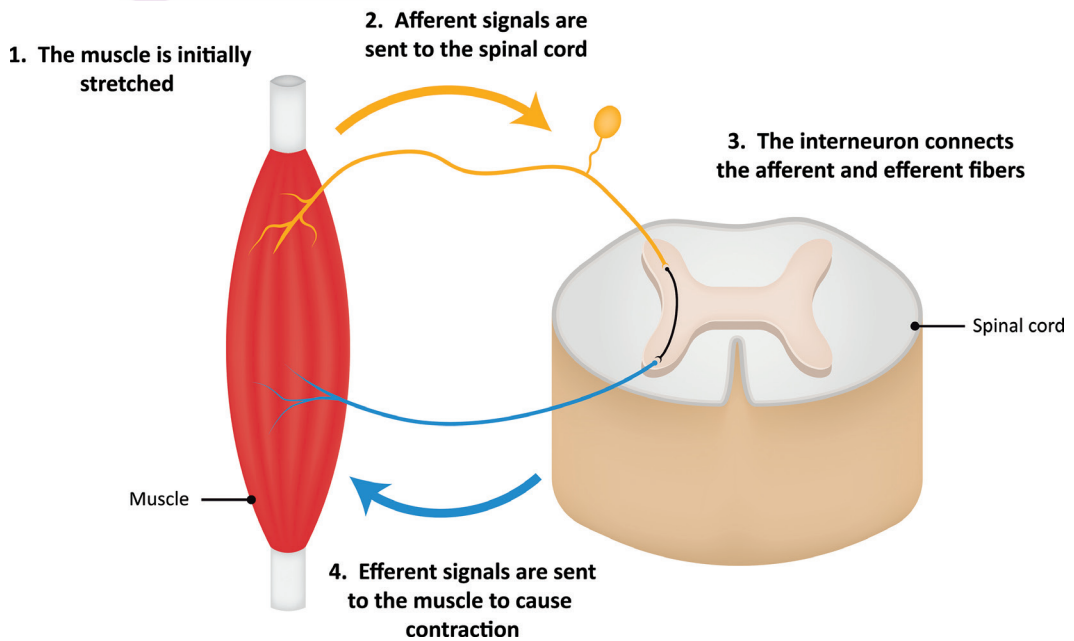

Figure 2.1 Anatomic structures of stretch reflex.

The "clasp knife phenomenon," is specific for spinal spasticity and describes the sudden disappearance of the velocity-dependent increase in resistance to passive stretch resulting in the affected limb moving akin to the opening of a clasp knife.[1]

Contractures

Contracture is defined to be the phenomenon that generates the loss of full active and passive range of motion in a limb, that results from limitations imposed by the joint, muscle or soft tissue.[21] The periarticular connective tissue restriction involving muscles, tendons, ligaments, and joint capsule. generate contracture in most of the situations but also could be the result of prolonged immobility of the limb and/or a lack of weight bearing in the lower limbs.[18] Much more the contracture is the feature of spasticity, dystrophic myopathies, neurologic disorders, trauma, burns, and generally any illness with resulting immobility place a patient at a high risk of developing contracture.[21]

The pathophysiology mechanisms of contractures start with immobilization in a shortened position and is correlated with loss of sarcomeres, accumulation of accumulation of connective tissue and fat in the muscle tissue.[21]

In the same time immobilization lead to decrease the muscle fiber diameter, the protein synthesizes and reduce the muscle volume. The loss of sarcomeres does not occur if the joint is in a neutral position. The remaining sarcomeres adapt to their shortened resting position and overlap optimally to potentiate maximal tension at the immobilized length. The following step from immobility to contracture formation is quantitative and qualitative changes in the intramuscular connective tissue.[21]

References

1. Yamada Y. Muscle mass, quality, and composition changes during atrophy and sarcopenia. Adv Exp Med Biol. 2018;1088:47-72. doi:10.1007/978-981-13-1435-3_3.
2. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; A quantitative review. Front Physiol. 2012;3:260. doi:10.3389/fphys.2012.00260.
3. Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci. 1995;50 Spec No:11-6. doi:10.1093/gerona/50a.special_issue.11.
4. Lee WS, Cheung WH, Qin L, Tang N, Leung KS. Age-associated decrease of type IIA/B human skeletal muscle fibers. Clin Orthop Relat Res. 2006;450:231-7. doi:10.1097/01.blo.0000218757.97063.21.
5. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. J Appl Physiol (1985). 2000;88(4):1321-6. doi:10.1152/jappl.2000.88.4.1321.
6. Visser M, Kritchevsky SB, Goodpaster BH, Newman AB, Nevitt M, Stamm E, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: The health, aging and body composition study. J Am Geriatr Soc. 2002;50(5):897-904. doi:10.1046/j.1532-5415.2002.50217.x.
7. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci. 1988;84(2-3):275-94. doi:10.1016/0022-510x(88)90132-3.
8. Greaves LC, Turnbull DM. Mitochondrial DNA mutations and ageing. Biochim Biophys Acta. 2009;1790(10):1015-20. doi:10.1016/j.bbagen.2009.04.018.
9. Wallwork TL, Stanton WR, Freke M, Hides JA. The effect of chronic low back pain on size and contraction of the lumbar multifidus muscle. Man Ther. 2009;14(5):496-500. doi:10.1016/j.math.2008.09.006.
10. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol (1985). 2000;89(1):104-10. doi:10.1152/jappl.2000.89.1.104.
11. Wokke BH, Bos C, Reijnierse M, van Rijswijk CS, Eggers H, Webb A, et al. Comparison of dixon and T1-weighted MR methods to assess the degree of fat infiltration in duchenne muscular dystrophy patients. J Magn Reson Imaging. 2013;38(3):619-24. doi:10.1080/01616412.2017.1314906.
12. Sattler M, Dannhauer T, Hudelmaier M, Wirth W, Sanger AM, Kwoh CK, et al. Side differences of thigh muscle cross-sectional areas and maximal isometric muscle force in bilateral knees with the same radiographic disease stage, but unilateral frequent pain – Data from the osteoarthritis initiative. Osteoarthritis Cartilage. 2012;20(6):532-40. doi:10.1016/j.joca.2012.02.635.
13. Wiggs MP. Can endurance exercise preconditioning prevention disuse muscle atrophy? Front Physiol. 2015;6:63. doi:10.3389/fphys.2015.00063.
14. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011;43(7):1334-59. doi:10.1249/MSS.0b013e318213fefb.
15. Glover EI, Phillips SM. Resistance exercise and appropriate nutrition to counteract muscle wasting and promote muscle hypertrophy. Curr Opin Clin Nutr. 2010;13(6):630-4. doi:10.1097/MCO.0b013e32833f1ae5.
16. Koltai E, Hart N, Taylor AW, Goto S, Ngo JK, Davies KJA, et al. Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. Am J Physiol-Reg I. 2012;303(2):R127-R34. doi:10.1152/ajpregu.00337.2011.
17. Kern H, Hofer C, Loefler S, Zampieri S, Gargiulo P, Baba A, et al. Atrophy, ultra-structural disorders, severe atrophy and degeneration of denervated human muscle in SCI and aging. Implications for their recovery by Functional Electrical Stimulation. Neurol Res. 2017;39(7):660-6. doi:10.1080/01616412.2017.1314906.

18. Carlson BM. The biology of long-term denervated skeletal muscle. Eur J Transl Myol. 2014;24:3293. doi:10.4081/ejtm.2014.3293.
19. Sajer S. Mobility disorders and pain, interrelations that need new research concepts and advanced clinical commitments. Eur J Transl Myol. 2017;27(4):7179. doi:10.4081/ejtm.2017.7179.
20. Kern H, Carraro U, Adami N, Biral D, Hofer C, Forstner C, et al. Home-based functional electrical stimulation rescues permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. Neurorehabil Neural Repair. 2010;24:709-21. doi:10.1177/1545968310366129.
21. Musculoskeletal Key. Spasticity and contractures. Accessed: https://musculoskeletalkey.com/spasticity-and-contractures.
22. Mitra R. Principles of rehabilitation (2019), McGraw-Hill Education. 1st ed. ISBN:978-0071793339.
23. PTdirect. Muscle Spindle and Stretch Reflex. Accessed: https://www.ptdirect.com/training-design/anatomy-and-physiology/muscle-spindles-the-stretch-reflex-and-force-generation.

Co-funded by the Erasmus+ Programme of the European Union

Denervated Muscle Physiology

MEHMET DURAY • GOKHAN BAYRAK

What is Denervation?

Denervation is a condition that usually develops due to damage to the central or peripheral nervous system. The skeletal muscles in human body, work voluntarily and contract or relax with the signals they receive from the nervous system. Skeletal muscle fiber contractions are controlled by alpha motor neurons in the anterior horns of the spinal cord and motor nuclei of the cranial nerves.[1] In healthy physiology, signal transmission is maintained between the peripheral nerve and skeletal muscle through the neuromuscular junction, ensuring muscle functions.[2] However, the denervation of skeletal muscles leads to loss of voluntary and reflex muscle activity, muscle atrophy, and changes in muscle excitability. Direct stimulation with a higher electrical stimulation (ES) intensity is required to achieve contraction in denervated muscles than in healthy muscles.[3]

The denervation that occurs after peripheral nerve injury varies in terms of recovery time depending on factors such as the type of injury, the age of the patient, and the distance between the lesion area and the cell body.[4] In denervation due to peripheral nerve damage, if the epineurium is intact, approximately 1 mm of regeneration occurs per day. Substantial atrophy and permanent loss of function can be observed in the denervated muscles if re-innervation is not achieved within the first year after denervation or if nerve repair is delayed too long to reach the muscle within this period.[5]

The activities of the muscle tissue depend on ES powered by neurotrophic factors from both the neuromuscular system and the autonomic nervous system. Therefore, after denervation, trophic, mechanical, and molecular changes occur as well as loss of muscle contractile function.[4] The first phase of denervation, which is characterized by rapid loss of muscle function, reduction in muscle mass, and atrophy of muscle fibers, begins immediately after the nerve impulse to the muscle is intercepted. In the second stage, sarcomere organization is disrupted and an increase in muscle atrophy is observed. In the last stage, the tissue architecture has thoroughly deteriorated and the process in which interstitial fibrous and adipocytes are dominant in the tissue begins. In this process, the number of muscle fibers decreases to a level that cannot sustain function, while functioning muscle fibers show little resemblance to healthy muscle fibers.[6,7]

Membrane Changes in Denervated Muscle

The cell membrane of a healthy muscle fiber has a resting membrane potential of -80 mV. After the denervation, myokymic and neuromyotonic discharges, muscle cramps, and fasciculations may develop due to functional impairment in the neural input to the muscle.[8] One of the first signs of denervation is the presence of spontaneous fibrillation in the muscle. This is associated with the spread of acetylcholine receptors along the muscle membrane from the early post-denervation period

and the deterioration in the general structure of the sarcoplasmic reticulum.[6] The membrane potential becomes more positive due to sodium ion flow to the cell membrane of the damaged muscle after denervation. The muscle cell tends to be less negative and therefore the denervated muscle cell is closer to the potential required for the generation of spontaneous action potentials. Spontaneous action potentials occur when the resting membrane potential in denervated muscle reaches -60 mV.[8] Besides, in the first 7 days after denervation, a decrease is observed in long-chain fatty acid transport to myocytes due to the decrease in the number of fatty acid and protein carriers.[9]

Cellular Changes in Denervated Muscle

As the protein breakdown in muscle is greater than protein synthesis, the process of muscle atrophy begins.[10] After denervation, collagen degradation is inhibited in skeletal muscle and this leads to collagen deposition in the muscle.[7] In addition, impairments are observed in the activation of the ability to produce new muscle fibers in denervated muscle.[6] Hence, the denervation not only causes atrophy in the muscle but also negatively affects the metabolic functions of the muscle cell.

Mitochondrial Activity

After the denervation, a complex plastic pattern initiates in the muscle, and slow-twitch oxidative type I muscle fibers start to transform into fast-twitch glycolytic type II muscle fibers. As a result of this transformation, greater dependence on anaerobic metabolism develops in the muscle cell. It is thought that this may be one of the reasons leading to a reduction in aerobic capacity and functional performance.[1] With the reduction in muscle fiber size after denervation, loss of mitochondrial content begins. The number of intracellular mitochondria drops, and their complex structural anatomy deteriorates and begins to exhibit a more primitive and simple structure.[6,11] This may explain the impairments in muscular endurance and muscle performance in skeletal muscles.[11]

Nutritional Functions

Skeletal muscle mass constitutes approximately 40% of the body weight, and having an optimal level of muscle mass is important for exercise and metabolic balance. The skeletal muscle is the main organ of not only physical movement in the body but also the body's glucose metabolism. Anabolic-catabolic balance, glucose metabolism, and maintenance and repair of muscle mass are under control in a healthy skeletal muscle.[1] In denervated muscles, on the other hand, glucose hypermetabolism is higher than in healthy muscles.[12] After the denervation, atrophic muscle fibers do not contain glycogen or RNA, do not show acid phosphatase activity, and succinate dehydrogenase activity is very low.[1] In addition, skeletal muscle denervation has adverse effects on insulin resistance. After 28 days of denervation, insulin resistance appeared in slow-twitch skeletal muscle fibers, whereas insulin sensitivity emerged in fast-twitch muscle fibers.[7]

Lysosomal Activities

Autophagy is a homeostatic mechanism used for the breakdown and subsequent recycling of the cytoplasm of the muscle and other intracellular organelles by the lysosomal mechanism. The autophagy lysosomal system is highly important for catabolism to control muscle volume. However, the autophagy lysosomal system requires homeostasis in the muscle fiber. With the loss of homeostasis after the denervation, deterioration in muscle cells arise. The autophagy lysosomal system shows low activity in healthy skeletal muscle tissue and aims to maintain this state. After denervation, the activation of the autophagy lysosomal system in skeletal muscle increases greatly and as a result, there is an increase in the destruction of proteins in the muscle.[1]

Changes in Muscle Tissue Level

After an axonal injury to a peripheral nerve, Wallerian degeneration occurs distal to the lesion site. This is due to the inability of the distal part of the peripheral nerve to receive structural proteins and neurotrophic substances from the soma.[13]

Atrophy

Muscle atrophy is basically a direct effect of protein degradation caused by pathophysiological conditions such as denervation, microgravity or immobilization, aging, disuse, and chronic diseases. Different degrees of muscle atrophy may occur after various pathologies affecting the peripheral system such as cancer, myopathies, and diabetes. Muscle atrophy is usually manifested by shrinkage of myofibrils, changes in muscle fiber and myosin isoforms, loss of cytoplasm, organelles, and proteins.[1]

Muscle atrophy due to denervation is observed when the neural connection to the muscle is interrupted or the muscle tissue cannot receive warning signals from the nervous system.[1] After denervation, the atrophy process begins rapidly as the muscle cannot receive the contractile stimuli necessary to maintain its normal size.[14] When skeletal muscle enters the atrophy process, fibrous connective tissue and adipose tissue take the place of functioning muscle tissue later on. At lower rates, a condition in which healthy muscle fibers are replaced by weakened muscle fibers is followed. The weakened muscle fibers are almost unrecognizable under the light microscope and are largely incapable of contraction.[6] Histopathologically, it reveals a characteristic appearance such as a very tight arrangement of myofibrils and their distribution around the nucleus. Loss of muscle mass and muscle strength occurs in all skeletal muscle atrophies (Figure 3.1).[1] In the initial stages of atrophy, the sarcomere structure is protected. In the second stage of atrophy, loss of actin and myosin monofilaments and disruptions in myofibrillar alignment are observed. Subsequently, changes occur in the size, number, and orientation of the sarcoplasmic reticulum system and its components. In the subsequent periods, significant declines in the number of capillaries associated with the muscle and losses in the nerve trunks innervating the muscle are followed.[6]

Despite all interventions for reinnervation, the recovery rate decreases after the first 3 months in the muscles that cannot achieve any innervation or have little recovery. It is stated that the probability of functional recovery of the muscle will be very low in the period from 1 to 2 years after denervation.[5,14]

Stem cells (satellite cells) in the muscle are responsible for the growth, repair, and regeneration of muscle tissue. Following denervation, an increase in stem cell proliferation appears in the muscle. However, as the denervation period extends, the number of stem cells in the muscle tends to decrease over time.[4] It is stated that long-term denervation periods such as 1 or 2 years will cause a reduction in the number of stem cells in the muscles and negatively affect the regeneration capability of the muscle. Depending on the level and

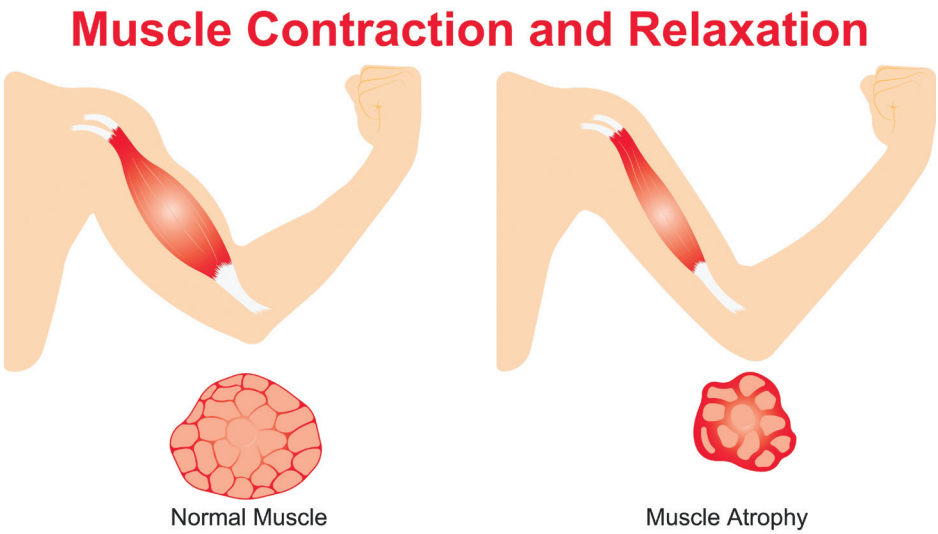

Figure 3.1 Muscle atrophy.

severity of injury in denervated muscles, the functional loss is seen first. It should be kept in mind that decreased muscle function will then lead to muscle atrophy and ultimately to contracture.[5] In long-term denervation, muscle fiber integrity is maintained with the activation and help of stem cells existing in the muscle. In this way, it is aimed to survive the atrophic skeletal muscle fibers and contributes to muscle recovery for reinnervation in the future.[7]

Age-related denervation arises with the loss of motor neurons in the spinal cord and/or loss of function of the neuromuscular junction. These losses increase with advancing age and after a point, permanent muscle denervation occurs in the skeletal muscles.[15] When this kind of denervation is analyzed microscopically, the sarcomere spacing within the muscle becomes irregular and the nuclei of muscle cells tend to converge to the center along the muscle fiber. In addition, there is a significant increase in adipose tissue in and around the muscle fibers.[1] In this respect, progressive denervation in old age is different from muscle denervation observed in relation to the central nervous system, peripheral nerve damage, or neuromuscular diseases.[15]

Disuse atrophy occurs in prolonged lying positions and lack of physical activity. It is frequently seen in conditions such as prolonged immobilization of joints, long bed rest, microgravity, dependence on mechanical ventilation devices, and coma. In such cases, muscle mass and myosin content in skeletal muscles are reduced and the muscle fiber type is converted from slow-twitch oxidative type I muscle fibers to fast-twitch glycolytic type II muscle fibers to adapt to the long-term reduction in physical activity.[1]

Fibrosis

After peripheral nerve damage, it takes a long time for impulses to pass through neuromuscular synapses and re-innervate the denervated muscles. In a such case, irreversible fibrous tissue is formed due to the extracellular matrix accumulating in the muscle.[1] In the final stage of muscle atrophy due to denervation, muscle fibers are fragmented, and muscle tissue is replaced by fibrous and adipose tissue. This is one of the most characteristic features seen in muscles after long-term denervation.[6,14] Muscle fibers that function after long-term denervation have a very low contractile capacity, and it is very hard to regenerate myofibrils even if the nerve impulse comes back.[14] Up to 4 months after the denervation, almost 80% of the muscle volume can be lost, and after approximately 2 years, irreversible muscle fibrosis and fat infiltration occur.[16]

Even if nerve fibers regenerate after the formation of fibrous tissue in skeletal muscle, effective neuromuscular synapses are unlikely to be formed.[2] Due to denervation atrophy, the length of the fibrous tissue that replaces the muscle fibers tends to continue to shorten in the subsequent period. This may lead to the formation of contractures in the muscle later on. Therefore, one of the most important goals in physiotherapy and rehabilitation is to prevent the development of contractures in atrophic muscles. In order to prevent the development of contracture, ES of atrophic muscles should be ensured after denervation until reinnervation begins, they should be kept in a tense position regularly with orthoses and assistive devices and supported by stretching exercises.[1,14] However, excessive stretching and incorrect positioning of the joints should be avoided.

Degeneration

Degeneration in the muscle is defined as a decrease in the cross-sectional area and density of the muscle, fatty infiltration and accumulation, and a decrease in muscle volume.[1] In the first 2 months after denervation, the degeneration process starts in the muscle fibers.[14] Muscle degeneration is closely related to myofibrillar protein fragmentation rates.[1] In addition, the degeneration process begins in cutaneous sensory receptors after denervation and sensory receptors may disappear 3 years after denervation.[16]

Muscle degeneration can be quantitatively evaluated as follows:

Grade 1: A normal muscle with fatty infiltration of 10% of the cross-sectional area of the muscle,

Grade 2: Moderate muscle degeneration and fatty infiltration of 10-50% of the cross-sectional area of the muscle,

Grade 3: Severe muscle degeneration and fatty infiltration of more than 50% of the cross-sectional area of the muscle.[1]

The Role of Genetic Factors in Denervated Muscle

The very high adaptability of skeletal muscle to environmental and intrinsic factors is due to secreted myokines and myometabolites. Denervation, disuse, feeding, etc. As atrophy begins to be observed after various reasons, a series of gene expression changes begin to be seen as well as biochemical and physiological processes. Although the examination of Deoxyribonucleic acid (DNA) microarrays in atrophic muscle provides detailed information about gene expression, it has not been clarified how genetic factors play a role from the onset of denervation atrophy. Recent data have divided the role of genetic factors on skeletal muscle into 4 phases during the denervation process, approximately between the first 30 minutes (min) and the 28th day. These phases are oxidative stress phase, inflammation phase, atrophy phase, and atrophic fibrous phase.[17]

Cytochrome P450 enzymes, which are activated in the oxidative stress phase, provide the conversion of toxic and estrogen metabolites into free oxygen radicals. Increased oxidative stress activates peroxisome proliferator activating receptor and hypoxia-inducible factor-1 signals. Thus, under the influence of genetic factors, the muscle begins to atrophy as a cellular adaptation mechanism to hypoxia.[17]

In the inflammatory phase, tumor necrosis factor (tnf), signal converter and transcription activator phosphorylated by the janus kinase enzyme, transforming growth factor-beta (TGF-beta), and nucleotide-binding oligomerization domain-like receptors (NOD-like) receptor and nuclear factor kappa B genes trigger the inflammatory response. With the onset of inflammation, the processes of atrophy formation and secondary changes in the denervated muscle manifest themselves more clearly.[17]

Denervated muscle, exposed to sufficient oxidative stress and inflammation, now begins to atrophy under the influence of up-regulated genes. Proteolysis occurs with the activation of phagosome, lysosome, endocytosis, and P53 signaling pathways. As protein degradation continues, muscle atrophy becomes evident. Meanwhile, sustained inhibition of the insulin signaling pathway during denervation also exacerbates muscle atrophy.[17]

Genes upregulated after the atrophic phase intensely induce fibrosis in denervated muscle for 14 days. While cyclic adenosine monophosphate and extracellular matrix synthesis further exacerbate denervated muscle fibrosis, increased ribosomal activity supports enzyme activity that plays a role in collagenation.[17] It has been stated that tumor necrosis factor-related weak inducer of apoptosis by binding to fibroblast growth factor-inducible 14 (Fn14) system, TGF-beta/myostatin pathway and matrix metalloproteinase (MMP) activity are among the genetic factors responsible for muscle atrophy, fibrosis, and performance loss during the denervation process. These activations, which disrupt the extracellular matrix modeling, contribute to fibrosis.[18]

Decreased chronic contractile activity reduces protein release from the nuclear and mitochondrial genome. In addition to the decrease in mitochondrial volume, proapoptotic protein secretion increases in all processes and programmed cell death is facilitated.[19]

Physiological Differences of Denervated and Healthy Muscles

The fact that denervated muscles exhibit different anatomical and physiological features than innervated muscles, the need to rehabilitate denervated muscles from the earliest time has arisen.[3] As explained in the previous sub-headings in the chapter, decrease in voluntary and reflex activity with muscle denervation, muscle atrophy, changes in muscle excitability and contractility, increase in

intramuscular fat and connective tissue, changes in the direction of muscle fiber bundles (especially in pennate muscles, the vectorial force that creates the contraction force decreases), muscle strength, muscle endurance, and cross-sectional area of the muscle, in short, the motor efficiency of the muscle decreases.[3,20] In the first minutes of axon damage, the increase in the concentration of Ca^{+2} ion within the axon, resulting in the increased Ca^{+2} amount closing the injury area, turns the injury area into an anastomosis site for many axon sprouts.[16] It should be kept in mind that all these changes will weaken the accommodation property of the denervated muscle and initiate the negative muscle plasticity process in the denervated muscle rapidly.[3,16] Table 3.1 summarizes the physiological differences between healthy and denervated muscle.[21,22]

The Importance of Electrical Stimulation of Denervated Muscle

In the treatment of denervated muscle, the primary goal of ES, which is one of the applications that has an important place apart from pharmacological treatments, is to provide physiological protection in the denervated muscle. If the appropriate modality is applied at the appropriate flow intensity, time, and frequency, denervated muscle can be protected from vascular congestion, trophic changes, and secondary complications such as progressive pain, atrophy, etc. that may occur over time.[23]

Events resembling "fuse burnout" in the first 12 hours after axonotmesis made it necessary to ensure the electrical functionality of the denervated muscle as soon as possible. After the acute process is taken under control, the damage and its effects must be eliminated.[16]

The importance of ES is emphasized and well-known. Appropriate ES is used to prevent progression of denervation-induced atrophy as well as atrophy treatment, restore contractility, facilitate a functional muscle stimulation rather than random innervation, to organize fiber type, convert maladaptive muscle plasticity to regenerative plasticity, increase the mitochondrial volume, increase the expression of antiapoptotic factors, decrease the release of proapoptotic proteins, and facilitate motor relearning.[19,20,24,25],

Table 3.1 Physiological differentiation in denervated muscles

	Denervated Muscle		Healthy Muscle
	Acute (0-3 months)	Chronic (+3 months)	
Axonal growth capacity	Yes, but decreased ⬇	Yes, but more decreased ⬇⬇	Yes
Atrophy	Yes ⬆	Yes, but further ⬆⬆	No
Infiltration of fat	Yes ⬆	Yes, but further ⬆⬆	No
Spontaneous activity	Yes ⬆⬆	Yes, but decreased ⬆	No
Muscle cell weight	Begins to decrease	Lower than 20% of healthy muscle weight	Normal
Cross-sectional area of the muscle	Begins to smaller ⬇	Smaller (Further) ⬇⬇	Normal
Decline of sensory input	Yes ⬇	Yes, but further ⬇⬇	No
Decline of motor input	Yes ⬇	Yes, but further ⬇⬇	No
Chronaxie (ms)	Higher (>0.08ms)	Higher (1-4 ms)	Normal (≦0.08 ms)

References

1. Xiao J. (2018), Muscle atrophy. 1st ed. Singapore: Springer Nature. ISBN:978-9811314353.
2. Xiang Y, Dai J, Xu L, Li X, Jiang J, Xu J. Research progress in immune microenvironment regulation of muscle atrophy induced by peripheral nerve injury. Life Sci. 2021;287:120117. doi:10.1016/j.lfs.2021.120117.
3. Pieber K, Herceg M, Paternostro-Sluga T, Schuhfried O. Optimizing stimulation parameters in functional electrical stimulation of denervated muscles: A cross-sectional study. J Neuroeng Rehabil. 2015;12(1):1-7. doi:10.1186/s12984-015-0046-0.
4. Jonsson S, Wiberg R, McGrath AM, Novikov LN, Wiberg N, Novikova LN, et al. Effect of delayed peripheral nerve repair on nerve regeneration, Schwann cell function and target muscle recovery. PLoS One. 2013;8(2):e56484. doi:10.1371/journal.pone.0056484.
5. Wong A, Pomerantz JH. The role of muscle stem cells in regeneration and recovery after denervation: A review. Plast Reconstr Surg. 2019;143(3):779-88. doi:10.1097/PRS.0000000000005370.
6. Carlson BM. The biology of long-term denervated skeletal muscle. Eur J Transl Myol. 2014;24(1):3293. doi:10.4081/ejtm.2014.3293.
7. Kostrominova TY. Skeletal muscle denervation: Past, present and future. Int J Mol Sci. 2022;23(14):7489. doi:10.3390/ijms23147489.
8. Weiss JM, Weiss LD, Silver JK. (2021), Easy EMG-e-book: A guide to performing nerve conduction studies and electromyography. 3rd ed. Philadelphia:Elsevier Health Sciences. ISBN:9780323796866.
9. Zoladz JA. (2018), Muscle and exercise physiology. 1st ed. Academic Press. Elsevier. ISBN:978-0128145937.
10. Sakuma K. (2013), Basic biology and current understanding of skeletal muscle. New York: Nova Biomedical. ISBN:9781628083675.
11. Tryon LD, Crilly MJ, Hood DA. Effect of denervation on the regulation of mitochondrial transcription factor: A expression in skeletal muscle. Am J Physiol Cell Physiol. 2015;309(4):C228-238. doi:10.1152/ajpcell.00266.2014.
12. Choi JS, Seo HG, Oh BM, Choi H, Cheon GJ, Lee SU, et al. 18F-FDG uptake in denervated muscles of patients with peripheral nerve injury. Ann Clin Transl Neurol. 2019;6(11):2175-85. doi: 10.1002/acn3.50899.
13. Pınar L. (2019), Basic information of nervous system and muscle physiology. Ankara: Akademisyen Yayınevi. ISBN:9786059354684.
14. Hall JE, Hall ME. (2020), Guyton and Hall textbook of medical physiology e-book. 14th ed. Philadelphia: Elsevier Health Sciences. ISBN:9780323640039.
15. Soendenbroe C, Andersen JL, Mackey AL. Muscle-nerve communication and the molecular assessment of human skeletal muscle denervation with aging. Am J Physiol Cell Physiol. 2021;321(2):C317-C329. doi:10.1152/ajpcell.00174.2021.
16. Phillips JB, Hercher D, Hausner T. (2022), Peripheral nerve tissue engineering and regeneration. 1st ed. Switzerland: Springer Nature. ISBN:9783030210519.
17. Shen Y, Zhang R, Xu L, Wan Q, Zhu J, Gu J, et al. Microarray analysis of gene expression provides new insights into denervation-induced skeletal muscle atrophy. Front Physiol. 2019;10(1298):1-14. doi:10.3389/fphys.2019.01298.
18. Pinheiro-Dardis CM, Russo TL. Electrical stimulation based on chronaxie increases fibrosis and modulates TWEAK/Fn14, TGF-β/myostatin, and MMP pathways in denervated muscles. Am J Phys Med Rehabil. 2017;96(4):260-7. doi:10.1097/PHM.0000000000000601.
19. O'Leary MF, Hood DA. Effect of prior chronic contractile activity on mitochondrial function and apoptotic protein expression in denervated muscle. J Appl Physiol. 2008;105(1):114-20. doi:10.1152/japplphysiol.00724.2007.
20. Bersch I, Fridén, J. Electrical stimulation alters muscle morphological properties in denervated upper limb muscles. EBioMedicine. 2012;74:103737. doi:10.1016/j.ebiom.2021.103737.
21. Tubbs RS, Rizk E, Shoja M, Loukas M, Barbaro N, Spinner RJ. (2015), Nerves and nerve injuries: Vol 2: Pain, treatment, injury, disease and future directions. 1st ed. Academic Press (E-book). ISBN:9780128026953.
22. Kimura J. (2013), Electrodiagnosis in diseases of nerve and muscle: Principles and practice. 4th ed. Oxford University Press (E-book). ISBN:978-0199738687.
23. Mazzanti M. (2022), Mechanisms in cell physiology. 1st ed. Newcastle: Cambridge Scholars Publishing. ISBN:978-1527582484.
24. Gera S, Gangadharan N, Navin BP, Tharion G, Chalageri PH, Thomas R, et al. Electrical stimulation and assessment of the induced force in the denervated muscle. IEEE. 2019;10:2046-50. doi:10.1109/TENCON.2019.8929282.
25. Salvini TF, Durigan JL, Peviani SM, Russo TL. Effects of electrical stimulation and stretching on the adaptation of denervated skeletal muscle: Implications for physical therapy. BJPT. 2012;16(3):175-83. doi:10.1590/s1413-35552012005000027.

CK4STEM

Co-funded by the Erasmus+ Programme of the European Union

Reinnervated Muscle Physiology

MEHMET DURAY • GOKHAN BAYRAK

Reinnervation Process

Reinnervation refers to the process by which the muscle becomes excitable again by the motor nerves after injury. In this process the anatomical and physiological neuromuscular connection between the muscle and the nerve must be restored, primarily. To provide this, it is aimed to achieve a healthy neuromuscular junction function during the reinnervation process.[1] The neuromuscular junction, which is the transition point of nerve conduction and expresses the interface of nerve and muscle, is the area of electrochemical connection between nerve and muscle (Figure 4.1).[1,2] The neuromuscular junction consists of three basic structures. 1) nerve ending containing acetylcholine vesicles released from the synaptic gap, 2) motor end plate covered with acetylcholine receptors, and 3) unmyelinated terminal Schwann cell or perisynaptic Schwann cells lining the nerve ending and synapse.[2] In the early stages of reinnervation, newly formed and immature neuromuscular junctions are often not successful in providing the correct impulse conduction. It has been suggested that this happened due to variability in motor endplate conduction or blockage of neural conduction along certain muscle fibers within the motor unit.[1]

There are many factors that may affect muscle reinnervation after nerve injuries, such as incision length, age, and local edema.[3] In a peripheral nerve, degenerative environmental factors near the injury location affect the survival of motor neurons. The

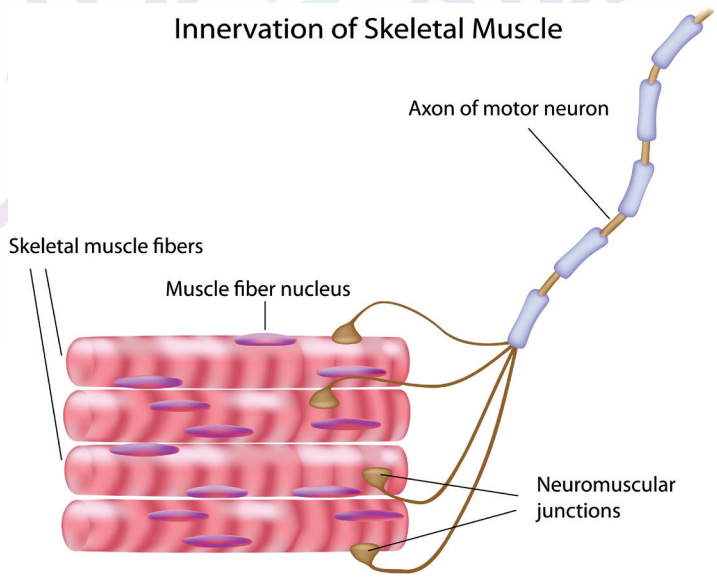

Figure 4.1 Neuromuscular junction.

fact that motor neurons struggle for survival rather than functioning sufficiently creates a restrictive effect on the formation and maturation of neuromuscular connections.[4] If there is a partial nerve injury, muscle reinnervation is endeavored by "axonal collateral sprouting".[1] To reach the muscle after injury, the peripheral nerve attempts to find a way toward the muscle by forming new sprouts from the proximal axon end to regenerate immediately. If no tissue such as necrosis that can occur in the terminal axon, newly developing neurites sprouts and head toward the muscle they innervated before.[3] However, cell death can be observed in muscles that experience loss of innervation after an axonal injury.[5] In order to prevent such a degenerative effect, an increase in the activity of the reinnervated muscle should be provided to contribute to this process as a positive regulator.[4]

In recent years, many surgical techniques have been developed for the reinnervation of denervated muscles. These techniques include direct nerve repair, nerve graft placement, nerve transfer, and neurotization methods.[6] However, despite the present knowledge regarding nerve regeneration mechanisms and newly developed surgical approaches, the desired muscular functional level cannot be achieved in most of the patients with peripheral nerve injury after surgical intervention. In peripheral nerve repair, various factors such as the type and location of the injury, the duration of muscle denervation after injury, and the age of the patient are important for restoring motor function. If nerve regeneration is not obtained within the first 1-1.5 years following peripheral nerve repair, degeneration begins at the neuromuscular junction, and as a result, potential muscle reinnervation is prevented.[2] At this stage, the stimuli with the potential to create new functional connections are sent to the muscle fibers in the maladaptive neural plasticity process that occurs in the proximal nerve fiber, and the muscle is endeavored to be reinnervated using a different pathway.[7] In addition, collateral sprouts (reactive synaptogenesis) formed in regenerated nerves and the resulting terminal branches may create new functional junction points more proximally to the neuromuscular junction.[1,5]

A reinnervated muscle has difficulty restoring its existing mass to a healthy level and regaining its former functional capacity. This may be due to the inability of neural connections to innervate the entire muscle fibers after injury.[7] It was stated that 87% of the damaged peripheral nerves are repaired with classical end-to-end anastomosis surgery. However, only half of these patients were found to be able to regain functional functions after nerve repair.[6] A reinnervated muscle will strengthen its neural connections over time, and thus the nerve will be able to reach a near-normal transmission velocity in motor units. However, when compared to the pre-injury state of the muscle, functional recovery of up to 70-80% may arise in reinnervated muscles.[7]

Membrane Changes

There are frequent changes in muscle membrane potential following the denervation. Fibrillation potentials emerge in the muscle due to this irregularity in the membrane potential. However, by reinnervation in the muscle, the fibrillation potential disappears over time. The reduction of muscle atrophy and the completion of autonomic axonal sprouting during the reinnervation process contribute to the reduction of fibrillation potentials, which are markers of degeneration.[8]

Calcium (Ca^{2+}) dependent processes increase the probability of neuron survival as the muscle cell membrane enters the depolarization process in the reinnervation phase. However, if the intracellular Ca^{2+} level rises much above normal during the reinnervation phase, this may cause neuronal death. Inhibition of intracellular electrical activity increases the risk of neuronal death during the continued reinnervation process and may delay reinnervation by reducing axonal growth.[9] Therefore, during the reinnervation phase, the functional status of the patient should be checked regularly by rehabilitation professionals and changes in the membrane level should be determined

Cellular Changes

As part of the reinnervation process, the muscle undergoes enzymatic and metabolic regeneration.

These processes vary according to the type of muscle fiber activated.

Enzymatic Activity Changes

After the newly formed nerve connections in the reinnervation process, rearrangements occur in the enzyme and histochemical models of the affected muscle fibers. "Fiber-type grouping", which refers to the grouping of histochemically alike fibers in a reinnervated muscle, is part of the reorganization that occurs in the motor unit structure.[10]

During the reinnervation, changes occur in myofibrillar protein isoforms, regulatory proteins, and enzyme activity patterns related to the proteins and energy sources involved in Ca^{2+}-ATPase and Ca^{2+} uptake. Accordingly, slow-twitch and oxidative or fast-twitch and glycolytic phenotypes of myofibrils are determined.[11]

Metabolic Changes

While the denervated muscle fibers resize and shape after reinnervation, the existing satellite cells in the cell nucleus are preserved. However, the inability of chronically denervated muscle fibers to fully return to their pre-injury size suggests that there may be a limit to the proliferation capacity of satellite cells.[12] The adequacy of axonal supply in the reinnervation process after peripheral nerve injury is seen as an important factor for the muscle to regain its strength-generating abilities.[6]

Muscular Reorganization in the Reinnervation Process

After the denervation, muscle fibers are reinnervated by collateral sprouts from intact motor units (Figure 4.2). Because these newly formed neural collateral sprouts are unmyelinated or thinly myelinated, nerve transmission is very slow. Reinnervation of muscle fibers is mainly dependent on motor unit action potential, which has a short transmission time and is easily generated.[1] To which tissue the sprouts formed in the regenerated motor axons will be directed is provided by a feature called "preferential motor reinnervation". The preferential muscular reinnervation process is supported by revealing some receptors and endogens under the influence of ectopic agents. With this feature, motor axons going in undesired directions are trimmed and only the desired muscle fiber is directed toward the desired muscle fiber.[13]

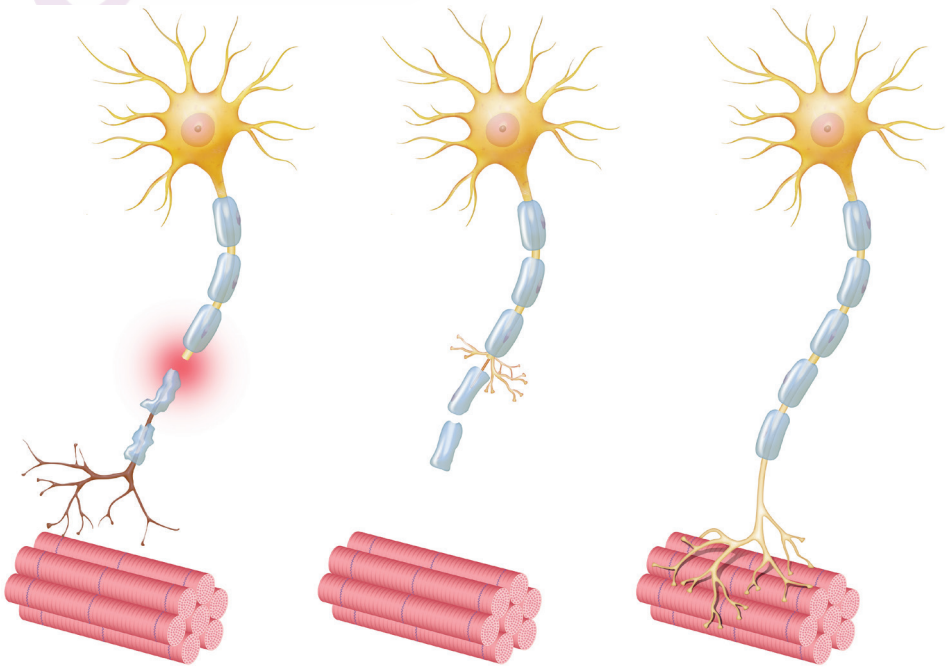

Figure 4.2 Axonal collateral sprouting.

As functional reinnervation of the muscle after peripheral nerve injury mainly focuses on motor function such as coordination of muscle function, muscle tone, and fine motor movements, little attention has been spent on signs of sensory recovery. However, in electromyographic and electroencephalographic evaluations, it has been proved that the sensory reflex of the muscle is preserved by the reinnervation of afferent fibers and muscle mechanoreceptors.[14] In addition, it has been reported that reinnervation directly affects the molecular structure of muscle myofibrils.[11] As a result of the reduction in the number of axons after denervation and the shrinkage of the muscle fibers due to denervation, a decrease in the strength of the muscle is observed. Therefore, early reinnervation is critical for muscle function. In addition, the large cross-sectional area of the muscle that can function in reinnervated muscle fibers may be due to the increase in neural activity.[15] This indicates the importance of physiotherapy and rehabilitation approaches such as physical activity and electrotherapy that support the physiological process of muscle reinnervation to increase neural activity after denervation.

Motor Innervation in Reinnervated Muscle

"Collateral sprouting" that appears during the reinnervation phase creates satellite potentials by extending from the healthy nerve axon to nearby denervated muscle fibers. However, new neuromuscular junctions that emerge with reinnervation cannot complete their maturation in a short time. Therefore, a decrease in the response to repetitive neural stimulation may be observed in newly formed neuromuscular junctions.[1] In addition, the regenerated nerve fibers included in the surgically repaired nerves after peripheral nerve damage may misdirect and lead to targets that they did not stimulate before. This situation continues to be one of the major challenges in terms of functional recovery in muscles after peripheral nerve injuries.[12]

After denervation, the nerve transmission velocity within the muscle fiber may decrease up to 0.5 m/s. As a result of a decrease in the nerve transmission velocity to these levels, improvement in nerve transmission velocity within the muscle fiber may not be achieved despite nerve regeneration. This indicates that atrophied small muscle fibers may not be receptive to regenerated axons.[8] Therefore, it is important to support the process of increasing nerve transmission velocity.

Muscle Spindle and Golgi Tendon Organ in the Reinnervation Process

Numerous changes are observed in motor unit properties during the reinnervation process. These changes, defined as muscle plasticity, include muscle contraction properties and biochemical changes.[16]

After the denervation, a rapid degeneration process initiates in the muscle spindles. Compared to the healthy controlled muscle, the number of tension receptors in the denervated muscle is considerably reduced. Interestingly, muscle spindles can be repaired without muscular reinnervation. However, an abnormal tension response is observed in reinnervated muscle spindles. Sometimes an increased stretch reflex is encountered, sometimes a decrease in the stretch reflex is observed.[14]

The Golgi Tendon Organ is a protective mechanism located at the muscle-tendon junction and reaching 1 mm in length. While information from the Golgi tendon organ is transmitted to the central nervous system, there is no direct efferent connection to the Golgi tendon organ, unlike the muscle spindle.[5] Therefore, the level of the functional recovery of the Golgi tendon organ in muscle after reinnervation is lower than that in muscle spindles.[14] In a reinnervated muscle, only the 50-75% of the muscle spindle and Golgi tendon organs can be reinnervated.[17] In this regard, the tension response of reinnervated muscles and a reduction in tension-induced tone are likely. For this reason, the inability to achieve an optimal tone in the reinnervation phase induces difficulties in daily living activities. It is necessary to be careful in the rehabilitation process during the reinnervation of the muscle, especially in stretching exercises.

Changes in Tissue Level in Reinnervated Muscle

Muscle cell apoptosis, an intracellular mechanism after denervation, plays a role in the atrophy process of muscle fibers. In post-denervation muscle atrophy, the release of apoptosis-related proteins increases particularly. Although the apoptosis response varies according to the type and duration of injury, it was stated that the apoptosis mechanism in the muscle approaches normal physiological levels in case of complete reinnervation.[18] Considering the destructive effect of the apoptosis mechanism in the post-denervation muscle atrophy process, the importance of reinnervation in terms of protecting the muscle structure becomes more prominent.

After the denervation, reinnervation does not occur in the whole muscle fibers, which results in muscle weakness.[19] Therefore, as the muscle becomes atrophic after denervation, both the tetanic strength of the muscle and the diameter of the muscle fibers lessen. The stimulation of muscle fibers in the process of reinnervation slows down the process of atrophy and muscle weakness. With the reinnervation, the maximal isometric tetanic strength is gradually restored firstly.[8]

In terms of muscle atrophy, it is of great importance to initiate the reinnervation process in the denervated muscle at the earliest period. A normal relationship between muscle fiber size and strength is not observed during the early stages of reinnervation. As soon as reinnervation is achieved, the muscle fibers a bit more atrophied, producing less power. While healthy motor units have a homogeneous structure in terms of their metabolic properties and contractile filaments (actin and myosin content), motor units in the reinnervation stage have heterogeneous properties. This is due to the activation of non-activated muscle fibers in the motor unit with reinnervation.[12]

If denervation is prolonged following the interruption of the impulse connection between the muscle and the nerve, the chances of full functional recovery are almost completely reduced. In addition to the loss of motor function, impaired collagenization, and an increase in fibrous tissue muscle atrophy in the denervated muscle can reduce the susceptibility of the muscle to reinnervation. Therefore, correct signaling should be provided from an early period in order to prevent inadequate and incorrect reinnervation.[20] Atrophied denervated muscle fibers do not lose their vitality for months or even years. In this respect, with the appropriate therapeutic interventions used in the reinnervation stage, a tremendous contribution is made to the functional and morphological recovery of the muscle. It should not be forgotten that even if the sarcomere structure of the muscle fibers is disrupted for reinnervation after short-term denervation, the fascicular architecture will be preserved. However, prolonged denervation makes intramuscular fatty infiltration apparent and muscle fiber necrosis is remarkable. Concomitant degeneration may continue due to the persistence of the long-term effects of denervation, as well as the regeneration of viable atrophic fibers in the reinnervation phase. Proper interventions reduce the effect of degeneration, which allows sarcomeres to become more regular. The findings show that post-injury muscle fibers regenerate while experiencing the necrosis process. Due to the existence of this cycle even in the degeneration phase, the need to recognize the physiological process and determine the proper therapeutic methods to increase the innervation of the muscle during the reinnervation phase is very important.[21]

Changes in Contraction Time and Intensity

Fasciculation potentials, which occur spontaneously, slowly, and irregularly in denervated muscle, are characterized by a firing potential of 1-2 Hertz (Hz). However, the motor unit potentials seen in a voluntary healthy muscle contraction are at the frequency level of 4-5 Hz and an ignition potential lower than this level cannot be realized. For this reason, motor unit action potentials fired at levels below 4-5 Hz in the muscle during the reinnervation process are fasciculation potentials that are not under voluntary control.[1]

The abnormal spontaneous electrical activity that occurs with denervation begins to be repaired

during the reinnervation process. During the reinnervation process, the first existing and spontaneously originating depolarizing fibrillations disappear.[8,13] New motor units formed after functional new connections can now perform voluntary contractions. While the intensity of contraction in the early stages of the reinnervation process is low, the intensity of contraction increases as a result of the growth in the motor unit over time. However, if a permanent loss occurred in some of the motor units, the size of the muscle fibers functioning in the active motor units may exceed the normal size.[13]

Muscle Fiber Type Changes

The proportion of type I muscle fibers is nearly half that of type II muscle fibers in a healthy person. This ratio may vary in some muscles in the body.[3] However, changes are observed in the ratios of type I and II fibers in the muscle during the reinnervation process after peripheral nerve injury.[14] In the reinnervation process, the proportion of type I and II muscle fibers in the muscle may be altered when fast-twitch muscles are reinnervated by a nerve whose transmission function is slowed or slow-twitch muscle fibers are reinnervated by a myelinated nerve. The roughly 65% reduction of myonuclei in type II muscle fibers after long-term (approximately 7 months) denervation indicates that type II muscle fibers may be less sensitive to reinnervation than type I muscle fibers.[11]

In the reinnervation process that arises after denervation due to old age, an expansion may occur in the activation level of thinly myelinated motor neurons with low-speed conduction. As a result of this process, the transformation from fast-twitch type II muscle fibers to slow-twitch type I muscle fibers emerges in muscles reinnervated by motor neurons with slow nerve conduction velocity.[22] Reinnervation capacity is quite higher in people under 20 years of age. It was stated that this was due to the need for a shorter nerve regeneration time due to axonal growth, less atrophy progression, and the strong regenerative capacity of people under 20 years of age.[10]

In addition to normalizing the physical properties of the muscle during the reinnervation phase, it is of great importance to provide a healthy neuronal activation pattern in the muscle and to support the optimal rate of fiber type change. In the clinical decision-making process, the duration of stimulation should be considered to reach healthy and functional muscles

- Stimulation given to the muscles for less than 5% of the day converts the heterogeneously distributed muscle fibers into fast glycolytic fibers (Type IIb),
- Stimulation given to the muscles for 5% of the day converts the heterogeneously distributed muscle fibers into fast glycolytic fibers (Type IIa),
- Stimulation given to the muscles 50% of the day transforms the heterogeneously distributed muscle fibers into slow oxidative fibers.[12,23]

The human neuromuscular system has impressive developmental flexibility. By choosing proper rehabilitation strategies, muscle fibers that are suitable for the healthy structure and physiology of the muscle can be obtained.[12]

The Role of Genetic Factors in the Reinnervation Process

In order to achieve normal muscle function in the reinnervated muscle, muscle evolution and differentiation programs must be initiated. This is possible with the introduction of some genetic factors.[24,25] In order to control the activation time of living Deoxyribonucleic Acid (DNA) genes in the fulfillment of cellular functions, transcription factors that attach to DNA are needed. Transcription factors show dynamic activation by interacting with different proteins, receptors, and hormones. Among the factors classified as basal and regulatory transcription factors, those with basal function are involved in gene synthesis for the biochemical and structural functionality required for the continuation of cell homeostasis.[26] Regulatory transcription factors control the Ribonucleic Acid function. The co-functioning of both transcription factors supports the evolution and differentiation programs of cells and tissues.[27] The Myoblast De-

termination (MyoD) protein plays a special role in the reinnervated muscle. Activation of MyoD in reinnervated muscle triggers muscle cell differentiation, initiating activation of Myogenic Factor 5, Myogenin, and Myogenic Regulatory Factor 4.[24,25] Triggered myogenesis facilitates the differentiation and regeneration of muscle tissue.[28]

Through therapeutic interventions such as electrical stimulation (ES), myogenic precursor cells are activated, promoting muscle regeneration and growth. In addition to increasing the concentration of free Ca^{2+} in the cytoplasm, ES also boosts the expression of the MYoD gene and myogenin.[30] Augmented MYoD gene, expression of myogenin, and increased oxygen utilization trigger muscle cell differentiation and muscular reinnervation.[24,25,28]

Therapeutic interventions in the reinnervation process reduce the activity of superoxide dismutase, which increases oxidative stress despite increased oxygen use. This leads to myotube hypertrophy and an increase in Mechanistic Target of Rapamycin complex and Extracellular Signal-Regulated Kinase 1/2 activity. Subsequently, while the transport of glucose transporter type 4 to the plasma membrane increases, glucose consumption also increases, and glucose metabolism changes.[29] Increased contractions during reinnervation activate Antimicrobial peptides (AMP)-activated protein kinase, raising glucose uptake.[29]

Supporting Muscle Reinnervation

Interventions to provide muscle reinnervation should aim to raise the size of innervated muscle fibers.[12,30] The reinnervated muscles may also have muscle fibers that are adversely affected by interventions such as ES, but this proportion does not exceed 5% when proper therapeutic intervention is executed. With proper interventions, the number of motor units in the muscle can be raised significantly in the first few months. In optimally supported muscle fibers, the reinnervation process is shortened, reaching a healthy muscle level in terms of the number of innervated motor units in the 3rd month.[30]

Augmenting Neuromuscular Plasticity in Muscle Reinnervation

There are two assumptions that a healthy neuromuscular structure can be achieved with the proper therapeutic stimulus of the reinnervated muscle. The first is to maintain and support the neuromuscular connection formed during embryonic development with appropriate therapeutic interventions. Motor units, which replace their most primitive state in denervation, can give healthy responses to environmental and ectopic stimuli during reinnervation.[31] The second is muscle plasticity that leads to synaptic changes with intramuscular fibrils.[31] Thus, muscle reinnervation mechanisms involve directing the damaged peripheral nerve to the stimulated muscle fibers. After nerve injury, randomly extending axons may begin to innervate different muscle fibers located other than the motor unit. Muscle reinnervation may be facilitated by avoiding synkinesis (misdirection of the nerve) with ES.[30] Due to the misdirection of the nerves in the regeneration phase, problems are experienced in the acquisition of fine motor movements in the reinnervated muscles and the normal functioning of the reinnervated muscles.[32]

References

1. Preston DC, Shapiro BE. (2020), Electromyography and neuromuscular disorders e-book: Clinical-electrophysiologic-ultrasound correlations. 4th ed. Elsevier. ISBN:978-0323661805.
2. Vannucci B, Santosa KB, Keane AM, Jablonka-Shariff A, Lu CY, Yan Y, et al. What is normal? Neuromuscular junction reinnervation after nerve injury. Muscle Nerve. 2019;60(5):604-12. doi:10.1002/mus.26654.
3. Tuncbay T, Tuncbay E. (2004), Nöromüsküler hastalıklar: motor nöron periferik sinir sistemi ve kas hastalıkları. 2nci baskı. İzmir: Nobel Kitabevi. ISBN:9789755670319.
4. Grumbles RM, Almeida VW, Casella GT, Wood PM, Hemstapat K, Thomas CK. Motoneuron replacement for reinnervation of skeletal muscle in adult rats. J Neuropathol Exp Neurol. 2012;71(10):921-30. doi:10.1097/NEN.0b013e31826cf69a.
5. Shumway-Cook A, Woollacott MH. (2016), Motor control: translating research into clinical practice. 5th ed. Lippincott Williams & Wilkins. ISBN:9780781766913.
6. Sobotka S, Mu L. Comparison of muscle force after immediate and delayed reinnervation using nerve-muscle-endplate band grafting. J Surg Res. 2013;179(1):117-26. doi:10.1016/j.jss.2012.02.055.
7. Kuiken TA, Feuser AES, Barlow AK. (2013), Targeted muscle reinnervation: A neural interface for artificial limbs. 1st ed. Boca Raton: CRC Press. ISBN:9780429065996.

8. Wu P, Chawla A, Spinner RJ, Yu C, Yaszemski MJ, Windebank AJ, et al. Key changes in denervated muscles and their impact on regeneration and reinnervation. Neural Regen Res. 2014;9(20):1796-809. doi:10.4103/1673-5374.143424.
9. Grumbles RM, Liu Y, Thomas CM, Wood PM, Thomas CK. Acute stimulation of transplanted neurons improves motoneuron survival, axon growth, and muscle reinnervation. J Neurotrauma. 2013;30(12):1062-9. doi:10.1089/neu.2012.2797.
10. Konofaos P, Wallace RD. Basic science of muscle neurotization: A review. J Reconstr Microsurg. 2015;31(7):481-6. doi:10.1055/s-0035-1554937.
11. Zhou Z, Cornelius C, Eichner M, Bornemann A. Reinnervation-induced alterations in rat skeletal muscle. Neurobiol Dis. 2006;23(3):595-602. doi:10.1016/j.nbd.2006.05.012.
12. Gordon T. Peripheral nerve regeneration and muscle reinnervation. Int J Mol Sci. 2020;21(22):8652-76. doi:10.3390/ijms21228652.
13. Zochodne DW. (2008), Neurobiology of peripheral nerve regeneration. Illustrated ed. Cambridge: Cambridge University Press. ISBN:9780511541759.
14. Adidharma W, Khouri AN, Lee JC, Vanderboll K, Kung TA, Cederna PS, et al. Sensory nerve regeneration and reinnervation in muscle following peripheral nerve injury. Muscle Nerve. 2022;66(4):384-396. doi:10.1002/mus.27661.
15. Grumbles RM, Sesodia S, Wood PM, Thomas CK. Neurotrophic factors improve motoneuron survival and function of muscle reinnervated by embryonic neurons. J Neuropathol Exp Neurol. 2009;68(7):736-46. doi:10.1097/NEN.0b013e3181a9360f.
16. Kapelner T, Jiang N, Holobar A, Vujaklija I, Roche AD, Farina D, et al. Motor unit characteristics after targeted muscle reinnervation. PLoS One. 2016;11(2):1-12. doi:10.1371/journal.pone.0149772.
17. Phillips J, Hercher D, Hausner T. (2022), Peripheral nerve tissue engineering and regeneration: 1st ed. Springer Cham. ISBN:978-3030210533.
18. Lim JY, Han TR. Effect of electromyostimulation on apoptosis-related factors in denervation and reinnervation of rat skeletal muscles. Muscle Nerve. 2010;42(3):422-30. doi:10.1002/mus.21719.
19. Grumbles RM, Wood P, Rudinsky M, Gomez AM, Thomas CK. Muscle reinnervation with delayed or immediate transplant of embryonic ventral spinal cord cells into adult rat peripheral nerve. Cell Transplant. 2002;11(3):241-50. PMID:12075989.
20. Willand MP, Holmes M, Bain JR, Fahnestock M, de Bruin H. Determining the effects of electrical stimulation on functional recovery of denervated rat gastrocnemius muscle using motor unit number estimation. Annu Int Conf IEEE Eng Med Biol Soc. 2011;1977-80. doi:10.1109/IEMBS.2011.6090557.
21. Schmalbruch H, al-Amood WS, Lewis DM. Morphology of long-term denervated rat soleus muscle and the effect of chronic electrical stimulation. J Physiol. 1991;441:233-41. doi:10.1113/jphysiol.1991.sp018748.
22. Zoladz JA. (2018), Muscle and exercise physiology. 1st ed. Academic Press: Elsevier. ISBN:978-0128145937.
23. Kernell D, Eerbeek O, Verhey BA, Donselaar Y. Effects of physiological amounts of high- and low-rate chronic stimulation on fast-twitch muscle of the cat hindlimb. I. Speed- and force-related properties. J Neurophysiol. 1987;58(3):598-613. doi:10.1152/jn.1987.58.3.598.
24. Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell. 1987;51(6):987-1000. doi:10.1016/0092-8674(87)90585-x.
25. Sabourin LA, Rudnicki MA. The molecular regulation of myogenesis. Clin Genet. 2000;57(1):16-25. doi:10.1034/j.1399-0004.2000.570103.x.
26. Reinberg D, Orphanides G, Ebright R, Akoulitchev S, Carcamo J, Cho H, et al. The RNA polymerase II general transcription factors: Past, present, and future. Cold Spring Harb Symp Quant Biol. 1998;63:83-103. doi:10.1101/sqb.1998.63.83.
27. Veenstra GJ, Wolffe AP. Gene-selective developmental roles of general transcription factors. Trends Biochem Sci. 2001;26(11):665-71. doi:10.1016/s0968-0004(01)01970-3.
28. Braun T, Rudnicki MA, Arnold HH, Jaenisch R. Targeted inactivation of the muscle regulatory gene Myf-5 results in abnormal rib development and perinatal death. Cell. 1992;71(3):369-82. doi:10.1016/0092-8674(92)90507-9.
29. Chu XL, Song XZ, Li Q, et al. Basic mechanisms of peripheral nerve injury and treatment via electrical stimulation. Neural Regen Res. 2022;17(10):2185-93. doi:10.4103/1673-5374.335823.
30. Willand MP. Electrical stimulation enhances reinnervation after nerve injury. Eur J Transl Myol. 2015;25(4):243-8. doi:10.4081/ejtm.2015.5243.
31. Zealear DL, Rodriguez RJ, Kenny T, Billante MJ, Cho Y, Billante CR, Garren KC, et al. Electrical stimulation of a denervated muscle promotes selective reinnervation by native over foreign motoneurons. J Neurophysiol. 2002;87(4):2195-9. doi:10.1152/jn.00451.2001.
32. Thomas CK, Stein RB, Gordon T, Lee RG, Elleker MG. Patterns of reinnervation and motor unit recruitment in human hand muscles after complete ulnar and median nerve section and resuture. J Neurol Neurosurg Psychiatry. 1987;50(3):259-68. doi:10.1136/jnnp.50.3.259.

Co-funded by the Erasmus+ Programme of the European Union

Healthy Nerve Physiology

EVA ILIE

Introduction

Neurobiology is a fascinating and dynamic field that has important implications for both clinical practice and technology. It encompasses a wide range of topics, from the composition of human personalities to the development of consciousness, and seeks to understand these complex phenomena through quantitative methods. At the heart of neurobiology is the study of neurons, which are responsible for transmitting information throughout the body via complex electrochemical signals. As the mysteries of the nervous system unraveled, the understanding of the brain and its role in shaping human behavior and cognition will continue to expand and evolve.[1]

The nervous system plays a critical role in information processing, which involves the transfer of electrochemical signals throughout the body (Figure 5.1). It is responsible for establishing and maintaining reliable relationships between input and output, which is essential for many life functions. The transmission of information in the nervous system involves complex mechanisms, including the release of neurotransmitters, the opening and closing of ion channels, and the modulation of synaptic strength. These mechanisms allow the precise and efficient processing of information, which is critical for the normal functioning of the body.[2]

Nervous System Overview

The nervous system's primary function is to process information through communication between neurons within neural networks and with effector cells in organs. Neural signaling is electro-chemical in nature and consists of intercellular and intracellular components. Chemical signals, such as neurotransmitters and neuropeptides, are

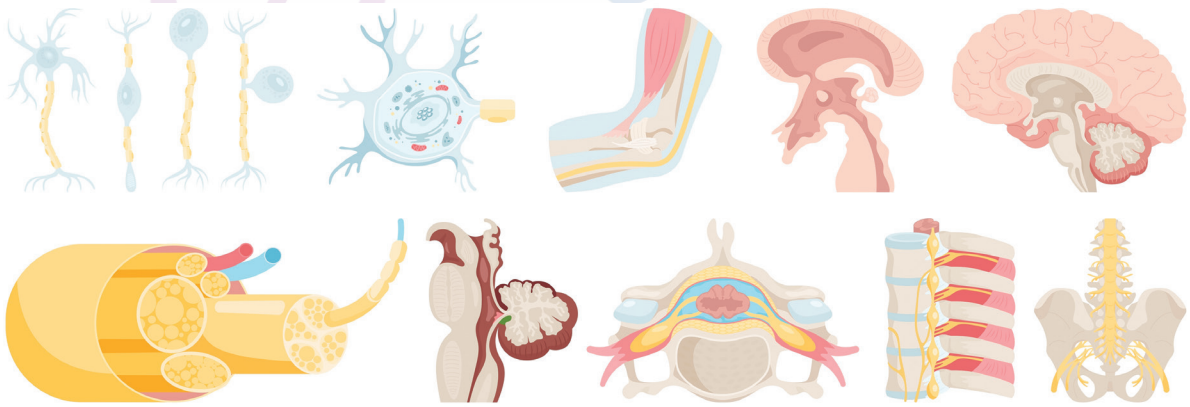

Figure 5.1 The nervous system.

transmitted through intercellular communication, which varies in intensity based on the strength of the stimulus.[3]

Anatomy and Physiology of the Central Nervous System

The central nervous system (CNS) is responsible for mediating all behavior, and it is comprised of the spinal cord and the brain (Figure 5.2). The brain can be divided into six major regions, each of which can be further divided into smaller, anatomically, and functionally distinct areas. These regions include the medulla, pons, cerebellum, midbrain, diencephalon, and cerebral hemispheres or telencephalon. Through these divisions, the brain is able to control a wide range of physiological and cognitive functions, allowing complex behaviors and actions. These divisions are present in both hemispheres of the brain, although they may differ in size and shape. The orientation of the CNS within the body is described by three axes, which refer to the rostral-caudal, dorsal-ventral, and medial-lateral directions. Understanding these axes is crucial while understanding the functions of the various components of the CNS.[4]

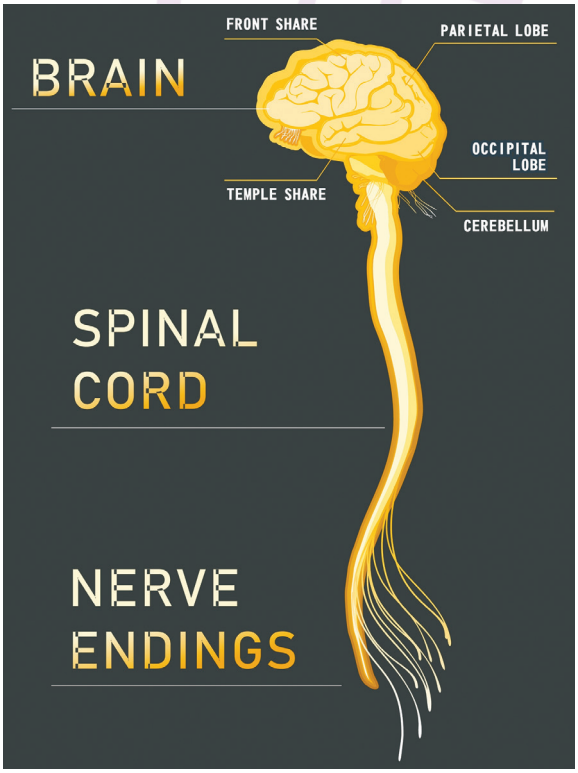

Figure 5.2 Central nervous system

Spinal Cord

The spinal cord is an essential part of the CNS that serves as a pathway for information to travel between the brain and the rest of the body. It plays a critical role in controlling reflexes and regulating motor movements. Additionally, the spinal cord is responsible for transmitting sensory information from the body to the brain, allowing us to perceive the world around us. While it may seem simple compared to the complex structures of the brain, the spinal cord is a vital component of the nervous system that enables to move, feel, and interact with the environment. The spinal cord extends from the base of the skull to the first lumbar vertebra, receiving sensory information from the skin, joints, and muscles and producing voluntary and reflex movements. It consists of gray matter containing nerve cell bodies, which are divided into dorsal and ventral horns, and white matter containing longitudinal tracts of myelinated axons that form ascending and descending pathways. Thirty-one pairs of spinal nerves are responsible for connecting the spinal cord to muscles and sensory receptors in the skin. The dorsal roots carry sensory information into the spinal cord, while the ventral roots carry motor commands out of the spinal cord.[4]

Medulla

The medulla is an extension of the spinal cord and regulates blood pressure and respiration.[4]

Pons

The pons is a structure located rostral to the medulla and protruding from the ventral surface of the brainstem. It contains pontine nuclei, which relay sensory and motor information between the cerebral cortex and the cerebellum. The dorsal part of the pons is involved in sleep, respiration, and taste.[4]

Midbrain

The midbrain is the smallest part of the brain stem and plays a crucial role in connecting different motor systems in the brain. The substantia nigra, a

nucleus in the midbrain, provides important input to the basal ganglia, a region that controls voluntary movements, and is implicated in Parkinson's Disease. The midbrain contains structures related to vision, hearing, and eye movements, and plays a crucial role in linking different motor systems in the brain.[4]

Cerebellum

The cerebellum, located above the pons, contains more neurons than any other part of the brain. It receives sensory input from the spinal cord, motor information from the cerebral cortex, and input about balance from the inner ear. The cerebellum is crucial for maintaining posture, coordinating head and eye movements, and fine-tuning muscle movements and motor skills.[4]

Diencephalon

The diencephalon has two subdivisions: the thalamus and hypothalamus. The thalamus relays sensory information (excluding olfactory) from peripheral receptors to the sensory processing regions in the cerebral hemispheres. The hypothalamus is located beneath the thalamus and regulates essential behaviors for homeostasis and reproduction, such as growth, eating, drinking, and maternal behavior, by controlling the pituitary gland's hormonal secretions.[4]

Cerebral Hemispheres

The largest region of the brain, the cerebral hemispheres, are responsible for perception, movement, memory, and emotion. They consist of the cerebral cortex, white matter, and three deep structures. The two hemispheres are connected by the corpus callosum.[4]

Anatomy and Physiology of the Peripheral Nervous System

Peripheral nerves allow nerve impulses to be conducted, enabling individuals to interact with the world while maintaining various postures of the trunk, head, and limbs. Peripheral nerve axons arise from various regions of the nervous system, including the sensory neurons in the dorsal root ganglia, autonomic neurons in the autonomic ganglia, and motor neurons in the ventral horn of the spinal cord or brainstem. To protect these lengthy extensions, axons are bundled together and insulated from each other by three connective tissue layers: the *endoneurium*, *perineurium*, and *epineurium*. This insulation allows efficient conduction of impulses along the axons and protects them from damage.[5]

Schwann cells are closely associated with axons within the *endoneurium*, where a single Schwann cell envelops a single myelinated axon to form an internode. Nodes of Ranvier are the points of separation between myelinating Schwann cells along a myelinated axon. The basal lamina provides a supportive matrix for the Schwann cells and helps to maintain the structural integrity of the nerve fibers. The loose connective tissue within the endoneurium contains various components such as type I and type II collagen fibrils in longitudinal orientation, fibroblasts, mast cells, macrophages, and endoneurial fluid.[5]

The *perineurium* is a dense connective tissue that surrounds and protects a bundle of axons to form a "fascicle". It is made up of up to 15 layers of flat perineurial cells, as well as collagen fibrils and elastic fibers that are arranged in different orientations to provide strength and flexibility to the structure. The perineurium also helps to regulate the exchange of substances between the blood vessels and the axons within the fascicle.[6] The perineurial diffusion barrier and blood-nerve barrier work together to regulate the exchange of substances between the endoneurium and surrounding tissues, protecting the nerve from harmful agents and maintaining proper nerve function. The layers of collagen and perineurial cells in the perineurium provide the nerve with mechanical strength, making it the primary load-bearing component of the nerve.[7]

The *epineurium* is the connective tissue layer that surrounds and holds together nerve fascicles. It contains collagen fibrils, elastic fibers, fibroblasts, mast cells, and fat cells. If the nerve has multiple fascicles, the epineurium may be divided into epifascicular and interfascicular layers.[8]

Anatomy and Physiology of Autonomic Nervous System

The anatomy of the Autonomic Nervous System (ANS) includes central control and feedback areas (such as the hypothalamus and brainstem), sensory receptors (such as baroreceptors and chemoreceptors), peripheral effectors (such as smooth muscle, cardiac muscle, and glands), and reflex conduction pathways (such as the sympathetic and parasympathetic pathways). The ANS also interacts with the endocrine system, with hormones (such as adrenaline and noradrenaline) playing a role in the sympathetic response.[9] Although there are no distinct centers of autonomic function in the cerebral cortex, higher cortical centers can modulate autonomic activity through cognitive processes (such as attention and decision-making). Additionally, reflexes that involve the ANS can be initiated at the level of the spinal cord or brainstem.[10]

External stimuli representing a threat or danger are detected by the senses and sent to the brainstem for reflex processing. The hypothalamus and limbic forebrain process these responses, and higher cortical centers provide descending input to the paraventricular nucleus of the hypothalamus. This nucleus then projects sympathetic and parasympathetic nuclei to initiate autonomic responses.[11]

The ANS is divided into two systems: the Sympathetic Nervous System (SNS) and the Parasympathetic Nervous System (PNS).[12] The SNS and PNS are anatomically and functionally divided components of the ANS (Figure 5.3). The thoracolumbar

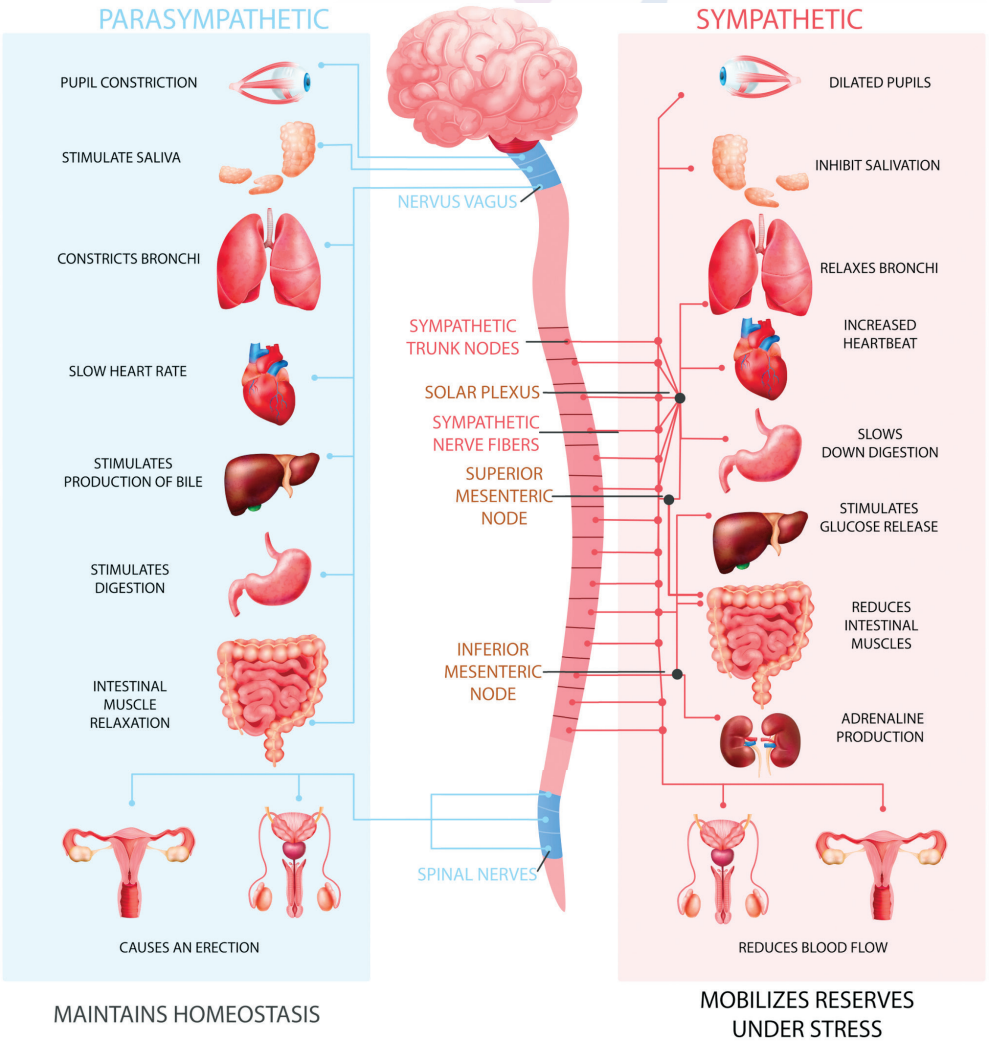

Figure 5.3 Peripheral autonomic nervous system.

spinal cord controls the peripheral SNS, whereas the brainstem nuclei and sacral segments control the PNS. The SNS produces widespread physiological responses, while the PNS exerts localized control over innervated organs. Both systems have efferent pathways that involve peripheral ganglia.[12,13]

Sympathetic Nervous System

The SNS consists of preganglionic fibers that originate in the thoracolumbar segments (T1-L3) of the spinal cord, specifically from the intermediolateral gray column. These myelinated fibers enter the paravertebral ganglia, and then they can travel up or down the sympathetic chain to synapse with the neuronal cell bodies of postganglionic sympathetic neurons. The unmyelinated postganglionic fibers of the SNS innervate their respective organs, allowing the physiological responses that result from sympathetic activation.[12]

Parasympathetic Nervous System

The PNS preganglionic fibers arise from the midbrain, medulla oblongata, and sacral segments of the spinal cord. They innervate organs through ganglia located near or directly in innervated tissues. Cranial nerves II, VII, IX, and X, particularly the vagus nerve (X), are major carriers of parasympathetic neuronal traffic. These fibers affect the heart, lungs, and abdominal organs, except for the distal portion of the colon, and provide innervation to the rectum and genitourinary tissues through sacral segments S2-S4.[13]

Nerve Structure

Neurons consist of a cell body called the soma, which contains organelles such as a nucleus, mitochondria, and lysosomes. At least one branch called a neurite extends from the soma, with axons relaying signals away from the soma and dendrites relaying signals towards it. The rough endoplasmic reticulum of a neuron is called Nissl substance and is exceptionally prominent due to its role in synthesizing the membrane for the neurites.[14]

During embryogenesis, the notochord induces the formation of the neural tube from the neuroectoderm, which gives rise to the CNS neurons, while the neural crest cells bordering the neural tube give rise to the PNS neurons (ganglion cells) and Schwann cells. Unlike some other cell types, neurons do not regenerate, so any neuronal loss is permanent. After birth, the nervous system is characterized by plasticity, which allows it to be shaped by experiences. Environmental factors such as sensory stimuli, relationships, hormones, and drugs can influence developmental processes like neural migration, maturation, and synaptogenesis.[15]

Myelin is a membrane that coats most axons and long dendrites to increase the speed of conduction of impulses. It is made by glial cells wrapping themselves tightly around a neurite, leaving gaps called "nodes of Ranvier". In the CNS, myelin is made by oligodendrocytes, whereas it is made by Schwann cells in the PNS. Each Schwann cell creates just one myelin bundle for an internode of a single axon.[16]

Nerves consist of bundles of axons from multiple neurons. Each individual axon is surrounded by a protective and connective tissue layer called the "endoneurium". Fascicles, or groups of axons, are then bundled together and surrounded by the "perineurium". Finally, the entire nerve is enclosed by the "epineurium", which also surrounds the blood vessels of the nerve. The endoneurium creates a fluid that protects the axons from injury. The perineurium and epineurium provide additional protection and support for the nerve.[17]

Innervation Process

Nerves send electrochemical signals to communicate throughout the body. There are three types of neurons: sensory, motor, and interneurons (Figure 5.4). Sensory neurons interpret sensory stimuli, motor neurons send messages to muscles or glands, and interneurons transmit signals between other neurons. Nerve fibers sending information to CNS are afferent, while fibers carrying information away from the central nervous system CNS are efferent.[18,19]

Sensory receptors are specialized structures within sensory neurons that detect changes in the environment and convert them into action po-

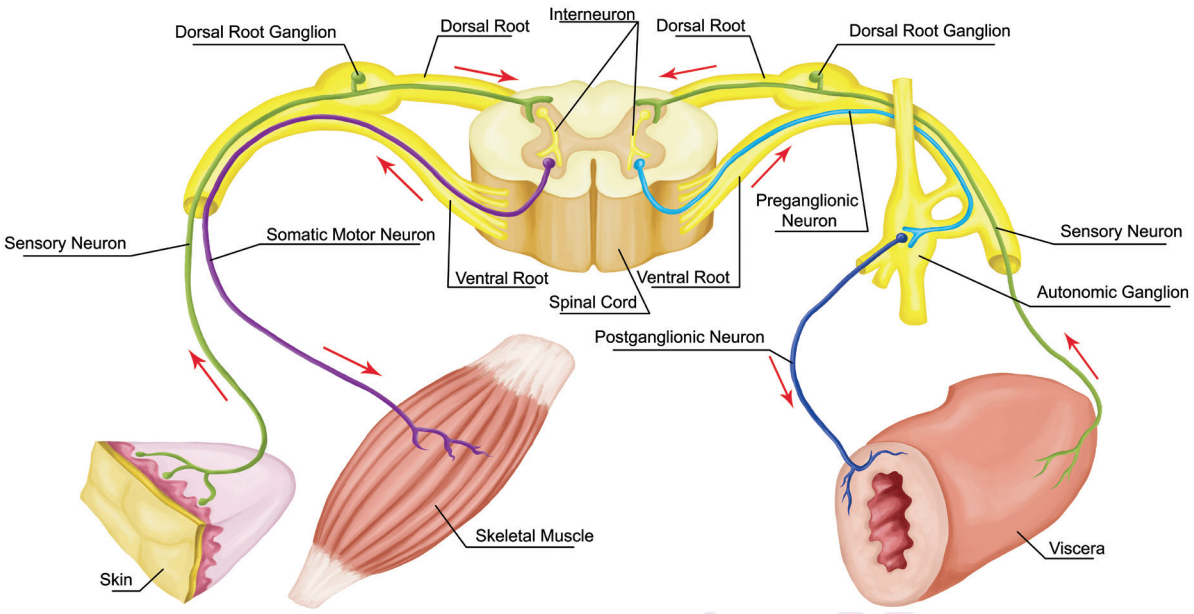

Figure 5.4 Innervation process.

tentials. These receptors are responsible for our five basic senses and other functions in the body. Nerve fibers are classified based on various factors, including diameter and myelination.[20]

Nerve impulses are transmitted through action potentials, which are brief changes in membrane potential. Neurons have a resting membrane potential due to separation of charge caused by the movement of sodium, potassium, and chloride ions into and out of the cell down their concentration and electrical gradients. The Na-K pump actively maintains this potential by pumping sodium and potassium against their concentration gradient. The neuron cell membrane is most permeable to potassium due to a large amount of potassium channels, making it most affected by relative potassium concentrations.[21]

When a neuron is stimulated, the opening of voltage-gated sodium channels causes a rapid influx of sodium ions into the cell, leading to depolarization and the generation of an action potential. This electrical signal then propagates down the axon and triggers the release of neurotransmitters at the synapse. Action potentials are all-or-none and require a minimum threshold of depolarization to occur.[22]

Voltage-gated ion channels mediate depolarization by allowing ions to flow across the cell membrane. Depolarization is caused by the influx of positively charged sodium ions, which triggers a rapid increase in membrane potential. Voltage-gated potassium channels then open, allowing potassium ions to flow out of the neuron, which brings the membrane potential back down. Sodium channels close first, followed by potassium channels, resulting in a brief hyperpolarization period. These channels have a refractory period where they cannot be reactivated, preventing redundant action potentials.[21]

Nerve impulses propagate down the length of the axon without losing amplitude through the inflow of sodium ions and the opening of adjacent voltage-gated ion channels. The speed of propagation is dependent on the concentration of sodium channels and the diameter of the axon. Myelin greatly increases conduction velocity by decreasing capacitance and increasing transmembrane resistance. Myelinated axons only develop action potentials at small gaps in myelin called nodes of Ranvier, allowing saltatory conduction. Voltage-gated sodium channels are much denser at nodes of Ranvier, allowing the neuron to focus its energy on opening channels in the nodes rather than wasting resources in the myelinated section.[21,22]

Neurites enable the conduction of action potentials, which must be transmitted to neighboring

neurons through synaptic transmission. This can occur either electrically or chemically. Electrical synapses allow the rapid transfer of information, while chemical synapses involve the release of neurotransmitters from the presynaptic neuron into the synaptic cleft to bind with the postsynaptic neuron, causing either an excitatory or inhibitory reaction.[21,22]

Linked Evaluation of Innervation Process

Peripheral nerve function can be tested clinically with *nerve conduction studies* (NCS) and *electromyograms* (EMG). NCS involves placing electrodes on a peripheral nerve and recording the electrical impulse generated by one electrode and recorded by the other. This test can provide information about amplitude, duration, and conduction velocity of the action potential. *The F and H responses* can provide information about more proximal segments of the nerve. NCS is used to diagnose neuropathies, demyelinating conditions, and radiculopathies.[23]

A needle EMG involves placing needle electrodes directly into a muscle and recording the electrical activity as the patient contracts the muscle. This test helps diagnose muscular conditions and those affecting the motor neuron unit and neuromuscular junction, such as muscular dystrophy, myasthenia gravis, and motor neuron loss in amyotrophic lateral sclerosis. Nerve conduction studies and electromyograms are often performed together in clinical practice.[24]

Importance of Healthy Nerve Innervation

Understanding nerve physiology is not only crucial for diagnosing and treating neurological conditions but also for appreciating the importance of healthy nerve innervation. Nerves are responsible for transmitting signals throughout the body, enabling us to perform various functions such as movement, sensation, and autonomic control. Healthy nerve function is critical while maintaining proper body function and can impact a range of systems, including the musculoskeletal, sensory, and autonomic systems. When nerves are damaged, it can lead to a range of problems, including pain, numbness, tingling, muscle weakness, and loss of function. For example, damage to the nerves that innervate the muscles can lead to atrophy and weakness, while damage to the sensory nerves can lead to loss of sensation and neuropathic pain. Peripheral neuropathy is categorized into axonal and demyelinating neuropathies. Axonal neuropathy is characterized by distal axonal loss and predominantly affects small fibers, while demyelinating neuropathy involves myelin sheath damage and affects larger and more proximal nerves. Nerve conduction studies can help distinguish between the two, with axonal neuropathy showing loss of nerve action potential amplitude and demyelinating neuropathy showing an early decrease in nerve conduction velocities. Understanding the physiology of nerve impulses is essential for interpreting these studies, making diagnoses, and treating neurological conditions effectively. Additionally, nerve damage can affect the autonomic system, leading to problems such as orthostatic hypotension, urinary and fecal incontinence, and sexual dysfunction. Maintaining healthy nerve innervation is, therefore, crucial for overall health and well-being. This can be achieved through a range of measures, including maintaining a healthy diet, exercising regularly, managing chronic conditions such as diabetes, and avoiding environmental toxins that can damage nerves.[1]

References

1. Ashley K, Lui F. (2023), Physiology, nerve. Treasure Island (FL): StatPearls Publishing. PMID: 31869116.
2. Raichle ME. Two views of brain function. Trends Cogn Sci. 2010;14(4):180-90. doi:10.1016/j.tics.2010.01.008.
3. Bishop GH. Natural history of the nerve impulse. Physiol Rev. 1956;36(3):376-99. doi:10.1152/physrev.1956.36.3.376.
4. Amaral, DG. The anatomical organization of the central nervous system. Principles of neural science. 2000:4(17);317-36.
5. Thomas PK. The connective tissue of peripheral nerve: An electron microscope study. J Anat. 1963;97(Pt 1):35-44. PMID:13981107.
6. Gamble HJ, Eames RA. An electron microscope study of the connective tissues of the human peripheral nerve. J Anat. 1964;98(Pt 4):655-63. PMID:14229996.

7. Rechthand E, Rapoport SI. Regulation of the microenvironment of peripheral nerve: Role of the blood-nerve barrier. Prog Neurobiol. 1987;28(4):303-43. doi:10.1016/0301-0082(87)90006-2.
8. Stolinski C. Structure and composition of the outer connective tissue sheaths of peripheral nerve. J Anat. 1995;186(Pt 1):123-30. PMID:7649808.
9. Glick DB. (2011), The autonomic nervous system. In: Miller RD, Eriksson LI, Fleisher LA, et al, eds. Miller's Anesthesia. 7th ed. Philadelphia: Elsevier.
10. Hainsworth R. Pathophysiology of syncope. Clin Auton Res. 2004;14(Suppl 1):18-24. doi:10.1007/s10286-004-1004-2.
11. Goldstein DS. Adrenal responses to stress. Cell Mol Neurobiol. 2010;30(8):1433-40. doi:10.1007/s10571-010-9606-9.
12. Johnson JO. (2019), Autonomic nervous system: Physiology. In Pharmacology and physiology for anesthesia. 2nd ed. Elsevier. ISBN:9780323568869.
13. Guyton AC, Hall JE. (1986), Textbook of medical physiology. Philadelphia: Saunders. ISBN:0-7216-0240-1
14. Ludwig PE, Reddy V, Varacallo M. (2022), Neuroanatomy, neurons. Treasure Island (FL): StatPearls Publishing. PMID:28723006.
15. Preuss CV, Kalava A, King KC. (2021), Prescription of controlled substances: Benefits and risks. Treasure Island (FL): StatPearls Publishing. PMID:30726003.
16. Kuhn S, Gritti L, Crooks D, Dombrowski Y. Oligodendrocytes in development, myelin generation and beyond. Cells. 2019;8(11):1424. doi:10.3390/cells8111424.
17. Banin VV, Gasymov EK. Distribution of proteins in neural membranes as a factor of reabsorption of endoneural (interstitial) fluid. Arkh Anat Gistol Embriol. 1990;99(9):47-54. PMID:2275614.
18. Kolb B, Gibb R. Brain plasticity and behaviour in the developing brain. J Can Acad Child Adolesc Psychiatry. 2011;20(4):265-76. PMID: 22114608.
19. Kumar P, Prabhakar NR. Peripheral chemoreceptors: Function and plasticity of the carotid body. Compr Physiol. 2012;2(1):141-219. doi:10.1002/cphy.c100069.
20. Wu C, Du YW, Huang L, Ben-Shoshan Galeczki Y, Dagan-Wiener, Naim M, et al. Biomimetic sensors for the senses: Towards better understanding of taste and odor sensation. Sensors (Basel). 2017;17(12):2881. doi:10.3390/s17122881.
21. Korrell KV, Disser J, Parley K, Vadisiute A, Requena-Komuro MC, Fodder H, et al. A. Differential effect on myelination through abolition of activity-dependent synaptic vesicle release or reduction of overall electrical activity of selected cortical projections in the mouse. J Anat. 2019;235(3):452-67. doi:10.1111/joa.12974.
22. Preuss CV, Kalava A, King KC. (2023), Prescription of controlled substances: Benefits and risks. Treasure Island (FL); StatPearls Publishing. PMID:30726003.
23. Herrmann DN. Noninvasive and minimally invasive detection and monitoring of peripheral neuropathies. Expert Rev Neurother. 2008;8(12):1807-16. doi:10.1586/14737175.8.12.1807.
24. Farina D, Fosci M, Merletti R. Motor unit recruitment strategies investigated by surface EMG variables. J Appl Physiol (1985). 2002;92(1):235-47. doi:10.1152/jappl.2002.92.1.235.

Co-funded by the Erasmus+ Programme of the European Union

Pathophysiology of the Nerve

LIGIA RUSU • OANA BIANCA BUDENACA BABOLEA

Overview About Nerve Damage

The nerve conducts information or commands in the form of an electrical impulse. The electrical impulse circulates in both directions, from the peripheral sensors to the brain, and from the Central Nervous System (CNS) to the periphery. The command from the brain to the periphery is most often represented by muscle control. Only through the nerve the body can move .[1]

The main symptoms of a peripheral nerve injury or peripheral neuropathy include anesthesia of the skin area (lack of sensitivity), numbness, tingling, and paresthesia. In some cases, patients complain of pain with burning sensations. The signs are reflected by the "melting" or decrease in volume of muscle mass – (amyotrophy). The abnormal position of the affected segment and the impossibility of performing specific movements are also specific signs of nerve damage.[1]

What Causes Nerve Damage?

A damaged nerve can no longer perform its function. This is how the signs and symptoms intended to raise an alarm appear, which means that the nerve tells that it is in pain.

Nerve injury can be classified into several types:[1]

- Section: Occurs when a nerve is cut.
- Crushing or stretching: Occurs with trauma, blows or strong contusions.
- Compression: Occurs pressure on an area due to the tissues that surround it.

Neuropathy is a group of diseases that affect nerves outside the brain and spinal cord (peripheral nerves). Mononeuropathy describes a condition in which only one nerve or nerve group is damaged. This disease negatively affects the part of the body associated with that nerve or nerve group, causing a loss of sensation, movement, or function in that part of the body. Mononeuropathy can affect any part of the body.[2]

Types of Neuropathy

There are two types of neuropathy. When symptoms develop slowly, it is called "chronic neuropathy". When symptoms suddenly appear, it is called "acute neuropathy".[2]

Neuropathy can be inherited. The most common form of "hereditary neuropathy" is Charcot-Marie-Tooth disease, in which a group of motor and sensory neuropathies that affect the arms and legs. "Acquired neuropathy" is much more common and is usually caused by a disease or injury. Nerve damage caused by Diabetes Mellitus is called "diabetic neuropathy". When the cause is unknown, it is called "idiopathic neuropathy".

Mononeuropathy can happen in any part of the body. There are more than hundred types of peripheral neuropathy. Some of the most common are:[2]

- The dysfunction of the Axillary nerve
- The dysfunction of the Radial nerve
- The dysfunction of the Ulnar nerve
- The dysfunction of the Sciatic nerve

- The dysfunction of the Common Peroneal nerve
- Carpal tunnel syndrome
- Cranial mononeuropathy
- Femoral neuropathy
- Thoracic/lumbar radiculopathy

Trauma is the most common cause of mononeuropathies. Violent muscle activity or forced overextension of a joint as well as small recurrent traumas (e.g. strong grasping of small tools, excessive vibrations of air hammers) can cause focal neuropathy. The prolonged exercising and uninterrupted pressure on bony prominences can cause pressure neuropathies that usually affect the superficial nerves (e.g. Ulnar, Radial, and Peroneal), especially in the case of weak people. Such pressures can be exerted during intoxication, cycling, or anesthesia.[3]

The compression of the nerves inside the narrow canals determines the occurrence of "trapping neuropathies" (e.g. Carpal Tunnel Syndrome). The compression of the nerve by a tumor, hyperostosis, or prolonged strained postures (e.g. those during gardening) can cause compression paralysis. Nerve hemorrhages, exposure to cold or radiation, or direct tumor invasion can also be causes of neuropathy.[3]

Multiple mononeuropathy (neuritis multiplex) is usually secondary to connective tissue disease (e.g. Polyarthritis Nodosa, Systemic Lupus Erythematosus, Sjogren's Syndrome, Rheumatoid Arthritis), Sarcoidosis, metabolic disease (e.g. Diabetes Mellitus, Amyloidosis) or nerve disease [e.g. Lyme Disease, Human Immunodeficiency Virus (HIV) infection, Leprosy, Herpes, Guillain-Barré Syndrome]. Diabetes Mellitus usually causes distal sensory and motor polyneuropathy. Other causes include:[2]

- Improper levels of vitamins E, B1, B6, B9, B12 and Niacin
- Some medications including chemotherapy
- Exposure to industrial chemicals, solvents and heavy metals such as mercury and lead
- Alcoholism.

Types of Nerve Damage

Peripheral nerve injuries can occur in the context of mechanical damage such as compression, traction, or direct injury, or in other contexts such as metabolic diseases, injuries caused by electric current, radiation, or poisoning. Secondary to nerve damage, nerve conduction capacity is completely or partially lost, so communication with the innervated peripheral organs disappears. These lesions were first described by H.J. Seddon in 1943 and divided into three categories: Neurapraxia, axonotmesis, and neurotmesis.[3,4]

Neurapraxia

It is the least severe nerve damage and is characterized by a focal nerve conduction block. The injury is located strictly at the level of the myelin sheath, the axon preserving its integrity in its entirety, so that there is a deficiency in the transmission of the action potential along the nerve fibers. Because of the limited involvement, this type of injury has a very good prognosis, with complete and spontaneous regeneration beginning after 2 or 3 weeks and lasting approximately 6 weeks. Meanwhile, there are cases where regeneration occurs within hours of the injury.[3,4]

This impairment is described in the form of a clinical syndrome, being represented by: predominantly motor-type paralysis, normal responsiveness to electrical stimulation of the muscle fibers in the innervation territory, alteration of subjectively expressed sensitivity (burning sensation, tingling, and numbness), and impairment of nerve sensitivity (minimal in terms of thermal, tactile and pain sensitivity, the loss of postural and vibratory sensitivity being much more important).

This type of injury is a dissociative one, because it is characterized by a predominantly motor and proprioceptive fiber damage. Neurapraxia type lesions appear after minor traumas, fact which allows the preservation of a variable number of functional nerve fibers. This type of injury can also occur as a result of maintaining an unnatural sleeping position that exerts additional pressure on the nerve fibers for a longer period of time.[1,2]

Axonotmesis

This type of injury involves the sectioning of the myelin sheath and axon. From a morphological and functional perspective, the connective tissue that covers these structures remains intact. This type of injury is characterized by preserving the continuity of the nerve path through a mass of tissue. Secondary to these lesioned particularities, spontaneous regeneration processes are present.[1,2]

Preserved structural continuity is the trigger for Wallerian Degeneration that follows this type of injury. The success of regeneration depends on the degree of damage to the surrounding structures, endonerve and perinerve, respectively. Preserving the integrity of the nerve sheaths plays the role of directing the regeneration process so that the new path completely coincides with the original one. The time required for nerve regeneration, provided the supporting structures are intact, varies between a few weeks and a few months. An example of such an injury occurs in the humerus fracture, which can be complicated by Radial nerve palsy and where there is a recovery of full nerve function. Many times, nerve injuries such as axonotmesis are secondary to a complete fracture in which the resulting bone fragments exert pressure directly on the nerve pathway.[1,2]

Neurotmesis

This type of injury is defined by the complete destruction of the nerve structure, both the myelin sheath and the axon together with the surrounding structures, i.e. connective tissue, being the most serious type of nerve injury. Although the external supporting structures may remain intact and macroscopically the nerve fibers may appear intact, the effect is identical to that of an anatomical disruption of the nerve fibers. Seddon defines this type of injury as being secondary either to a mechanical interruption of the nerve path, as in the case of a knife stroke, or to a complete disorganization of the nervous structure secondary to the formation of scar tissue, so that spontaneous regeneration is no longer possible.[1,2]

Due to the impossibility of spontaneous regeneration, this type of injury always requires surgical treatment to restore the integrity of the nerve structure. The signs of regeneration appear with the restoration of the integrity of the nerve pathway but the quality of the reconstruction process never being perfect. From a functional point of view, the nerve fibers lose their function distal to the sectioning point, so that clinical paralysis occurs and the innervated muscle groups progressively degenerate and atrophy.[1,2]

Nerve Composition

The nerve is a whitish cylindrical cord made up of nerve fibers. Nerves, together with nerve ganglia (small swellings along the path of the nerves), constitute the peripheral nervous system, unlike the central nervous system, which is made up of the brain and spinal cord.[1,2]

- Structure: Nerves are made up of parallel nerve fibers, which are themselves extensions (axons or dendrites) of nerve cells (neurons). In addition to nerve fibers, nerves include Schwann's cells, which form a sheath (myelin) around certain fibers. A protective tissue (connective tissue) surrounds the bundles of fibers (perineurium) in the set of nerves (epineurium).
- Function: In a nerve, two types of fibers coexist. The fibers that carry the information to the organs and tissues and the sensitive fibers that carry the information to the central nervous system.

Among the fibers, there are, moreover, somatic fibers (belonging to the nervous system of relational life, conscious) that innervate the skeletal muscles, skin, joints. The vegetative fibers (belonging to the autonomic nervous system) innervate the wall and muscles of the viscera and glands. Depending on the part of the central nervous system to which they are connected, spinal nerves (connected to the spinal cord) and cranial nerves (connected to the brain) are distinguished.[1,2]

Pathology: Nerves can be damaged during various circumstances:

- Neuritis (inflammatory, toxic or infectious touch)

- Compression (of the Median nerve in the carpal tunnel of the wrist)
- Tumor (neuroma, neurinoma)
- Trauma (often by cutting with a bladed weapon or a bullet).

Nerve Damage During Aging

Aging is a normal process of physical changes over time. Human being can age passively without paying much attention to the process or actively by consciously making certain choices and taking great care of the body and mind. For people with chronic diseases, for those with disabilities, or for those who feel tired, the adoption of healthy measures can have a great impact on physical and mental health. Regardless of the moment of adopting a healthy lifestyle, it improves the quality of life and can extend life expectancy. The normal signs of aging are generally the same for everyone, although they do not necessarily appear at a certain age. As the body ages, it is expected to undergo certain changes at its own pace. The way the body ages depends partly on certain genetic (inherited) factors. However, the choice of lifestyle has a stronger impact on the way of aging. Fortunately, lifestyle can be controlled.[1,2]

Starting with the third decade of life, the weight of the brain, the length of the nervous network, and the cerebral blood flow start to decrease. However, the brain manages to adapt to the new conditions by forming new synapses at the nerve endings. Changes in memory are a normal part of the aging process, certain more recent events are forgotten and names and details are more difficult to recall. The brain can be kept active through regular social activities, mental exercises (such as crossword puzzles and reading), and physical activities that increase the flow of blood and oxygen to the brain.

Measurements of Nerve Atrophy

Atrophy of brain tissue represents a progressive decrease in the number of nerve cells and interneuronal connections that, over time, leads to the loss of brain volume (shrinking of the brain) and the reduction of cognitive functions. Progressive degeneration of the brain is a process that occurs physiologically with aging, but it can also be encountered in the evolution of certain pathologies represented by:[4]

- Neurodegenerative diseases: Alzheimer's Disease (severe hippocampal atrophy), Pick's Disease (frontotemporal dementia), Parkinson's Disease, Huntington's Disease (condition that causes movement disorders and dementia)
- Craniocerebral trauma (boxers, head trauma)
- Stroke (ischemic or hemorrhagic)
- Cerebral infections (encephalitis, neurosyphilis, HIV)
- Chronic inflammatory diseases (Multiple Sclerosis)
- Hereditary metabolic diseases, including leukodystrophy (affecting the white matter)
- Mitochondrial encephalomyopathies.

The treatment of cerebral atrophy is individualized and instituted depending on each individual case in order to slow down the atrophy process and manage the associated symptoms. Non-drug treatment options for cerebral atrophy include physical therapy, speech therapy, and psychological counseling. In the case of cerebral infections that cause cerebral atrophy, antibiotics (bacterial infections) and antivirals (viral infections) are used successfully. Cerebral atrophy occurring after acute nerve tissue ischemia is treated etiologically and can include, in addition to anticoagulant and antiplatelet medication, lifestyle changes with the adoption of a diet low in saturated fat and regular physical exercise.[4]

Diagnosis and Tests

History and neurological examination are basic elements in the diagnosis of neuromuscular disorders leading to specific diagnostic tests. Neuropathies and radiculopathies are usually caused by local trauma and thus blood tests are not indicated but not excluded. Therefore, a complete and correct diagnosis of neuropathies may include checks such as:[4]

- Blood tests are used less often and are intended to identify certain systemic diseases that can cause or put the patient at risk for a nerve condition.
- Genetic tests are required only if there is a family history of neuropathy.
- Electrodiagnostic tests allow a complete electrodiagnostic assessment and include two complementary tests such as nerve conduction study and needle electromyography.
- Cervical, thoracic, or lumbar magnetic resonance imaging (MRI) can identify expansive processes that compress nerve roots or even root anomalies, can reveal hypertrophy or contrast uptake in patients with polyradiculopathies. Brachial or lumbosacral plexus MRI examination can reveal neoplastic or infectious infiltration or structural damage.
- Nerve biopsy involves the examination of a fragment of a nerve, usually a sensory nerve.
- Skin biopsy allows the evaluation of nerve endings and is a standard type of check to establish a diagnosis for small fiber neuropathies.
- Autonomic function testing.

Management and Treatment

The primary goal in evaluating neuropathies is to identify the etiology and, if possible, treat the underlying cause of the neuropathies. However, even when neuropathy has a treatable cause (such as Diabetes Mellitus, vitamin B12 deficiency, exposure to toxins), treatment is aimed at preventing the progression of neuropathic symptoms. Symptoms present at the start of treatment or when the toxic agent is removed may improve, or occasionally even be cured. However, in most patients, healing of the neuropathy is quite difficult and symptoms persist due to pretreatment neurogenic injury. In the case of peripheral neuropathy, the treatment involves electrical stimulation and physiotherapy and rehabilitation approaches in addition to medication.[5,6]

Etiological treatment of the condition associated with neuropathy:[5,6]

- The treatment of symptoms: The first step in the treatment of painful polyneuropathy is to quantify the severity of the pain and establish the therapeutic target.
- The treatment of nerve damage: Each type of injury benefits from the specific treatment. In the case of compression, we have an irreversible and progressive situation, that is, once the peripheral compression neuropathy is triggered, it worsens over time. The section of an obligate nerve is treated with neurorrhaphy. This involves the nerve reconstruction through a microsurgical technique. Microsurgery requires both an overspecialization of the surgeon and a special equipment. Neurorrhaphy is performed under a microscope or magnifying glass.[7,8]
- Pharmacological treatment: Medicines used to relieve the symptoms of painful peripheral neuropathy include non-steroidal anti-inflammatory drugs, antidepressants, anticonvulsants, opioids (medicines containing opioids lead to dependence and addiction - generally prescribed when other treatments fail), topical treatments (creams or patches).[9]
- Non-pharmacological treatment of neuropathic pain:
  - Physical activity: Due to the multiple benefits of physical activity, including the improvement of other health parameters, it should be encouraged in all patients with painful polyneuropathy. Specialists recommend 30 minutes of moderate physical activity daily.
  - Spinal cord stimulation: The premises of spinal cord stimulation are that electrical stimulation of nerve roots and spinal columns through an electrode placed in the epidural space will act as a gate and block pain perception without ablation.
  - Acupuncture: Along with chiropractic massage and Yoga, acupuncture is recommended for the treatment of peripheral neuropathy.
- Treatment of diabetic and prediabetic neuropathy: The main goal is the treatment of modifiable risk factors such as hyperglycemia, hyperlipidemia, hypertension, obesity and smoking, treatment of symptoms, and prevention of complications.[7,8]

- Treatment of incarceration neuropathies:
  - Median nerve - surgical dissection of the carpal ligament with decompression of the nerve is curative, but is only necessary in severe and prolonged cases.
  - Immobilizing the fist in splints to limit flexion relieves discomfort.

References

1. Eid, S, Sas, KM, Abcouwer, SF, Feldman EL, Gardner TW, Pennathur S, et al. New insights into the of diabetic complications: Role of lipids and lipid metabolism. Diabetologia. 2019;62(9):1539-49. doi:10.1007/s00125-019-4959-1.
2. Feldman, EL, Callaghan, BC, Pop-Busui R, Zochodne DW, Wright DE, Bennet DE, et al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5(1):41. doi:10.1038/s41572-019-0092-1.
3. Stino, AM, Smith, AG. Peripheral neuropathy in prediabetes and the metabolic syndrome. J Diabetes Investig. 2017;8(5):646-55. doi:10.1111/ jdi.12650.
4. Rajagopalan, V, Pioro, EP. Unbiased MRI analyses identify micropathologic differences between upper motor neuron-predominant ALS phenotypes. Front Neurosci. 2019;13:704. doi:10.3389/fnins. 2019.00704.
5. Peltier AC, Wood D. Management of Neuropathic Pain in Polyneuropathy. Continuum. 2020;26(5):1299-322. doi:10.1212/CON.0000000000000928.
6. Pop-Busui R, Boulton, AJM , Feldman EL, Bril, V, Freeman R, Malik RA, et al. Diabetic neuropathy: A position statement by the American Diabetes Association. Diabetes Care. 2017;40(1):136-54. doi:10.2337/dc16-2042.
7. Zeng L, Alongkronrusmee D, van Rijn RM. An integrated perspective on diabetic, alcoholic, and drug-induced neuropathy, etiology, and treatment in the US. J Pain Res. 2017;10:219-28. doi:10.2147/JPR.S125987.
8. American Stroke Association. Stroke. Accessed: https://www.stroke.org/en/about-stroke/effects-of-stroke/physical-effects-ofstroke/physical-impact/spasticity
9. Saebo. How to Treat Spasticity After Stroke. Accessed: https://www.saebo.com/blog/treat-spasticity-stroke/.

Co-funded by the Erasmus+ Programme of the European Union

Degenerated Nerve Physiology

MEHMET DURAY • GÖKHAN BAYRAK

Cellular and Molecular Changes in the Degenerated Nerve

Following a traumatic injury to a peripheral nerve, a series of molecular and functional changes occur in the peripheral and central nervous systems. These changes appear in the axonal region of peripheral nerve injury, the neuronal cell body in the dorsal root ganglion, the presynaptic terminals and postsynaptic environment of the dorsal horn in the medulla spinalis, and supraspinal regions including the dorsal column nuclei, thalamus, and cortex. Peripheral molecular and functional changes are induced by Wallerian Degeneration.[1] Examinations made with a light microscope determined that axonal fragmentation started nearly 44-46 hours after the peripheral nerve injury.[2] Anterograde axonal fragmentation emerges at rates ranging from 10 to 24 mm/hour after nerve injury.[1] The most significant difference in axonal degeneration occurring in peripheral nerves compared with other tissue types in the body is that Wallerian Degeneration of the distal axonal segment continues toward the proximal of the trauma site.[3]

When the molecular mechanisms and changes that occur are examined, the connection between the injury in the lesion area and fragmentation of the axons along the distal segment of the nerve has not yet been fully clarified.[1] However, some enzymatic activities [nicotinamide mononucleotide adenylyl transferase 1 (Nmnat1)] are known to protect axons by inhibiting the self-destruct mechanism of Nmnat isoforms produced in neuronal cell bodies and transported anterogradely following nerve injury.[4] In other words, activation or deactivation of the Nmnat1 enzyme after nerve injury leads to axonal neuroprotection or axonal destruction.[1] Therefore, such cellular changes may arise due to axonal fragmentation and subsequent loss of neural/axonal control over the phenotype of Schwann cells. However, the molecular mechanism that causes the breakdown of axonal myelin has not been fully clarified, but a quick increase in activation of the Erb2 receptor in Schwann cells was detected approximately 1 hour after nerve injury.[5]

Inadequate healing and regeneration following a peripheral nerve injury may be caused by various cellular and metabolic activities. Molecular structures such as myofibroblasts are closely associated with the presence of painful neuroma in humans after nerve injury.[1] The fact that the nerve endings are properly ruptured instead of fragmented, has positive effects in terms of the control mechanism on suppressing the factors that trigger the formation of a painful neuroma.[6]

Inflammatory Process

After axon and myelin sheath damage, fibroblast and Schwann cells proliferate and macrophages work together with Schwann cells to try to remove the degenerated axons and myelin sheath from the region. Nearly 2 days after the injury, the my-

elin sheath begins to separate from the nerve and ruptures, and banding proliferation of Schwann cells is observed at the injury site (Figure 7.1).[1] Schwann cells migrating to the injury site begin phagocytosis to clear axon and myelin residues formed during Wallerian Degeneration.[7,8] Endoneural macrophages proliferating simultaneously at the injury site support the phagocytic activity of Schwann cells during the first month of peripheral nerve injury.[1] Monocytes, which come to the injury site by passing through the blood-nerve barrier, are activated by some cytokines and chemokines, differentiate and turn into macrophages, increasing their number significantly within 2 weeks after injury.[9] Monocytes and macrophages directed by Schwann cells help to remove the residues formed as a result of nerve degeneration.[10,11] Schwann cells located in the damaged distal part spread out growth factors in this area after complete degeneration is achieved. These growth factors attract new axonal branches sprouting from the proximal part and try to help regeneration.[10] It is also stated that macrophages and immune system cells can directly alter gene expression in neurons following a peripheral nerve injury.[12] Recent studies indicated that accelerating Wallerian Degeneration in the injury area following a nerve injury can provide a faster and more promising repair, and accordingly, the possibility of regeneration may increase.[1]

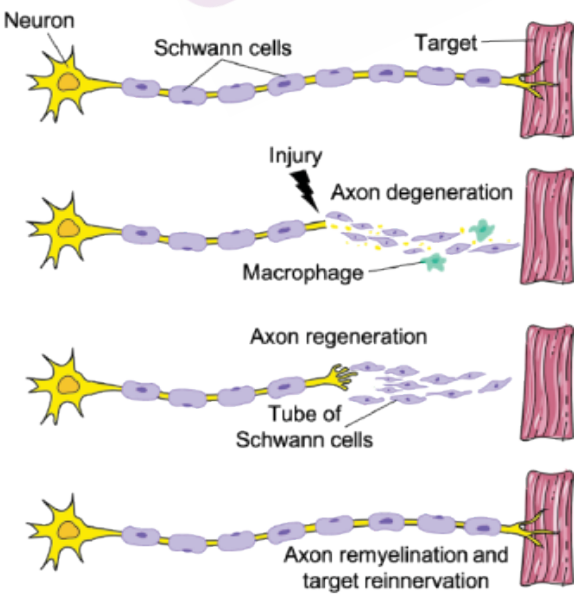

Figure 7.1 Axonal degeneration (Taken from "https://search.creativecommons.org/").

Trophic Changes After Degeneration

During degeneration after a peripheral nerve injury, some structural and dynamic changes occur in the injury site to provide a favorable microenvironment for axonal regeneration.[11] Released neurotrophic factors regulate neuronal survival, axonal growth, and synapse formation mechanisms after peripheral nerve injury.[1] In particular, the nerve growth factor supports neuronal survival and axonal growth of sympathetic and sensory dorsal root neurons in degenerated nerve cells.[10]

Changes in Nerve Excitability after Degeneration

In nerve conduction studies performed after sensory axonal degeneration, electrodes placed on the damaged area indicate a decrease in the sensory action potential. There is also a central slowing of sensory and/or motor conduction along the damaged part of the nerve after demyelination.[1]

The myelin sheath located in peripheral nerves contains myelin-associated glycoprotein molecules that prevent the regrowth of severed axon endings.[13] Molecular mechanisms triggering the degeneration of myelin, which arises as a result of peripheral nerve degeneration, have not been fully clarified.[5]

Early Changes

It has been determined that within seconds following a peripheral nerve injury, Ca^{+2} ions enter the nerves via Ca^{+2} channels proximal and distal to the injury site. Within hours following this process, ions mediate proteolysis and cause the degeneration of the axon segments proximal and distal to the injury site. Later, Ca^{+2} ions spread anterogradely in the proximal axon membrane, preventing cell membrane passage, and starting the degeneration process.[1] The disintegration of cytoskeletal structures occurs through Ca^{+2}-dependent endogenous proteolytic activity.[14] In addition, mitochondria are accumulated at points

close to the injury site in the early post-injury period.[10]

However, even in such conditions, nerve conduction continues for a certain period of time. Therefore, the capacity to generate an action potential in the process following nerve injury is of great importance in terms of synaptic transmission and muscle function.[1] Current evidence indicates that axonal degeneration is an active response programmed to maintain the connection between the cell body and the cell and the target organ, rather than a passive process.[15]

Late Changes

Axonal regeneration ability is determined by many factors, including the presence or absence of an environment that allows axon sprouting, the influence of non-neuronal cells, and the neuron's autonomic intrinsic growth capacity.[1] One of the late signs of degeneration is the decrease in mitochondrial membrane potential.[16] Significantly myelinated by Schwann cells in chronic denervations.[17] This indicates that degeneration after peripheral nerve injury may be beneficial in terms of late recovery.

Action Potential

In other types of tissue injury in the body, there is a Ca^{+2} flow to that area for the repair of the damaged area. In the first 30 minutes after a peripheral axonal injury, Ca^{+2} can lead to degeneration at the damaged ends and cause acute axonal degeneration.[10,12] It is a factor that suppresses the axonal growth of electrical activity and Ca^{+2} flow in the cell, especially in sensory neurons.[12] In addition, a decrease in resting membrane potential and an increase in acetylcholine sensitivity are changes that occur in response to degeneration after peripheral nerve injury.[18]

After peripheral nerve injury, the motor action potential usually remains constant for several days after injury and fades away 3-5 days after injury. The sensory action potential, on the other hand, decreases and disappears within 5 to 7 days after injury. This situation can be defined as an indication of the beginning of the biological process of Wallerian Degeneration.[18]

Evaluation of Nerve Degeneration

Electrodiagnostic tests are important markers for diagnosing and creating a treatment plan after peripheral nerve injuries. Electrodiagnostic evaluations consist of two separate parts: nerve conduction velocity studies and needle electrode examination. Although it is not clear when and how to use electrodiagnostic tests, they can sometimes produce inconclusive results.[19] However, nearly 3 days after the beginning of peripheral nerve damage, the first electrodiagnostic tests can be performed and repeated in 3–5-day periods.[20] In the electrodiagnostic evaluations performed after the peripheral nerve injury, it is not likely to detect or distinguish the types of nerve injury from each other. In the electrodiagnostic evaluations made in the first week of the injury, the motor action potential obtained from the distal side seemed to remain normal. Axonal degeneration can be detected at the earliest and most accurately approximately 21 days after peripheral nerve injury using needle electrodes with electrodiagnostic tests.[19]

In sensory nerve conduction studies, a recording electrode is placed on the skin over the sensory nerve. An electrical impulse distally generates a waveform defined as the sensory nerve action potential at the proximal recording electrode. Motor nerve conduction studies are performed by proximal stimulation of the nerve by placing a recording electrode directly on the muscle. A waveform, defined as depolarization and then motor action potential, is produced in muscle fibers. If there is damage to the motor axons, this potential can be achieved at a lower level. Motor nerve conduction studies are preferred as a reliable test method to evaluate the amount of axon loss.[19]

In the examinations made with electromyography, signs of muscle degeneration are not detected before the first 10-14 days after the injury. Within 21 days after peripheral nerve injury, nerve excitability is lost and absolute nerve degeneration is complete. Electrodiagnostic examinations can be complicated by early regeneration of nerve axons. Therefore, the most accurate assessment is provided in detecting nerve damage if electrodiagnostic tests are performed 72 hours af-

ter the beginning of peripheral nerve damage and repeated several times until the 14th-21st day. Muscular degeneration seen after neurotmesis in peripheral nerve injury may occur over a much longer period of time.[20]

In physiotherapy and rehabilitation, in case of partial degeneration, a decrease in the response to Faradic current is seen in the peripheral nerve and therefore more current density is required to contract the muscle. In partial degeneration, the response of the nerve to the Galvanic current is normal. Nerve regeneration is expected within a period of nearly 1.5 months after injury. If there is an absolute degeneration in the peripheral nerve, there is no response to the Faradic current. However, a normal response to the Galvanic current can be obtained in the first 7 and 21 days of complete degeneration. In this case, the nerve recovery period may be 6 months or more. If definite degeneration is detected in the peripheral nerve at the end of the first 21 days, the nerve cannot respond to Faradic and Galvanic currents. After definitive nerve degeneration, the nerve recovery period may take up to 2 years and in some cases recovery does not occur.[21]

The presence of an abnormal spontaneous activity such as fibrillation after peripheral nerve injury also starts from the 4th day after injury and the fibrillation potential increases over time. Although it is widely accepted that electrodiagnostic tests should be performed between 8 and 10 days after the injury in the clinical management of peripheral nerve injury, given the varying degrees of peripheral nerve injuries, the length of the clinical evaluation process, and the scarcity of resources, it is recommended to repeat the test at the end of 21 days to clarify the picture.[18,21]

Connective Tissue Activity

After peripheral nerve injury, connective tissue accumulation occurs in nerve endings.[1,10] This accumulation has a therapeutic effect on preserving the space between distal and proximal nerve endings after nerve injury, optimizing the ratio of nerve and connective tissue, and inhibiting the growth of neuroma at the proximal nerve end.[1]

Immune Response

In the first 3-5 hours following peripheral nerve injury, Schwann cells and fibroblasts initiate the inflow of cytokines and chemokines to the damaged area to strengthen the inflammatory response against peripheral nerve injury. In this process, denervated Schwann cells synthesize various proinflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukin (IL) 1α, IL-6, macrophages inflammatory protein-1, and monocyte chemoattractant protein-1α. For the same goal, the synthesis of IL-β is completed within the first day after injury.[1,22] Myelin destruction appears in denervated Schwann cells over time and differentiation occurs in their cellular cycles and mitosis occurs in an immature manner. This reaches its highest level in the first month following peripheral nerve injury. Afterward, if the denervation period continues for more than nearly 1 month, the release of neurotrophic factors and receptors no longer continues and tends to decrease over time.[1]

Different novel interventions are used nowadays, such as the use of antisense oligonucleotides, to block Wallerian Degeneration to increase axonal substance transport and prevent axonal degeneration.[23] However, there is a need for studies to consider these advances and new developments in terms of physiotherapy and rehabilitation, to review them by integrating and combining them with existing information, and to develop physiotherapists' perspectives on post-peripheral nerve rehabilitation.

Genetic Changes

As a result of insufficient activation or inhibition in gene signaling pathways such as Mitogen-Activated Protein Kinase, Erk1 and Erk2, c-jun N-terminal kinase (JNK) and p38 kinase required for nerve recovery (will be explained in the physiology of nerve regeneration section), a regenerative response will not be initiated and degeneration will be observed.[24] The inactivation of rapamycin, one of the autophagy activators, also prevents nerve healing. Insufficient activation of Autophagy relat-

ed (ATG-7) gene, one of the autophagy regulators, causes a delay in myelin clearance and contributes to degeneration.[25]

After axon injury, there is a downregulation of the genetic coding of neurotransmitters and proteins. Changes in the damaged axon and its projections are accompanied by changes in the neuropeptide expression. Neuropeptides give active reactions when the nerve is under stress. In particular, decrease in choline acetyltransferase (ChAT) level is observed. No increase in ChAT activity is observed without regeneration in the nerve. However, 3-10 days after the axonal lesion, a rapid increase in calcitonin gene-related peptide immunoreactivity is observed before ChAT activity.[24]

Neuroplasticity

After nerve injuries, the anatomical connections between the distal axons and the neuron body are lost and degeneration occurs. Degeneration towards the distal continues after a while retrograde. Schwann cells that differentiate during degeneration form Bungner bands by lining up within the axons to provide structural support to the axons.[1,25,26] Axon and myelin proteins formed as a result of the activation of Schwann cells reach significant sizes as early as 2 weeks after injury.[25]

Growth cones sprouting from damaged axons proximal to the lesion extend towards the target or area they find suitable. In the distal part, since there is no directing signal from the neuron body, newly sprouting immature axon fibers and connective tissue form a neuroma. If the necessary prerequisites for regeneration are not met, sufficient trophic substance expression cannot be achieved.[24]

Symptoms such as allodynia and hyperalgesia are among the common sensory symptoms after peripheral nerve injury. The excessive response of A-delta and C fibers to harmless stimuli, which are normally inactive against innocuous stimuli, leads to peripheral sensitivity. This situation changes the excitability properties of sodium, potassium, and Ca^{+2} ion channels. As the sodium ion concentration increases, the decrease in potassium flows is evident. In particular, the accumulation of ions as a result of changes in sodium flow has been held responsible for neuropathic pain, and the increase in Ca^{+2} ion concentration has been held responsible for allodynia.[24] These ionic changes stimulate long-term and abnormal firing of nociceptors.[27]

The plasticity mechanisms observed after nerve incision without therapeutic intervention are complex, and beneficial adaptive functional changes that support healing can be observed (Figure 7.2), as well as findings that result in negative neuroplasticity such as pain, dysesthesia, and hyperreflexia.[24]

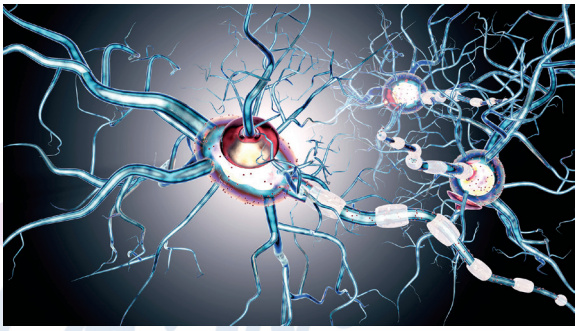

Figure 7.2 Complex remodeling after nerve injury.

Neurotrophic Factors

Neurotrophic factors play an important role in providing appropriate signaling from the neuron body to the damaged ends and supporting growth in order to ensure regeneration after nerve damage. Neuronal survival and axonal growth cannot be supported if neurotrophic factors, especially the nerve growth factor (NGF), do not have sufficient effect. For NGF to act, activation of 140 kD tyrosine kinase receptor A and 75 kD neurotrophin receptor (p75NTR) is required. The loss of NGF binding to p75NTR with high affinity in Schwann cells during the denervation process creates an inhibitory factor in terms of cell proliferation, myelination, and other positive plastic changes.[25]

Inadequate activation of some reactors, such as IL-1 and apolipoprotein E, along with growth factors also leads to negative results of neuronal survival and regeneration. However, long-term activation of cytokines such as TNFα, TGFβ and Leukemia Inhibitory Factor, which play a role in the proinflammatory process, prolongs the proinflammatory process. This situation leads to the accumulation of molecules such as Chondroitin Sulfate

Proteoglycans, which inhibit axonal growth, and cause scar tissue formation within the nerve.[28]

Loss of Biotensegrity

The idea that healthy axonal connections should be reestablished and strengthened after nerve damage has increased the interest in the mechanobiology of axon growth. Recently, mechanical tension on the axon has become one of the focal points of axon healing, especially after the axons merge with the terminal point. Mechanical stretching, which is a natural growth and development stimulant, is an important concept. In the process after nerve damage, the ability of mechanical stretching against biomechanical forces should be strengthened so that the newly formed neural networks do not leave the reached targets. While increasing axonal tension in the normal physiological process leads to axonal development, a decrease in tension causes the growth process to slow down and disappear. The lack of adequate mechanotransduction signals after nerve damage inhibits the growth of neurons. However, deviation from the optimal voltage level also leads to disturbances in neuronal activity.[29]

Normal biotension that is lost after nerve damage leads to the formation of a gap between the nerve tissues. This is because the damaged fibers lose their pre-tension and the damaged ends are pulled back. If surgical intervention is not performed in the early period, scar tissue formation is observed between the cut ends. If the tension concentration is not well-adjusted during surgery ischemia, inflammation, and fibrosis may occur between the suture and nerve.[30]

Ischemia

In the degenerated nerve, the blood nerve barrier, which provides the passage of substances necessary for the maintenance of tissue hemostasis and holds the endothelial cells together, is disrupted (Figure 7.3). The endothelial cells within the barrier regulate its bi-directional flow in blood flow, other plasma, and tissue fluids, minimizing the toxic effects of exogenous agents. Disruption of the blood nerve barrier can lead to further increase in nerve damage. The most important mechanism for this

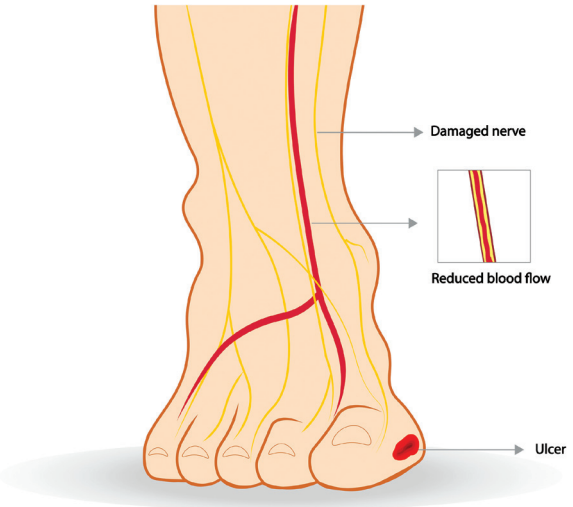

Figure 7.3 Reduced blood flow after nerve damage.

is the low bioavailability of Nitric Oxide (NO). As a result of low bioavailability, in addition to altered NO production and signaling, impaired vascular tone modulation and decreased vasodilation, oxidative stress increases and endothelial cell apoptosis and inflammation are triggered. Increased reactive oxygen species stimulate JNK and p38 activity, leading to increased proapoptotic signaling. Oxygen free radicals, which must be neutralized by antioxidant systems under normal physiological conditions, increase endothelial permeability due to their increased activity, allowing toxin passage.[31]

Types of Degeneration

In order to understand the degeneration process and physiology that will occur after nerve damage, it is necessary to remember the types of nerve injury. According to the Seddon classification, traumatic nerve injuries are divided into three categories: neuropraxia, axonotmesis, and neurotmesis.[1] In neuropraxia, which causes the slightest dysfunction in the nerve, there is no physical damage to the nerve and connective tissue, but only a physiological conduction block. Axotomy or Wallerian Degeneration is not seen in neuropraxia, and spontaneous remyelination and recovery are expected. If only the axon of a nerve is injured but the integrity of the nerve membranes is preserved, the injury is called axonotmesis. In axonotmesis,

rupture of axons, damage to myelin sheaths, and Wallerian Degeneration are observed in the distal parts of the axon. If the supporting connective tissue cells remain intact despite the damage to the axons, healing is exhibited through the axonal connections that remain intact. In mild axonotmesis, the endoneurium and perineurium membranes are intact. In severe axonotmesis, these inner membranes are damaged and only the outer epineurium membrane remains intact. Neurotmesis is the most severe peripheral nerve injury and refers to the complete rupture of axons, myelin sheaths, endoneurium, perineurium, and epineurium in terms of neural connectivity. Neurotmesis, which indicates complete paralysis of the peripheral nerve, causes atrophy of the muscles innervated by the damaged nerve and complete loss of sensation in the relevant dermatome area. As a result of neurotmesis injury, gaps form between the proximal and distal ends of the nerve and spontaneous healing does not occur. Therefore, surgical repair of the nerve is required after neurotmesis, and despite great advances in microsurgical techniques and closing the gaps in the nerve endings, reinnervation is slow and full function is difficult to reach.[1,32,33]

Wallerian Degeneration

It is the type of degeneration seen in the distal region of the lesion following peripheral nerve injury. In Wallerian Degeneration, myelin is further fragmented the following axon destruction. Increasing fibroblasts and Schwann cells in the medium are activated together with monocyte-derived macrophages taken from the circulation to clear degenerated axons and myelin.[1,34] Wallerian Degeneration is progressive, not limited to the damaged area, and extends to target tissues. In particular, the late onset of axon fragmentation and a longer error propagation of degeneration are the main obstacles to functional recovery.[1]

The fragmentation of axons after injury shows anterograde development at a speed of about 10-24 mm/hour. At first, distal fragmentation is an unexpected event, while degeneration suddenly moves backwards. Inactivation of Nmnat, which is a natural protection mechanism of axons and provides neuroprotection after nerve injury, disrupts axon nutrition from the cell body, causes Nmnat depletion in axons and increases axon destruction.[1,24]

After Wallerian Degeneration in the demyelination process, Schwann cells are activated and separate from the myelin sheath, which is a special extension of their plasma membranes. The myelin sheath, which is separated from the Schwann cell, is further fragmented. It is unclear why both Schwann cells and the myelin sheath undergo such drastic changes distal to the nerve without direct physical injury. The possible mechanism is that disintegrating axons disrupt the signaling of Schwann cells, with which they are closely related, causing Schwann cells to lose neural control. About 1 hour after nerve injury, neuregulins can activate the Erb2 receptor in Schwann cells rapidly but transiently. Neuregulin-Erb interactions play an important role in myelination-demyelination balance.[1]

Neuropathic Pain

The term neuropathic pain, which gathers more than a hundred conditions under its umbrella, is defined by the International Association for the Study of Pain as “pain arising from a lesion or disease of the somatosensory nervous system”. Neuropathic pain may occur as a result of a central nervous system lesion or may be induced by peripheral mechanisms.[35]

Damage to thin myelinated A-delta and unmyelinated C fibers from any cause can lead to neuropathic pain. The altered membrane composition, synapse properties, and disturbances in nerve conduction after existing damage led to ectopic firing and faulty signal transmission from the peripheral neuron to the target structure. Ectopic discharge of A-delta fibers begins within a few hours after damage to nociceptive afferents, while C fibers begin to discharge within a few days-weeks on average. Increasing ectopic discharge triggers the activation of sodium and Ca^{+2} ion channels and creates a hyperexcitation response. Activation of transient receptor potential ankyrin 1 (TRPA1),

one of the transient receptor potential channels, by mediators associated with nerve cell damage, such as Reactive Oxygen/Nitrogen Species, increases sensitivity to mechanical and thermal stimuli. Increasing sympathetic system activation over time leads to more severe pain after peripheral nerve injury.[27,35,36]

Energy Consumption

As it is known, the energy demand for the nervous system to exhibit a healthy physiology is huge and this energy is oxygen dependent. It has been determined that myelin sheath damage after nerve damage is an obstacle to meet the energy needs. It has been thought that one of the underlying reasons for the loss of myelin that causes axonal degeneration is that myelin may also have a trophic role. In later studies conducted for this purpose, the presence of proteins used in aerobic respiration in myelin tissue was determined. After nerve damage, dysfunction of both axonal mitochondria and myelin tissue disrupts the oxidative phosphorylation mechanism, leading to deficiencies in Adenosine Triphosphatase (ATP) activity, oxygen consumption and ATP synthesis.[37]

Mutants resistant to cholinesterase inhibitors after peripheral nerve injury increase the number of dysfunctional mitochondria by disrupting the mitochondrial structure of the axon. While this reduces the energy production, it also leads to an increase in oxidative stress.[38] Mitochondrial activity is essential for axon regeneration. Mitochondrial dysfunction leads to calpain-induced deficiencies in the cytoskeleton and axonal degeneration.[39]

Degeneration after Incorrect Injection

In mechanical injury, there is contact of the needle with the nerve or injection into the nerve. The mechanical force acting on the nerve destroys the nerve and the injection into the nerve causes nerve compression and nerve conduction block. Myelin and axonal damage may accompany the rupture of the perineurium. Increased intraneural pressure after injection may lead to ischemia of the nerve over time. Damage to the vasa nervorum can also be caused by injection. These traumas that cause direct damage, occlusion, or bleeding into the perineurium of the artery can lead to varying degrees of ischemia. If the epineural circulation is impaired, the blood flow in the nerve is reduced by 50%. Another type of injury seen after injection is chemical injury. The injected solution may cause tissue toxicity. Contact of the nerve or adjacent tissues with the chemical may first lead to acute inflammation and then to chronic fibrosis over time. It should be noted that local anesthetics have varying degrees of myotoxic, neurotoxic and cytotoxic effects.[40,41]

Degeneration After Toxic Injuries

Toxic injuries can be environmental and occupational, such as exposure to heavy metals, arsenic, and organophosphorus compounds, recreationally due to alcohol use, or iatrogenic due to the use of drugs such as anti-arrhythmic and anticancer. While acute or delayed toxicities can be seen in nerves, the most common type of toxicity is cumulative toxicities.[42]

Toxic substances usually reach the peripheral nerve parenterally. Toxicity that leads to more symmetrical axonal degeneration can also damage myelin. Depending on the substance exposed, sensory, motor, or autonomic fibers may be affected.[42,43]

New Perspectives on Degeneration

Although the etiology of nerve degeneration is very diverse, from a new perspective, nerve degeneration is pathogenetically divided into 6 subclasses. These include:

- Metabolic dysregulation,
- Covalent modifications,
- Altered mitochondrial and endoplasmic reticulum function leading to overproduction of reactive oxygen species,

- Altered intracellular and inflammatory signaling,
- Impaired axonal transport,
- Channel dysfunction.

Each of these subunits generally causes Wallerian Degeneration from distal to proximal. After degeneration, inflammatory deposits in the area are cleared. This process is certainly not associated with apoptosis and is not associated with caspase activity and apoptotic signaling. Recently, two factors responsible for degeneration have been identified. One is nikotinamid adenin dinükleotid (NAD+) deficiency and the second is sterile alpha and TIR motif containing 1 (SArm 1) molecule. It is stated that NAD+ and its isoforms are involved in the protection of the axon, and SArm1 supports axonal degeneration by neutralizing the NAD+ function. SArm1 expression can cause cell death as a result of depolarization of the mitochondrial membrane. Either way, SArm1 causes both toxic and metabolic peripheral nerve damage.[18]

References

1. Tubbs RS, Rizk E, Shoja M, Loukas M, Barbaro N, Spinner RJ. (2015), Nerves and nerve injuries: Vol 2: Pain, treatment, injury, disease and future directions. 1st ed. Academic Press. ISBN:9780128026953.
2. Cullen MJ. Freeze-fracture analysis of myelin membrane changes in Wallerian Degeneration. J Neurocytol. 1988;17(1):105-15. doi:10.1007/BF01735383.
3. Grinsell D, Keating C. Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. Biomed Res Int. 2014;2014:1-14. doi:10.1155/2014/698256.
4. Sasaki Y, Vohra BPS, Baloh RH, Milbrandt J. Transgenic mice expressing the Nmnat1 protein manifest robust delay in axonal degeneration in vivo. J Neurosci. 2009;29(20):6526-34. doi:10.1523/JNEUROSCI.1429-09.2009.
5. Guertin AD, Zhang DP, Mak KS, Alberta JA, Kim HA. Microanatomy of axon/glial signaling during Wallerian Degeneration. J Neurosci. 2005;25(13):3478-87. doi:10.1523/JNEUROSCI.3766-04.2005.
6. Chim H, Miller E, Gliniak C, Cohen ML, Guyuron B. The role of different methods of nerve ablation in prevention of neuroma. Plast Reconstr Surg. 2013;131(5):1004-12. doi:10.1097/PRS.0b013e3182879ec2.
7. Brück W. The role of macrophages in Wallerian Degeneration. Brain Pathol. 1997;7(2):741-52. doi:10.1111/j.1750-3639.1997.tb01060.x.
8. Perry V, Tsao J, Feam S, Brown M. Radiation-induced reductions in macrophage recruitment have only slight effects on myelin degeneration in sectioned peripheral nerves of mice. Eur J Neurosci. 1995;7(2):271-80. doi:10.1111/j.1460-9568.1995.tb01063.x.
9. Bendszus M, Stoll G. Caught in the act: In vivo mapping of macrophage infiltration in nerve injury by magnetic resonance imaging. J Neurosci. 2003;23(34):10892-6. doi:10.1523/JNEUROSCI.23-34-10892.2003.
10. Carp S. (2015), Peripheral nerve injury: an anatomical and physiological approach for physical therapy intervention. 1st ed. FA Davis Company. ISBN:978-0803625600.
11. Li X, Zhang T, Li C, Xu W, Guan Y, Li X, et al. Electrical stimulation accelerates Wallerian Degeneration and promotes nerve regeneration after sciatic nerve injury. Glia. 2023;71(3):758-74. doi:10.1002/glia.24309.
12. DeFrancesco-Lisowitz A, Lindborg J, Niemi J, Zigmond R. The neuroimmunology of degeneration and regeneration in the peripheral nervous system. Neuroscience. 2015;302:174-203. doi:10.1016/j.neuroscience.2014.09.027.
13. Bähr M, Przyrembel C. Myelin from peripheral and central nervous system is a nonpermissive substrate for retinal ganglion cell axons. Exp Neurol. 1995;134(1):87-93. doi:10.1006/exnr.1995.1039.
14. Coleman MP, Perry VH. Axon pathology in neurological disease: A neglected therapeutic target. Trends Neurosci. 2002;25(10):532-7. doi:10.1016/s0166-2236(02)02255-5.
15. Campbell WW. Evaluation and management of peripheral nerve injury. Clin Neurophysiol. 2008;119(9):1951-65. doi:10.1016/j.clinph.2008.03.018.
16. Loreto A, Di Stefano M, Gering M, Conforti L. Wallerian Degeneration is executed by an NMN-SARM1-dependent late Ca(2+) influx but only modestly influenced by mitochondria. Cell Rep. 2015;13(11):2539-52. doi:10.1016/j.celrep.2015.11.032.
17. Sulaiman OAR, Gordon T. Effects of short-and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia. 2000;32(3):234-46. doi:10.1002/1098-1136(200012)32:3<234::aid-glia40>3.0.co;2-3.
18. Phillips JB, Hercher D, Hausner T. (2022), Peripheral nerve tissue engineering and regeneration. 1st ed. Switzerland: Springer. ISBN:978-3-030-21051-9.
19. Chémali KR, Tsao B. Electrodiagnostic testing of nerves and muscles: When, why, and how to order. Cleve Clin J Med. 2005;72(1):37-48. doi:10.3949/ccjm.72.1.37.
20. Guntinas-Lichius O, Volk GF, Olsen KD, Mäkitie AA, Silver CE, Zafereo ME, et al. Facial nerve electrodiagnostics for patients with facial palsy: A clinical practice guideline. Eur Arch Otorhinolaryngol. 2020;277(7):1855-74. doi:10.1007/s00405-020-05949-1.
21. Kırdı N. (2016), Elektroterapide temel prensipler ve klinik uygulamalar. 2nd ed. Ankara: Hipokrat Kitabevi. ISBN:9786059160032.
22. Boivin A, Pineau I, Barrette B, Filali M, Vallières N, Rivest S, et al. Toll-like receptor signaling is critical for Wallerian Degeneration and functional recovery after peripheral nerve injury. J Neurosci. 2007;27(46):12565-76. doi:10.1523/JNEUROSCI.3027-07.2007.
23. Babetto E. (2020), Axon degeneration: Methods and protocols. 1st ed. Humana. ISBN: 978-1071605844.
24. Navarro X, Vivó M, Valero-Cabré A. Neural plasticity after peripheral nerve injury and regeneration. Prog Neurobiol. 2007;82(4):163-201. doi:10.1016/j.pneurobio.2007.06.005.
25. Li R, Li D, Wu C, Ye L, Wu Y, Yuan Y, et al. Nerve growth factor activates autophagy in Schwann cells to enhance myelin debris clearance and to expedite nerve regeneration. Theranostics. 2020;10(4):1649-77. doi:10.7150/thno.40919.
26. Salonen V, Aho H, Röyttä M, Peltonen J. Quantitation of Schwann cells and endoneurial fibroblast-like cells after experimental nerve trauma. Acta Neuropathol. 1988;75(4):331-6. doi:10.1007/BF00687785.

27. Osborne NR, Anastakis DJ, Davis KD. Peripheral nerve injuries, pain, and neuroplasticity. J Hand Ther. 2018;31(2):184-94. doi:10.1016/j.jht.2018.01.011.
28. Li R, Li DH, Zhang HY, Wang J, Li XK, Xiao J. Growth factors-based therapeutic strategies and their underlying signaling mechanisms for peripheral nerve regeneration. Acta Pharmacol Sin. 2020;41(10):1289-300. doi:10.1038/s41401-019-0338-1.
29. Pfister BJ, Grasman JM, Loverde JR. Exploiting biomechanics to direct the formation of nervous tissue. Curr Opin Biomed Eng. 2020;14:59-66. doi: 10.1016/j.cobme.2020.05.009.
30. Nassimizadeh M, Nassimizadeh AK, Power D. Managing the nerve gap: New tools in the peripheral nerve repair toolbox. JMSR. 2019;3(1):4-8. doi:10.4103/jmsr.jmsr_98_18.
31. Maiuolo J, Gliozzi M, Musolino V, Carresi C, Nucera S, Macrì R, et al. The role of endothelial dysfunction in peripheral blood nerve barrier: Molecular mechanisms and pathophysiological implications. Int J Mol Sci. 2019;20(12):3022. doi:10.3390/ijms20123022.
32. Pottorf TS, Rotterman TM, McCallum WM, Haley-Johnson ZA, Alvarez FJ. The role of microglia in neuroinflammation of the spinal cord after peripheral nerve injury. Cells. 2022;11(13):2083. doi:10.3390/cells11132083.
33. Kaplan B, Levenberg S. The role of biomaterials in peripheral nerve and spinal cord injury: A review. Int J Mol Sci. 2022;23(3):1244. doi:10.3390/ijms23031244.
34. Gebhart GF, Schmidt RF. (2013), Encyclopedia of pain. 2nd ed. Springer. ISBN:978-3-642-28753-4.
35. Meacham K, Shepherd A, Mohapatra DP, Haroutounian S. Neuropathic pain: Central vs. peripheral mechanisms. Curr Pain Headache Rep. 2017;21(6):28. doi:10.1007/s11916-017-0629-5.
36. Baron R. Peripheral neuropathic pain: From mechanisms to symptoms. Clin J Pain. 2000;16(2):12-20. doi:10.1097/00002508-200006001-00004.
37. Ravera S, Nobbio L, Visigalli D, Bartolucci M, Calzia D, Fiorese F, et al. Oxydative phosphorylation in sciatic nerve myelin and its impairment in a model of dysmyelinating peripheral neuropathy. J Neurochem. 2013;126(1):82-92. doi:10.1111/jnc.12253.
38. Rawson RL, Yam L, Weimer RM, Bend EG, Hartwieg E, Horvitz HR, et al. Axons degenerate in the absence of mitochondria in C. elegans. Curr Biol. 2014;24(7):760-5. doi:10.1016/j.cub.2014.02.025.
39. Wang B, Huang M, Shang D, Yan X, Zhao B, Zhang X. Mitochondrial behavior in axon degeneration and regeneration. Front Aging Neurosci. 2021;13:650038. doi:10.3389/fnagi.2021.650038.
40. Brull R, Hadzic A, Reina MA, Barrington MJ. Pathophysiology and etiology of nerve injury following peripheral nerve blockade. Reg Anesth Pain Med. 2015;40(5):479-90. doi:10.1097/AAP.0000000000000125.
41. Hogan QH. Pathophysiology of peripheral nerve injury during regional anesthesia. Reg Anesth Pain Med. 2008;33(5):435-41. doi:10.1016/j.rapm.2008.03.002.
42. Grisold W, Carozzi VA. Toxicity in peripheral nerves: An overview. Toxics. 2021;9(9):218. doi:10.3390/toxics9090218.
43. Sioka C, Kyritsis AP. Central and peripheral nervous system toxicity of common chemotherapeutic agents. Cancer Chemother Pharmacol. 2009;63(5):761-7. doi:10.1007/s00280-008-0876-6.

Co-funded by the Erasmus+ Programme of the European Union

Regenerated Nerve Physiology

MEHMET DURAY • GÖKHAN BAYRAK

Regenerative Events Forming in the Early Stage

In the body of a healthy neuron, there are functional homeostasis processes such as neurotransmitter synthesis, transport and storage, perception, and transmission of nerve impulses. After Wallerian Degeneration, which occurs in the early period after peripheral nerve injury, is completed, the peripheral nerve begins to transform into a regenerative phenotype to create axonal regeneration and axonal migration. In this process, some events occurring inside the cell result in an increase in the volume of the cell body (Figure 8.1).[1,2] Signals from

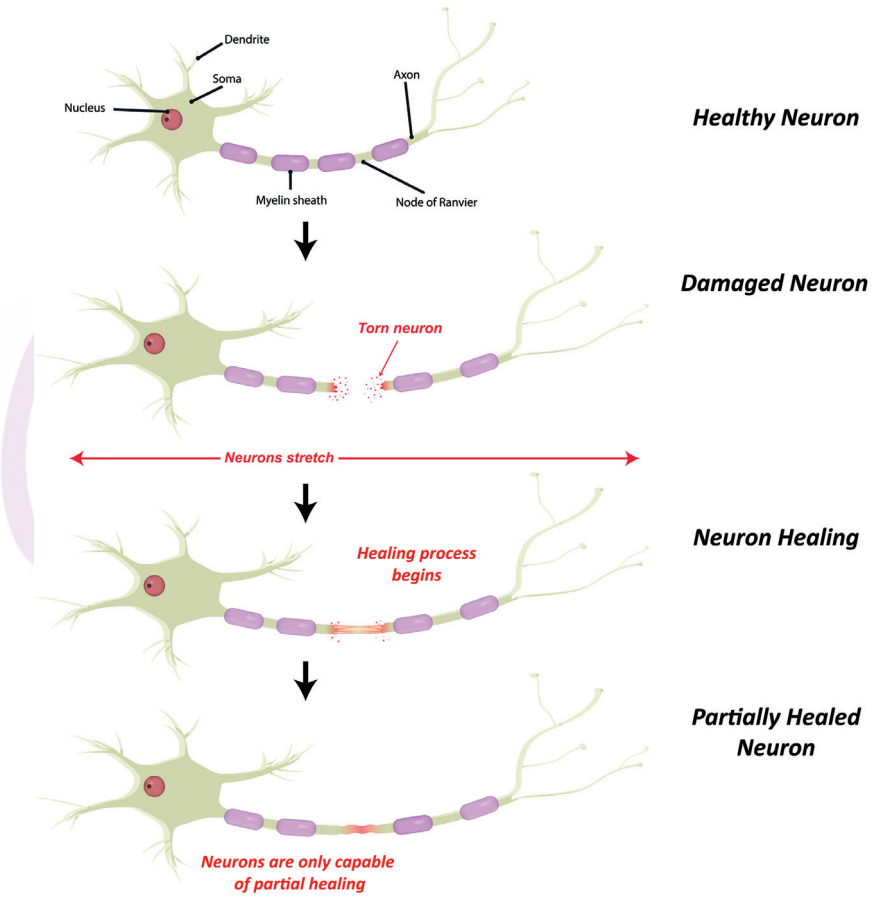

Figure 8.1 Nerve regeneration process.

the injury site are now ready to initiate this regenerative phenotype by altering gene expression to initiate regeneration.[2,3]

The first signal the neuronal cell body receives from the injury site is antidromic electrical activity, which can open calcium channels, affect cellular conduction, and is characterized by the explosion of high-frequency action potentials.[4] In the early period after injury, the neurotrophic activity of the peripheral nerve to innervate the target organ decreases. This decrease in neurotrophic activity may be of great importance in terms of neuron survival and peripheral nerve regeneration.[3,4] The resulting gene and protein expression differentiation initiates early regenerative events required for neuron survival.[4] In the next stage of regeneration, the process accompanying the transport of newly synthesized structural proteins in the cell body to multiple shoots emerging from the central axon is initiated.[5]

The regeneration capacity of axons and the growth support that Schwann cells can provide for regrowth decrease as the time elapsed after injury and the distance between nerve endings after injury increases. This means that the individual struggles against time regarding nerve regeneration and functional gain after peripheral nerve injury.[6]

Healing Mechanism

Following peripheral nerve injury, some changes are observed at the molecular and cellular levels, defined as Wallerian Degeneration and described in the previous sections, in the neuron body, injury site, and organs innervated by the nerve.[4] These changes continue the process of clearing the myelin sheath and myelin-associated glycoproteins from the environment in order to maintain axonal regeneration. In the first few hours after injury, the expression of phospholipases secreted from Schwann cells into the injury area is initiated to destroy myelin.[6] Additionally, after Schwann cells and macrophage activation and cleaning of waste materials in the environment, Schwann cells form an array in the form of Bungner bands, and a suitable environment for regeneration is prepared.[1,2,4,6]

The mechanisms that terminate the inflammatory response, which occurs as a healing mechanism after peripheral nerve injury, have not yet been fully elucidated. However, the transition to the anti-inflammatory process can be achieved due to the release of anti-inflammatory cytokines such as interleukin (IL)-10.[6] Despite the developments achieved for many years, it has not yet reached the desired level regarding clinical rehabilitation applications in terms of nerve neuropathophysiology.[4,6]

Stem Cell Treatment

The aim of stem cell therapy applied after peripheral nerve injury is to create an ideal environment for axonal regeneration and to maintain this environment for a long time. Regarding stem cell therapy, the ideal stem cells should be easily accessible and survive in vivo.[2] After stem cell treatment is applied to the damaged peripheral nerve area, these stem cells begin to proliferate and differentiate into the targeted cell type under appropriate environmental conditions (Figure 8.2A and 8.2B).[7] Stem cell treatment also supports the regenerative environment in the damaged area.[8] Stem cells have the potential to regenerate damaged neurons and increase the number of glial support cells.[2] There are different treatment sources and application forms for stem cell therapy. These include embryonic, neural, mesenchymal, and artificial stem cell treatments.[2,8]

Although embryonic stem cell therapy has significant advantages, it also has disadvantages. The most important is the risk of developing tumors called teratoma and neuroblastoma. In addition, the limited resources of embryonic stem cell treatment applications cause problems in accessing this method. It has been determined that embryonic stem cells can differentiate into Schwann cells with the application of embryonic stem cells to the sciatic nerve after peripheral nerve injury, thus providing axonal regeneration. In addition, immunological measurements have shown that embryonic stem cells survive and are a potential treatment method for regeneration after nerve injury.[2,8]

As an alternative method, neural stem cells have the potential to differentiate into neurons and glia. However, neural stem cells are located in

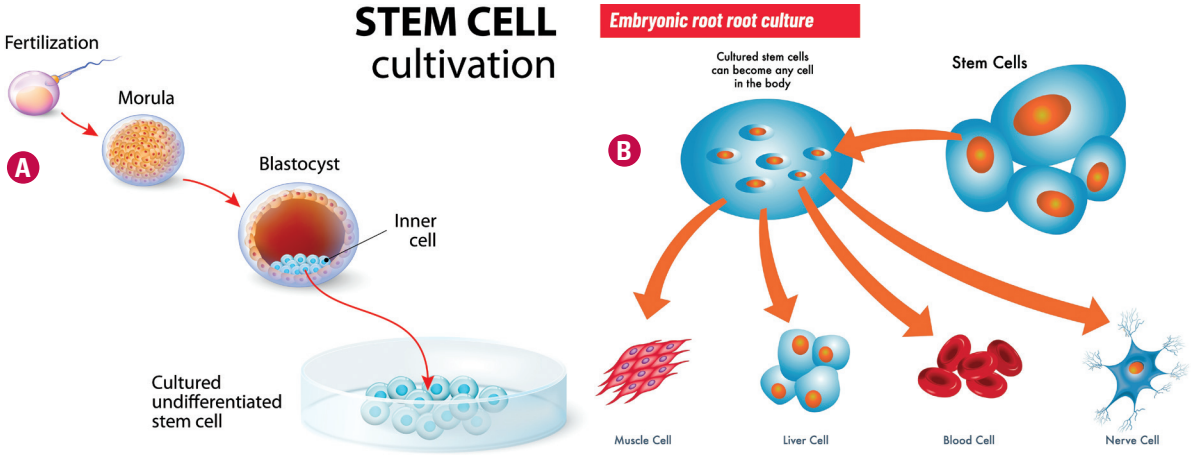

Figure 8.2 **A.** Stem cell cultivation, **B.** Stem cell differentiation

the subventricular region and hippocampus of the human brain and have limited differentiation abilities. In addition, removing neural stem cells due to the risks posed by access to the area where they are located can also cause significant problems. In addition, neural stem cell therapy can potentially cause tumoral conditions such as neuroblastoma.[2,8]

Mesenchymal stem cells can be obtained from various sources such as blood, adipose tissue, umbilical cord, tendon, hair root, and dental pulp. Today, mesenchymal stem cell applications originate from bone marrow, adipose tissue, fetus, hair follicle, skin, and dental pulp. Mesenchymal stem cells have the ability to differentiate into a wide variety of types. The capacity of mesenchymal stem cells to transform into neurons or Schwann cells with the proper application creates an essential potential for nerve regeneration.[2,8]

The use of artificial stem cell therapy has been increasing in recent years. Artificial stem cells have the potential to differentiate into neural cells in addition to differentiation into somatic cells. However, artificial stem cell therapy is currently only used in animal models due to its low efficiency. Artificial stem cell therapy may have a substantial potential for applications in humans in the future.[2,8]

Neurochemistry

Due to the polypeptides produced by non-neuronal cells after peripheral nerve injury, it is aimed to survive, grow, and direct the nerves trying to regenerate. Various factors aid peripheral nerve regeneration, including nerve growth factor, glial cell-derived neurotrophic factor, vascular endothelial growth factor, fibroblast growth factor, neuregulins, pleiotrophin, insulin-like growth factors, IL-1, and IL-6.[3]

Axonal Regeneration

In a healthy peripheral nerve, axons and Schwann cells send signals to each other. This signaling ensures that the link remains functional. Therefore, re-establishment of this healthy signaling is essential for successful nerve regeneration.[1] For nerve regeneration to occur after denervation, retrograde proximal degeneration of the axon must occur. Nerve fibers that regenerate within a few weeks regenerate from the injury site to the proximal or retrograde region of degeneration.[9]

Many factors, including the presence of an environment conducive to the extensibility of axons, the influence of non-neuronal cells, and the intrinsic growth capacity of the neuron, determine the axonal regeneration ability.[10] Besides these, neural tension, physical activity level including the injury site, chemotherapy drugs used for cancer disease, high blood sugar concentration, and biomechanical, physiological, and metabolic variables are also effective on regeneration.[9] When the effects of testosterone and progesterone on nerve healing after

peripheral nerve injury were compared in men and women, axonal growth was found to be higher in men. In addition, it has been reported that the axonal regeneration capacity is preserved mainly with advancing age. However, the Wallerian Degeneration capacity and the nerve endings' clearance capacity decrease.[1]

Axons that begin to regenerate extend to the target tissue or the distal nerve target in 3 different ways. First, an extension may occur within the Schwann tubes surrounding the central axon. Second, the extension can be seen with the help of tubes in the autograft or allograft at the distal end of the peripheral nerve. Third, an axonal extension oriented from proximal to distal can occur in a non-neural environment.[1]

However, sometimes, even if nerve regeneration occurs, it is difficult for peripheral nerve fibers to continue their regular duties.[9] During extremity movements, a normal peripheral nerve adapts to differences in length and tension and glides toward the surrounding tissues. However, peripheral nerves trying to maintain normal tension due to the fibrous tissue developing in long-term degeneration may be exposed to insufficient microcirculation because they cannot have adequate tension.[1] This situation should be considered as a barrier to prevent axonal regeneration in the rehabilitation process.

Vascular Changes

The vascular structure also has critical importance in supporting nerve regeneration. The sensitivity of peripheral nerves to low oxygen conditions is due to their vasculature characteristics due to the greater distance between the capillaries around the peripheral nerve compared to other body tissues.[11]

Neurovascular compliance, which develops for nerve regeneration after a peripheral nerve injury, is of great importance in vascularization before the migration of Schwann cells to the injury site. Glial cells in the peripheral nervous system use the newly formed vessels to guide the axonal shoots of the nerve that are trying to regenerate to the relevant target organs. This indicates that both the vascular and neuronal cell systems are in close relationship with each other from the early stage of nerve regeneration.[1]

Myelin Sheath Regeneration

Another condition that affects the nerve regeneration and functional recovery is myelination.[8] Myelin in the peripheral nerve contains molecules such as myelin-related glycoprotein, preventing disrupted axon development. Therefore, cleaning the myelin sheath as early as possible for regeneration after denervation is vital for faster regeneration.[12]

As is known, Schwann cells have the potential to form myelin by synthesizing myelin essential proteins and various myelin proteins in neuronal cells. It has been determined that tissues that differentiate into Schwann-like cells with stem cell applications have the potential to myelinate regenerating peripheral nerves.[2,8] Similarly, bone marrow-derived stem cell application has been found to support myelination in axons by releasing myelin factors and messenger ribonucleic acid during regeneration after peripheral nerve injury.[11]

Growth Factors

After peripheral nerve damage, there is a tendency for a decrease in the synthesis of substances associated with nerve conduction in the cell body and an increase in the synthesis of proteins associated with axonal growth and substances that make up the cell structure. In addition, in this process, there is an increase or decrease in the release of some genes that cause changes in ion and membrane excitability.[1] The use of growth factors in peripheral nerve regeneration has yet to be sufficiently clarified. While neurotrophin-3 increases neurite outgrowth in peripheral nerves, it is ineffective in enhancing functional recovery after severing the lingual or sciatic nerves. Similarly, brain-derived and neural growth factors do not affect developing peripheral nerve regeneration.[12]

Scar tissue, which may occur due to the proliferation of fibroblasts in the epineurium, perineurium, or endoneurium at the injury site, creates a

mechanical barrier for axons trying to regenerate. Because of scar tissue, the shrinkage of endoneurial sheaths containing axons and the formation of neuroma in the injury area prepares the ground. However, in terms of peripheral nerve regeneration, melatonin secretion has stimulatory effects on axonal regeneration with its anti-inflammatory effect on the injury site and its neuroprotective and scar-reducing effect on the damaged area.[3] Similarly, it has been determined that glial cell-derived neurotrophic factor and neurotrophin 4-5 support regeneration in the peripheral nerve, increase the number of regenerated axons, and thus improve functional recovery at the injury site.[12] Doxorubicin, a chemotherapy product applied to the injury site, reduces the scar tissue formation index and scar density in the injury site.[3] In addition, stem cell treatment upregulates the activity of Schwann cells by increasing growth factor secretion and extracellular matrix production.[2] This situation reveals the importance of melatonin and potential chemotherapy products in healing mechanisms and nerve regeneration after peripheral nerve injuries.

Electrophysiological Measurements

The previous section explained the importance of performing electrodiagnostic tests at regular intervals after denervation regarding the treatment and injury prognosis. The primary purpose of electrodiagnostic tests is to understand the likely outcome of a nerve injury and thus to help the treating physiotherapist adjust their treatment options.[13]

Understanding all stages of nerve regeneration after peripheral nerve injury is essential to determine the most appropriate treatment modalities and interpret the results correctly. From nerve injury to complete recovery, there are stages such as axonal regeneration, reinnervation of target organs, and recovery of functions.[14] The first of these stages, the axonal regeneration process, usually occurs in the first few months after injury. As it is known, if there is complete nerve degeneration in a peripheral nerve after the acute period, a contraction response cannot be obtained from the muscle with the application of the Faradic current. However, the response to the Galvanic current is protracted. Interim evaluations are made in the subacute and chronic periods and are tried to determine whether axonal regeneration is achieved.[15] Regenerated axons that reach the appropriate target generally regain their standard conduction properties, eventually resulting in an increase in axon diameter and myelin thickness. In the final stage of regeneration, it is aimed to restore complex functions such as fine motor control and sensory discrimination to their pre-injury state and to optimize the functional status.[14] However, although peripheral nerve regeneration has been achieved, nerve conduction velocity increases slowly and gradually. In the examinations, nerve conduction velocity increases slowly after peripheral nerve regeneration, reaching only 60% of the average conduction velocity value within four years and 85% on average after 16 years.[5]

Factors Affecting Regeneration

In order to accelerate recovery after peripheral nerve injury, it is essential to know the factors affecting regeneration and to determine the appropriate surgical and therapeutic approaches. In this context, the factors affecting the extent of axon regeneration and neurological recovery are;[16-19]

- Type of nerve trauma
- Gender and age
- Promoting axon regeneration through damaged nerves
- Providing restoration of function without surgical intervention
- Restoration of function by surgical intervention – anastomosis
- Neurotropic/trophic environment
- Physiotherapy and rehabilitation protocol
- Application of electrical stimulation with appropriate current type and parameters (e.g., frequency, duration, amplitude)
- The length of the space between the damaged nerve endings
- Whether or not autograft is used.

The following factors are at the forefront of the factors limiting nerve recovery;[17,19,20]

- Loss of sensory nerve function
- False Schwann cell phenotype
- Inflammation
- Necrosis
- Decreased improvement with patient age
- Smoking
- Concomitant vascular injuries
- Aging of Schwann cells
- Increased gap length
- Increased time between nerve injury and repair loss of neuron regeneration ability
- Excessive or incorrect application of electrical stimulation
- Nutritional deficiency.

Function of Neurotrophic Factors

It is vital for Schwann cells to direct regenerated axons to the target after peripheral nerve injury. One of these important roles of Schwann cells is being the source cell for neurotrophic factors.[19,21] It should be noted that newly sprouted axotomized motor neurons and denervated Schwann cells have a sufficient amount of endogenous neurotrophic factor to support nerve regeneration.[20] The distal stump in contact with regenerating axons further increases neurotrophic factor release, stimulating a second phase of Schwann cell proliferation. Schwann cells originating from the peripheral nerve fragment distal to the injury are the most effective substrates for regeneration. All growth factors, including Nerve Growth Factor (NGF) secreted by Schwann cells, support regeneration by upregulating neuronal cell adhesion molecules (NCAM).[21]

The mechanism by which neurotrophic factors affect axons is not clearly known. However, axons respond selectively to these neurotrophic stimuli and grow toward the motor or sensory target organ.[16,21] Although cytokines and growth factors are similar, cytokines are inducible, whereas growth factors are constitutive. Of the growth factors, neurotrophic factors promote development and maturation and affect the regeneration of neuronal activity. Trophic proteins, especially NGF, brain-derived neurotrophic factor (BDNF), Neurotrophin-3, and Neurotrophin-4, are synthesized in target tissues and transported retrogradely to the neuron's posterior surface.[19,22] Addition of exogenous trophic factors to the medium may contribute to regeneration after degeneration in which transport is severely damaged. Neurotrophic factors are internalized by binding to low and high affinity tyrosine kinase (trk) receptors in the same neuron. NGF binds to trkA, Neurotrophin-3 to trkC, and BDNF and Neurotrophin-4 to trkB.[21]

When peripheral nerve fibers, whether sensory or motor, are injured, some neurotrophic factors are upregulated.[20] Ciliary Neurotrophic Factor ensures the survival of motor neurons following axon destruction, while BDNF is upregulated.[20,21] Leukemia Inhibitory Factor, another neurotrophic factor, ensures the survival of sensory and sympathetic neurons[16]. Glial Growth Factor myelinates sensory and motor Schwann cells, stimulates cell proliferation and ensures their survival.[20,21]

Functional Sensory Recovery

After peripheral nerve injuries, sensory axons as well as motor fibers enter the reinnervation process by creating a regeneration response. Reinnervation due to misdirection of axons is very common in afferent nerves, and it remains unclear why it is so common in sensory nerves compared with motor nerves. Unfortunately, studies do not show the necessary interest in sensory recovery and focus more on motor function.[16,22] However, the regeneration of the sensory afferents of the muscle is also required for the coordination of muscle function, regulation of reflex activity and tone. The most important factor in the sensory feedback process is the presence of the sensory target. While axons are directed to the target through neurotrophic factors, axons that are regenerated in the absence of the target either form neuromas or cause abnormal stimulation of faulty targets.[16] The influence of autonomic fibers together with neuroma and faulty target stimulation may collectively lead to hypersensitization.[17] Increased Euregulin-1/ErbB signal

and gabapentin secretion increase remyelination and reduce hyperalgesia.[18] With correct sensory guidance, an increase in somatosensory evoked potential is observed in the nerve after nerve injuries.[16] Sensory neurons do not contain the trk receptor but instead express Ret ribonucleic acid (mRNA), the signal transduction component of the Glial-Derived Neurotrophic Factor (GDNF) receptor from the Transforming Growth Factor-b family, during the regeneration process. Ret receptors are found especially in small sensory neurons.[21]

Cortical changes also occur after peripheral nerve damage. Therefore, the progressive cortical regulation is required within the sensory retraining process for functional sensory return. In the process, it is aimed both to protect healthy sensory areas and to restore them to their pre-damage state. With education, it is tried to prevent the sensory cortical area from shrinking and being occupied by other areas.[23,24]

Functional Motor Recovery

In the motor nerve regeneration process, not only neurotrophic factors are involved but also the expression of regeneration-related genes. However, the link between increased Growth Associated Protein 43 (Gap43), Cap23, tubulin beta 2A (Tubb2a), small proline rich protein 1A (Sprr1a), fibroblast growth factor-inducible 14 (Fn14), neurodevelopment protein 1 like 1 (Ndel1) and transient receptor potential channel 4 (Trpc4) gene expression and transcription factors that support regeneration after injury has not been established.[25] However, the presence of Heat shock protein 27 (Hsp27) after injury has been described in the soma and axons of both motor and sensory neurons. Induction of the Hsp27 protein prevents apoptosis of these neurons and promotes axonal growth.[25] P2X receptors, one of the purinergic receptors that play a role in the process after peripheral nerve pathologies, are involved in synaptic transmission, neuromodulation, and neuroinflammatory response. In particular, they are upregulated in response to nerve damage.[18] In addition to forming myelin sheaths, Schwann cells regulate the balance of their microenvironment by providing support and nutrition to motor neurons. The secretion of neurotrophic factors by Schwann cells promotes neuronal survival and positive synaptic plasticity. In particular, BDNF accelerates cellular myelination and induces the formation of a thick myelin sheath. The main factor that increases BDNF secretion is the expression of P2X4R, which is enriched in the lysosomes of Schwann cells.[18]

Long Term Changes-Events in the Regeneration Process

In the previous section, we described the peripheral nerve degeneration process in detail. Although the degeneration process is a routine result of the spontaneous process that is not intervened, axonal regeneration occurs spontaneously to a limited extent, and supportive stimuli are needed. Axons advancing to the target with axonal regeneration reinnervate the target and normalize the damaged distal stump structurally and physiologically by establishing functional connections.[26]

There is a latent delay before axon shoots emerging from the proximal stump enter the denervated tubes and initiate regeneration. One reason for this is the slow regeneration of proteins by axonal transport, and the other is the inhibition of chondroitin sulfate proteoglycan. If the endoneurial tube is also impaired, it takes time for Schwann cells to migrate to the injury site. Axon shoots may not be able to decide in which direction to branch between the proximal and distal parts, and reaching the distal stump may be quite slow and gradual, as it is sometimes directed toward the proximal stump.[27]

Two-three weeks after injury, motor neurons randomly sprout their axons, sometimes sprouting to appropriate motor neurons and sometimes to inappropriate sensory fibers. This inappropriate combination remains fixed after 3 weeks if it cannot be changed. After RNA levels of motor neuron-specific neurotrophic factors peak within two weeks, motor neurons that remain intact sprout toward the appropriate motor neuron within 10 weeks.[27] However, processes that hinder regeneration continue in order to gain functionality. One of the dif-

ficulties of functional recovery after nerve injury is the inability to correct axons' misdirection to the wrong target, and the second is that long-term deterioration in neuronal regenerative capacity and chronic denervation reduce axonal regeneration. The growth-promoting phenotypes of denervated Schwann cells decrease over time.[27]

Neuroplasticity in the Regeneration Process

After peripheral nerve injury, recovery is not only supported by sprouting intact axons. At the same time, functional and structural changes occur in the spinal cord, brain stem, raphe nuclei, thalamus, and cortex at different levels, where mapping is rearranged from periphery to the center.[23] Therefore, in the acquisition of motor and sensory functions, it is essential to integrate alternative mechanisms beyond a simple definition of regeneration. In order to correctly manage this process, the neuroplastic changes that will occur should be well known. Axon sprouting, which occurs rapidly after nerve damage, establishes both appropriate and inappropriate connections. Even in the complete regeneration process, which indicates that all connections are properly established, permanent and rapid changes occur in the central nervous system as well. This central reorganization in the regeneration process tries to compensate for innervation errors. If denervation occurs and the compensatory mechanism is not activated, the central representation of faulty connections becomes stronger and the function in motor control and sensation becomes chronic. This situation is called denervation plasticity and it reflects very negatively on regeneration. Especially in adults, the weak plastic capacity reduces the healing after reinnervation and regeneration mistakes. Therefore, negative neuroplasticity should be transformed into regenerative plasticity as soon as possible with appropriate techniques.[28] Applications such as vagal nerve stimulation, nerve growth factors, and electrical stimulation, which stimulate the release of neuromodulators including acetylcholine, are frequently used for local nerve regeneration.[23,28]

The axon growth mechanism, which is present in the neural development process, loses its effectiveness in the adult nervous system and is reactivated by the peripheral nerve lesion. Correct orientation of axons by activating intrinsic growth programs contributes to nerve regeneration and functional recovery.[25,29] In this process, there is ante- and retrograde conduction for the formation, growth, elongation and direction of new axons.[25] While Schwann cells facilitate axonal regeneration, stem cells that differentiate into structures similar to Schwann cells can support neuron survival.[30,31] Secreted growth factors [c-Jun, GDNF, BDNF, neurotrophin-3, artemin, NGF, Vascular Endothelial Growth Factor etc.] support angiogenesis by facilitating not only axonal sprouting but also capillary sprouting.[30,31] The degree of nerve regeneration depends on the functional support of the local environment of the nerve, but the nerve repair program is very complex.[21,30,31]

High amount of protein synthesis is essential for the development of growth cones that facilitate axonal sprouting.[21,29] The fact that an axon contains 3000 mRNA for repair and regeneration, as well as ribosomes and the Golgi apparatus, shows that this will not be too difficult. However, cleaning the inflammatory residues in the post-injury area accelerates the regeneration process. While regeneration progresses at a rate of 1 mm per day, it can fill 1 cm gaps. While Schwann cells stop responding to regeneration signals over time, the chance of distal regeneration decreases, in many cases only proximal regeneration can be achieved.[29]

Energy Consumption and Mitochondrial Behavior

After peripheral nerve injury, axons exhibit strong regenerative capacity. However, one of the most essential organelles for the supply of necessary energy in this process is mitochondria.[32] During the regeneration process, mitochondrial density increases, reducing oxidative stress.[32,34] Along with the increased mitochondrial division, the effect capacity of the mentioned effects in damaged axons is increased and axon healing is accelerated.[32]

Electrical Stimulation in the Nerve Regeneration Process

Although peripheral nerve regeneration is much more successful than central regeneration, factors that inhibit reinnervation complicate the regeneration process in the peripheral nerve. Surgical applications alone are not sufficient for absolute regeneration, and accelerating interventions are needed for the newly formed axon shoots to travel long distances to reach the target organ.[35] Electrical stimulation, which is an alternative method used to accelerate regeneration after nerve damage, is used especially in the treatment of motor, sensory and autonomic disabilities after surgery.[36]

Along with electrical stimulation, action potentials generated retrogradely from the stimulated area increase intraneuronal cyclic adenosine monophosphate (AMP), accelerating the expression and upregulation of regenerative genes and neurotrophic factors. Therefore, axonal sprouting accelerates in the damaged area, myelination increases and as a result, regeneration is achieved.[35,36]

It is important to use electrical stimulation in the early post-injury period. In addition, it is reported that electrical stimulation supports regeneration after delayed nerve repair. Even single-session electrical stimulation applications in the early period can have a regenerative effect.[37,38] Electrical stimulation applied to the repair site should be short-term and low-frequency to stimulate regeneration.[20,27,37] It has been reported that 20 Hertz electrical stimulation applied for 1 hour facilitates axonal regeneration.[35,37] The duration of the stimulation applied to the motor nerves is also important, as explained in the "Reinnervated Muscle Physiology" section. If motor nerve stimulation is applied less than 5% of the day, muscle fibers become fast glycolytic fibers, and if motor nerve stimulation is applied more than 50% of the day, muscle fibers convert to slow oxidative fibers.[27]

Delayed Regeneration

After nerve injuries, fibrous tissue formed during the delay of its regeneration due to reasons such as prolongation of denervation, collagenization of the distal stump, loss of motor endplate reduces the regeneration potential and leads to a weak functional recovery.[16] After chronic denervation of Schwann cells, the growth environment at the injury site deteriorates and degenerative changes occur in target organs.[38] Schwann cells normally ensure that the distal fragmented axonal structures are cleared together with the macrophages in the region. If axon fragments cannot be cleared, these fragments inhibit axonal regeneration, leading to delayed axonal growth.[31] The peripheral nerve regeneration rate, which already has a slow rate of 1 mm per day, slows down even more and the low regenerative capacity of neurons decreases even more.[16,37,39]

Long nerves that require greater functional recovery after proximal injuries require axonal growth at a longer distance, delaying regeneration. If axotomy is prolonged and becomes chronic, the regenerative capacity of neurons decreases to 33% of normal in a 6-month period.[37] Similarly, long-term denervation is one of the biggest obstacles to functional recovery.[40]

Does Electrical Stimulation Prevent or Delay Regeneration?

Activation of neurotrophic factors is essential for axon growth to occur. Therefore, the presence of neurotrophic factors such as BDBF, neurotrophin-4, neurotrophin-5 is required for electrical stimulation to result in nerve regeneration. Absence of neurotrophic factors or activation of sodium channel blockers in the environment of injury negates the therapeutic effects of electrical stimulation, as it inhibits retrograde signal transmission.[41]

However, the inability to optimize electrical stimulation in terms of parameters such as current intensity, frequency, and duration may also have regeneration-inhibiting effects. For example, high-frequency electrical stimuli can increase neuron damage and even lead to peripheral nerve degeneration. In Galvanic current applications, the current intensity of more than 4 milliamperes (mA) also prevents axonal sprouting. The application time of electrical stimulation is also one of the determining factors on regeneration. Electrical

stimulation should be applied briefly and intermittently to achieve therapeutic benefit. As long-term electrical current applications will increase the dosage, they prevent regeneration in the peripheral nerve and may even increase neuronal damage. Therefore, in post-injury electrical stimulation applications, current characteristics should be arranged by considering the regeneration process in the peripheral nerve.[41]

References

1. Phillips JB, Hercher D, Hausner T. (2022), Peripheral nerve tissue engineering and regeneration. 1st ed. Springer. ISBN:978-3030210533.
2. Fairbairn NG, Meppelink AM, Ng-Glazier J, Randolph MA, Winograd JM. Augmenting peripheral nerve regeneration using stem cells: A review of current opinion. World J Stem Cells. 2015;7(1):11-26. doi:10.4252/wjsc.v7.i1.11
3. Benga A, Zor F, Korkmaz A, Marinescu B, Gorantla V. The neurochemistry of peripheral nerve regeneration. Indian J Plast Surg. 2017;50(1):5-15. doi:10.4103/ijps.IJPS_14_17.
4. Faroni A, Mobasseri SA, Kingham PJ, Reid AJ. Peripheral nerve regeneration: Experimental strategies and future perspectives. Adv Drug Deliv Rev. 2015;82-83:160-7. doi:10.1016/j.addr.2014.11.010.
5. Kimura J. (2013), Electrodiagnosis in diseases of nerve and muscle: Principles and practice. 4th ed. Oxford University Press. ISBN:978-0199738687.
6. Chan KM, Gordon T, Zochodne DW, Power HA. Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets. Exp Neurol. 2014;261:826-35. doi:10.1016/j.expneurol.2014.09.006.
7. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol. 2000;164(2):247-56. doi:10.1006/exnr.2000.7389.
8. Jiang L, Jones S, Jia X. Stem cell transplantation for peripheral nerve regeneration: Current options and opportunities. Int J Mol Sci. 2017;18(1):94. doi:10.3390/ijms18010094.
9. Carp S. (2015), Peripheral nerve injury: An anatomical and physiological approach for physical therapy intervention. 1st ed. FA Davis Company. ISBN:978-0803625600.
10. DeFrancesco-Lisowitz A, Lindborg JA, Niemi JP, Zigmond RE. The neuroimmunology of degeneration and regeneration in the peripheral nervous system. Neuroscience. 2015;302:174-203. doi:10.1016/j.neuroscience.2014.09.027.
11. Muangsanit P, Shipley RJ, Phillips JB. Vascularization strategies for peripheral nerve tissue engineering. Anat Rec (Hoboken). 2018;301(10):1657-67. doi:10.1002/ar.23919.
12. Tubbs RS, Rizk E, Shoja M, Loukas M, Barbaro N, Spinner RJ. (2015), Nerves and nerve injuries: Vol 2: pain, treatment, injury, disease and future directions. 1st ed. Academic Press. ISBN:9780128026953.
13. Robinson LR. How electrodiagnosis predicts clinical outcome of focal peripheral nerve lesions. Muscle Nerve. 2015;52(3):321-33. doi:10.1002/mus.24709.
14. Navarro X. Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: A critical overview. Eur J Neurosci. 2016;43(3):271-86. doi:10.1111/ejn.13033.
15. Kırdı N. (2016), Elektroterapide temel prensipler ve klinik uygulamalar. 2nd ed. Ankara: Hipokrat Kitabevi. ISBN:978-605-9160-03-2.
16. Adidharma W, Khouri AN, Lee JC, Vanderboll K, Kung TA, Cederna PS, et al. Sensory nerve regeneration and reinnervation in muscle following peripheral nerve injury. Muscle Nerve. 2022;66(4):384-96. doi:10.1002/mus.27661.
17. Bulut T, Akgün U, Cıtlak A, Aslan C, Şener U, Şener M. Prognostic factors in sensory recovery after digital nerve repair. Acta Orthop Traumatol Turc. 2016;50(2):157-61. doi:10.3944/AOTT.2015.15.0140.
18. He B, Zhu Z, Zhu Q, Zhou X, Zheng C, Li P, et al. Factors predicting sensory and motor recovery after the repair of upper limb peripheral nerve injuries. Neural Regen Res. 2014;9(6):661-72. doi:10.4103/1673-5374.130094.
19. Kuffler DP, Foy C. Restoration of neurological function following peripheral nerve trauma. Int J Mol Sci. 2020;21(5):1-23. doi:10.3390/ijms21051808.
20. Gordon T. The physiology of neural injury and regeneration: The role of neurotrophic factors. J Commun Disorder. 2010;43(4):265-73. doi:10.1016/j.jcomdis.2010.04.003.
21. Terenghi G. Peripheral nerve regeneration and neurotrophic factors. J Anat. 1999;194(1):1-14. doi:10.1046/j.1469-7580.1999.19410001.x.
22. Anand P, Birch R. Restoration of sensory function and lack of long-term chronic pain syndromes after brachial plexus injury in human neonates. Brain. 2002;125(1):113-22. doi:10.1093/brain/awf017.
23. Li C, Liu SY, Pi W, Zhang PX. Cortical plasticity and nerve regeneration after peripheral nerve injury. Neural Regen Res. 2021;16(8):1518-23. doi:10.4103/1673-5374.303008.
24. Saunders NR, Dziegielewska KM. (2005), Degeneration and regeneration in the nervous system. 1st ed. Harwood Academic Publishers. ISBN:0-203-30448-9.
25. Ma CHE, Omura T, Cobos EJ, Latrémolière A, Ghasemlou N, Brenner GJ, et al. Accelerating axonal growth promotes motor recovery after peripheral nerve injury in mice. J Clin Invest. 2011;121(11):4332-47. doi:10.1172/JCI58675.
26. Leite APS, Pinto CG, Tibúrcio FC, Sartori AA, de Castro Rodrigues A, Barraviera B, et al. Heterologous fibrin sealant potentiates axonal regeneration after peripheral nerve injury with reduction in the number of suture points. Injury. 2019;50(4):834-47. doi:10.1016/j.injury.2019.03.027.
27. Gordon T. Peripheral nerve regeneration and muscle reinnervation. Int J Mol Sci. 2020;21(22):8652-76. doi:10.3390/ijms21228652.
28. Meyers EC, Kasliwal N, Solorzano BR, Lai E, Bendale G, Berry A, et al. Enhancing plasticity in central networks improves motor and sensory recovery after nerve damage. Nat Commun. 2019;10(1):1-14. doi:10.1038/s41467-019-13695-0.
29. Nagappan PG, Chen H, Wang DY. Neuroregeneration and plasticity: A review of the physiological mechanisms for achieving functional recovery postinjury. Mil Med Res. 2020;7(1):1-16. doi:10.1186/s40779-020-00259-3.
30. Saffari S, Saffari TM, Ulrich DJ, Hovius SE, Shin AY. The interaction of stem cells and vascularity in peripheral nerve regeneration. Neural Regen Res. 2021;16(8):1510-17. doi:10.4103/1673-5374.303009.
31. Nocera G, Jacob C. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. Cell Mol Life Sci. 2020;77(20):3977-89. doi:10.1007/s00018-020-03516-9.
32. Wang B, Huang M, Shang D, Yan X, Zhao B, Zhang X. Mitochondrial behavior in axon degeneration and regeneration. Front. Aging Neurosci. 2021;13(650038):1-17. doi:10.3389/fnagi.2021.650038.

33. Han SM, Baig HS, Hammarlund M. Mitochondria localize to injured axons to support regeneration. Neuron. 2016;92(6):1308-23. doi:10.1016/j.neuron.2016.11.025.
34. Kuo CC, Su HL, Chang TL, Chiang CY, Sheu ML, Cheng FC, et al. Prevention of axonal degeneration by perineurium injection of mitochondria in a sciatic nerve crush injury model. Neurosurg. 2017;80(3):475-88. doi:10.1093/neuros/nyw090.
35. Geremia NM, Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. Exp Neurol. 2007;205(2):347-59. doi:10.1016/j.expneurol.2007.01.040.
36. Zuo KJ, Gordon T, Chan KM, Borschel GH. Electrical stimulation to enhance peripheral nerve regeneration: Update in molecular investigations and clinical translation. Exp Neurol. 2020;332:1-12. doi:10.1016/j.expneurol.2020.113397.
37. Gordon T, Eva P, Borschel GH. Delayed peripheral nerve repair: Methods, including surgical 'cross-bridging' to promote nerve regeneration. Neural Regen Res. 2015;10(10):1540-44. doi:10.4103/1673-5374.167747.
38. Jivan S, Novikova LN, Wiberg M, Novikov LN. The effects of delayed nerve repair on neuronal survival and axonal regeneration after seventh cervical spinal nerve axotomy in adult rats. Exp Brain Res. 2006;170(2):245-54. doi:10.1007/s00221-005-0207-7.
39. Su WF, Wu F, Jin ZH, Gu Y, Chen YT, Fei Y, et al. Overexpression of P2X4 receptor in Schwann cells promotes motor and sensory functional recovery and remyelination via BDNF secretion after nerve injury. Glia. 2019;67(1):78-90. doi:10.1002/glia.23527.
40. Jonsson S, Wiberg R, McGrath AM, Novikov LN, Wiberg M, Novikova LN, et al. Effect of delayed peripheral nerve repair on nerve regeneration, schwann cell function and target muscle recovery. PloS One. 2013;8(2):1-13. doi:10.1371/journal.pone.0056484.
41. Javeed S, Faraji AH, Dy C, Ray WZ, MacEwan MR. Application of electrical stimulation for peripheral nerve regeneration: Stimulation parameters and future horizons. Interdiscip Neurosurg. 2021;24:1-12. doi:10.1016/j.inat.2021.101117.

CK4STEM