Biomechanics and Electrophysiology

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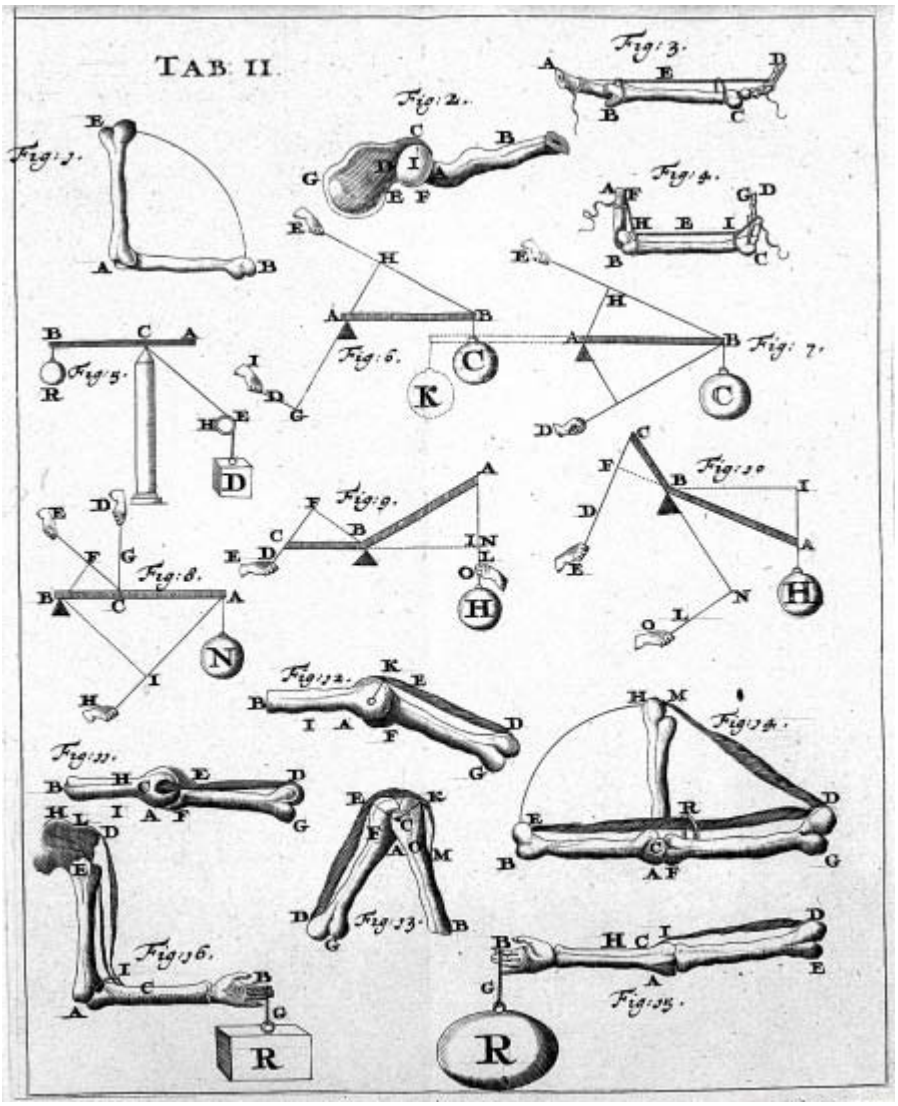
Chapter 1

Biomechanics



Page of one of the first works of Biomechanics (*De Motu Animalium* of Giovanni Alfonso Borelli)

Biomechanics (from Ancient Greek: βίος "life" and μηχανική "mechanics") is the application of mechanical principles to biological systems, such as humans, animals, plants, organs, and cells. Perhaps one of the best definitions was provided by Herbert Hatze in 1974: "Biomechanics is the study of the structure and function of biological systems by means of the methods of mechanics". The word biomechanics developed during the early 1970s, describing the application of engineering mechanics to biological and medical systems. In Modern Greek, the corresponding term is εμβιομηχανική.

Biomechanics is closely related to engineering, because it often uses traditional engineering sciences to analyse biological systems. Some simple applications of Newtonian mechanics and/or materials sciences can supply correct approximations to the mechanics of many biological systems. Applied mechanics, most notably mechanical engineering disciplines such as continuum mechanics, mechanism analysis, structural analysis, kinematics and dynamics play prominent roles in the study of biomechanics.

Usually biological system are more complex than man-built systems. Numerical methods are hence applied in almost every biomechanical study. Research is done in a iterative process of hypothesis and verification, including several steps of modeling, computer simulation and experimental measurements.

Subfields

Plant biomechanics

The application of biomechanical principles to plants and plant organs has developed into the subfield of plant biomechanics.

Sport biomechanics

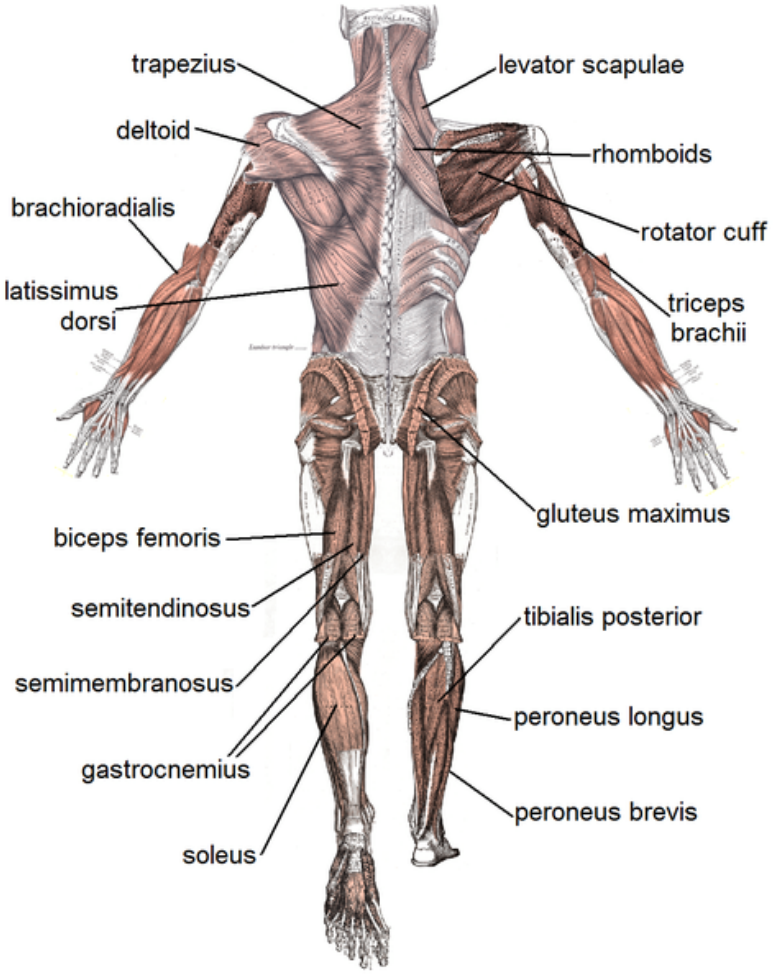
In **sports biomechanics**, the laws of mechanics are applied in order to gain a greater understanding of athletic performance and to reduce sport injuries as well. Elements of mechanical engineering (e.g., strain gauges), electrical engineering (e.g., digital filtering), computer science (e.g., numerical methods), gait analysis (e.g., force platforms), and clinical neurophysiology (e.g., surface EMG) are common methods used in sports biomechanics.

Continuum biomechanics

This research and analysis can be carried forth on multiple levels, from the molecular, wherein biomaterials such as collagen and elastin are considered, all the way up to the tissue and organ level. Some simple applications of Newtonian mechanics can supply correct approximations on each level, but precise details demand the use of continuum mechanics. A further look into continuum mechanics is provided in a textbook by W. Michael Lai, David Rubin, and Erchard Krempl titled: *Introduction to Continuum Mechanics*.

The study of biomaterials is of crucial importance to biomechanics. For example, the various tissues within the body's organs, such as skin, bone, and arteries each possess unique material properties. The passive mechanical response of a particular tissue can be attributed to characteristics of the various proteins, such as elastin and collagen, living cells, ground substances such as proteoglycans, and the orientations of fibers within the tissue. For example, if human skin were largely composed of a protein other than collagen, many of its mechanical properties, such as its elastic modulus, would be different.

It has been shown that applied loads and deformations can affect the properties of living tissue. There is much research in the field of growth and remodeling as a response to applied loads. For example, the effects of elevated blood pressure on the mechanics of the arterial wall, the behavior of cardiomyocytes within a heart with a cardiac infarct, and bone growth in response to exercise, and the acclimative growth of plants in response to wind movement, have been widely regarded as instances in which living tissue is remodelled as a direct consequence of applied loads.



Human muscleskeletal system

It is often appropriate to model living tissues as continuous media. For example, at the tissue level, the arterial wall can be modeled as a continuum. This assumption breaks down when the length scales of interest approach the order of the micro structural details of the material. The basic postulates of continuum mechanics are conservation of linear and angular momentum, conservation of mass, conservation of energy, and the entropy inequality. Solids are usually modeled using "reference" or "Lagrangian" coordinates, whereas fluids are often modeled using "spatial" or "Eulerian" coordinates. Using these postulates and some assumptions regarding the particular problem at hand, a set of equilibrium equations can be established. The kinematics and constitutive relations are also needed to model a continuum.

Second- and fourth-order tensors are crucial in representing many quantities in electromechanical. In practice, however, the full tensor form of a fourth-order constitutive matrix is rarely used. Instead, simplifications such as isotropy, transverse isotropy, and incompressibility reduce the number of independent components. Commonly-used second-order tensors include the Cauchy stress tensor, the second Piola-Kirchhoff stress tensor, the deformation gradient tensor, and the Green strain tensor. A reader biomechanical literature would be well-advised to note precisely the definitions of the various tensors which are being used in a particular work.

Chemistry, molecular biology, and cell biology have much to offer in the way of explaining the active and passive properties of living tissues. For example, in muscle contractions, the binding of myosin to actin is based on a biochemical reaction involving calcium ions and ATP.

Bones are anisotropic but are approximately transversely isotropic. In other words, bones are stronger along one axis than they are along a pivotal (i.e., normal or orthogonal) axis, and are approximately the same strength no matter how they are rotated around the one axis.

The stress-strain relations of bones can be modeled using Hooke's law, in which they are related by elastic moduli, e.g., Young's modulus, Poisson's ratio, or the Lamé parameters. The constitutive matrix, a fourth-order tensor, depends on the isotropy of the bone.

$$\sigma_{ij} = C_{ijkl}\varepsilon_{kl}$$

There are three main types of muscles:

- Skeletal muscle (striated): Unlike cardiac muscle, skeletal muscle can develop a sustained condition known as tetany through high frequency stimulation, resulting in overlapping twitches and a phenomenon known as wave summation. At a sufficiently high frequency, tetany occurs, and the contracticle force appears constant through time. This allows skeletal muscle to develop a wide variety of forces. This muscle type can be voluntary controlled. Hill's Model is the most popular model used to study muscle.

- Cardiac muscle (striated): Cardiomyocytes are a highly specialized cell type. These involuntarily contracted cells are located in the heart wall and operate in concert to develop synchronized beats. This is attributable to a refractory period between twitches.
- Smooth muscle (smooth, lacking striations): The stomach, vasculature, and most of the digestive tract are largely composed of smooth muscle. This muscle type is involuntary and is controlled by the enteric nervous system.

Soft tissues such as tendon, ligament and cartilage are combinations of matrix proteins and fluid. In each of these tissues the main strength bearing element is collagen, although the amount and type of collagen varies according to the function each tissue must perform. Elastin is also a major load-bearing constituent within skin, the vasculature, and connective tissues. The function of tendons is to connect muscle with bone and is subjected to tensile loads. Tendons must be strong to facilitate movement of the body while at the same time remaining compliant to prevent damage to the muscle tissues. Ligaments connect bone to bone and therefore are stiffer than tendons but are relatively close in their tensile strength. Cartilage, on the other hand, is primarily loaded in compression and acts as a cushion in the joints to distribute loads between bones. The compressive strength of cartilage is derived mainly from collagen as in tendons and ligaments, however because collagen is comparable to a "wet noodle" it must be supported by cross-links of glycosaminoglycans that also attract water and create a nearly incompressible tissue capable of supporting compressive loads.

Recently, research is growing on the biomechanics of other types of soft tissues such as skin and internal organs. This interest is spurred by the need for realism in the development of medical simulation.

Viscoelasticity is readily evident in many soft tissues, where there is energy dissipation, or hysteresis, between the loading and unloading of the tissue during mechanical tests. Some soft tissues can be preconditioned by repetitive cyclic loading to the extent where the stress-strain curves for the loading and unloading portions of the tests nearly overlap. The most commonly used model for viscoelasticity is the Quasilinear Viscoelasticity theory (QLV). In addition, soft tissues exhibit other viscoelastic properties, including creep, stress relaxation, and preconditioning.

Hooke's law is linear, but many, if not most problems in biomechanics, involve highly nonlinear behavior, particularly for soft tissues. Proteins such as collagen and elastin, for example, exhibit such a behavior. Some common material models include the Neo-Hookean behavior, often used for modeling elastin, and the famous *Fung-elastic* exponential model. Non linear phenomena in the biomechanics of soft tissue arise not only from the material properties but also from the very large strains (100% and more) that are characteristic of many problems in soft tissues.

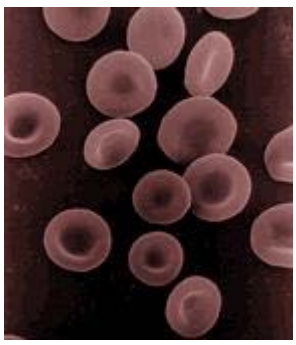
Comparative Biomechanics

Chinstrap Penguin leaping over water

Comparative biomechanics is the application of biomechanics to non-human organisms, whether used to gain greater insights into humans (as in physical anthropology) or into the functions, ecology and adaptations of the organisms themselves. Common areas of investigation are Animal locomotion and feeding, as these have strong connections to the organism's fitness and impose high mechanical demands. Animal locomotion, has many manifestations, including running, jumping and flying. Locomotion requires energy to overcome friction, drag, inertia, and gravity, though which factor predominates varies with environment.

Comparative biomechanics overlaps strongly with many other fields, including ecology, neurobiology, developmental biology, ethology, and paleontology, to the extent of commonly publishing papers in the journals of these other fields. Comparative biomechanics is often applied in medicine (with regards to common model organisms such as mice and rats) as well as in biomimetics, which looks to nature for solutions to engineering problems.

Biofluid mechanics



Red blood cells

Under most circumstances, blood flow can be modeled by the Navier-Stokes equations. Whole blood can often be assumed to be an incompressible Newtonian fluid. However, this assumption fails when considering flows within arterioles. At this scale, the effects of individual red blood cells becomes significant, and whole blood can no longer be modeled as a continuum. When the diameter of the blood vessel is slightly larger than the diameter of the red blood cell the Fahraeus–Lindquist effect occurs and there is a decrease in wall shear stress. However, as the diameter of the blood vessel decreases further, the red blood cells have to squeeze through the vessel and often can only pass in single file. In this case, the inverse Fahraeus–Lindquist effect occurs and the wall shear stress increases.

Biotribology

The main aspects of tribology are related with friction, wear and lubrication. When the two surfaces come in contact during motion i.e. rub against each other, friction, wear and lubrication effects are very important to analyze in order to determine the performance of the material. Biotribology is a study of friction, wear and lubrication of biological systems especially human joints such as hips and knees. For example, femoral component and tibial component of knee implant rub against each other during daily activity such as walking or stair climbing. If the performance of tibial component needs to be analyzed, the principles of biotribology are used to determine the wear performance of the implant and lubrication effects of synovial fluid. In addition, the theory of contact mechanics also becomes very important for wear analysis.

History

Antiquity

Aristotle wrote the first book on biomechanics, *De Motu Animalium*, or On the Movement of Animals. He not only saw animals' bodies as mechanical systems, but pursued questions such as the physiological difference between imagining performing an action and actually doing it. Some simple examples of biomechanics research include the investigation of the forces that act on limbs, the aerodynamics of bird and insect flight, the hydrodynamics of swimming in fish, and locomotion in general across all forms of life, from individual cells to whole organisms. The biomechanics of human beings is a core part of kinesiology.

Renaissance

Probably Leonardo da Vinci could be recognized as the first true biomechanician, because he was the first to study anatomy in the context of mechanics. He analyzed muscle forces as acting along lines connecting origins and insertions and studied joint function. He also intended to mimic some animal features in his machines. For example, he studied the flight of birds to find means by which humans could fly. Because horses were the principal source of mechanical power in that time, he studied their muscular

systems to design machines that would better benefit from the forces applied by this animal.

Galileo Galilei was interested in the strength of bones and suggested that bones are hollow for this affords maximum strength with minimum weight. He noted that animals' masses increase disproportionately to their size, and their bones must consequently also disproportionately increase in girth, adapting to loadbearing rather than mere size the bending strength of a tubular structure such as a bone is increased relative to its weight. This surely was one of the first grasps of principles of biological optimization.

In the 16th century, Descartes suggested a philosophic system whereby all living systems, including the human body (but not the soul), are simply machines ruled by the same mechanical laws, an idea that did much to promote and sustain biomechanical study. Giovanni Alfonso Borelli embraced this idea and studied walking, running, jumping, the flight of birds, the swimming of fish, and even the piston action of the heart within a mechanical framework. He could determine the position of the human center of gravity, calculate and measured inspired and expired air volumes, and showed that inspiration is muscle-driven and expiration is due to tissue elasticity. Borelli was the first to understand that the levers of the musculoskeletal system magnify motion rather than force, so that muscles must produce much larger forces than those resisting the motion. Influenced by the work of Galileo, whom he personally knew, he had an intuitive understanding of static equilibrium in various joints of the human body well before Newton published the laws of motion.

Industrial era

In the 19th century Étienne-Jules Marey used cinematography to scientifically investigate locomotion. He opened the field of modern 'motion analysis' by being the first to correlate ground reaction forces with movement. In Germany, the brothers Ernst Heinrich Weber and Wilhelm Eduard Weber hypothesized a great deal about human gait, but it was Christian Wilhelm Braune who significantly advanced the science using recent advances in engineering mechanics. During the same period, the engineering mechanics of materials began to flourish in France and Germany under the demands of the industrial revolution. This led to the rebirth of bone biomechanics when the railroad engineer Karl Culmann and the anatomist Hermann von Meyer compared the stress patterns in a human femur with those in a similarly shaped crane. Inspired by this finding Julius Wolff proposed the famous Wolff's law of bone remodeling.

Applications

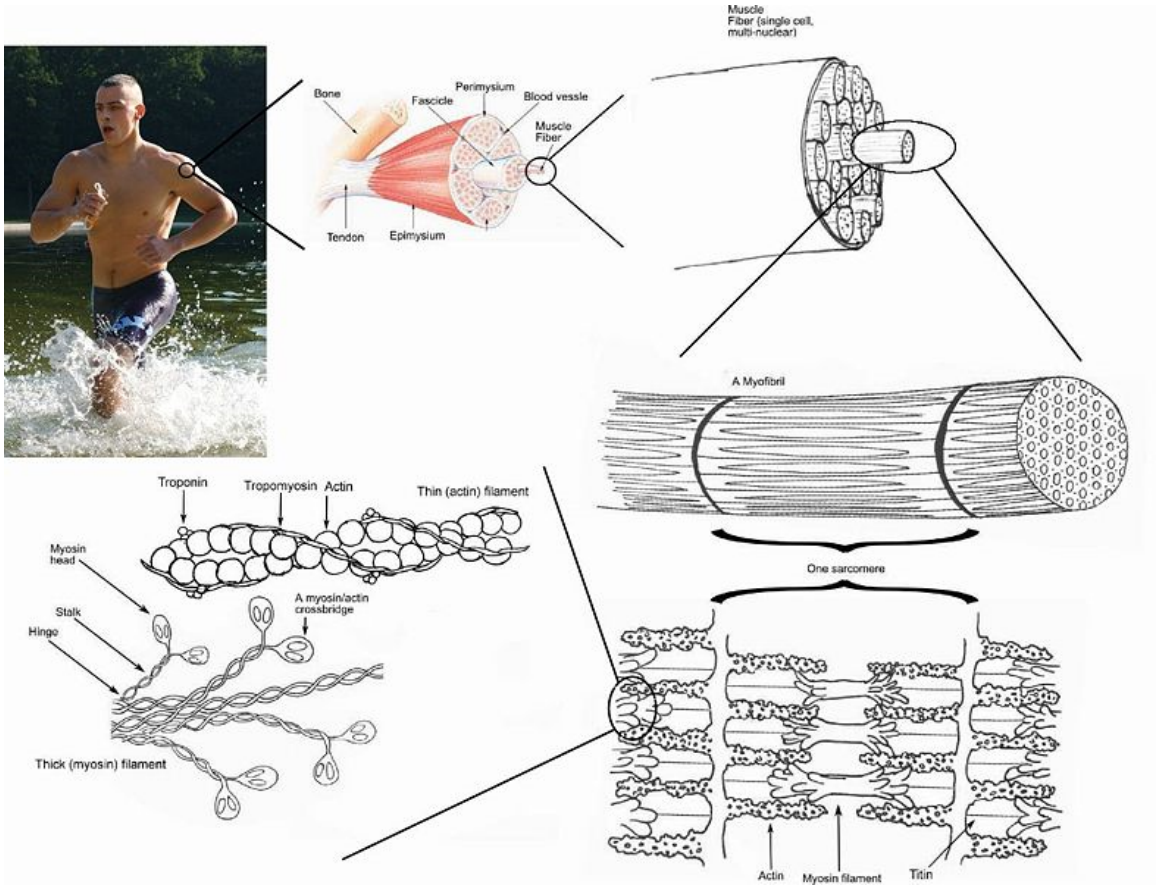
The study of biomechanics ranges from the inner workings of a cell to the movement and development of limbs, to the mechanical properties of soft tissue, and bones. As we develop a greater understanding of the physiological behavior of living tissues, researchers are able to advance the field of tissue engineering, as well as develop improved treatments for a wide array of pathologies.

Biomechanics is also applied to studying human musculoskeletal systems. Such research utilizes force platforms to study human ground reaction forces and infrared videography to capture the trajectories of markers attached to the human body to study human 3D motion. Research also applies electromyography (EMG) system to study the muscle activation. By this, it is feasible to investigate the muscle responses to the external forces as well as perturbations.

Biomechanics is widely used in orthopedic industry to design orthopedic implants for human joints, dental parts, external fixations and other medical purposes. Biotribology is a very important part of it. It is a study of the performance and function of biomaterials used for orthopedic implants. It plays a vital role to improve the design and produce successful biomaterials for medical and clinical purposes.

Chapter 2

Skeletal Muscle



A top-down view of skeletal muscle

Skeletal muscle is a form of striated muscle tissue existing under control of the somatic nervous system. It is one of three major muscle types, the others being cardiac and smooth muscle. As its name suggests, most skeletal muscle is attached to bones by bundles of collagen fibers known as tendons.

Skeletal muscle is made up of individual components known as *muscle fibers*. These fibers are formed from the fusion of developmental myoblasts (a type of embryonic progenitor cell that gives rise to a muscle cell). The myofibers (muscle fiber) are long,

cylindrical, multinucleated cells composed of actin and myosin myofibrils repeated as a sarcomere, the basic functional unit of the cell and responsible for skeletal muscle's striated appearance and forming the basic machinery necessary for muscle contraction. The term muscle refers to multiple bundles of muscle fibers held together by connective tissue.

Muscle fibers

Individual muscle fibers are formed during development from the fusion of several undifferentiated immature cells known as myoblasts into long, cylindrical, multi-nucleated cells. Differentiation into this state is primarily completed before birth with the cells continuing to grow in size thereafter. Skeletal muscle exhibits a distinctive banding pattern when viewed under the microscope due to the arrangement of cytoskeletal elements in the cytoplasm of the muscle fibers. The principal cytoplasmic proteins are myosin and actin (also known as "thick" and "thin" filaments, respectively) which are arranged in a repeating unit called a sarcomere. The interaction of myosin and actin is responsible for muscle contraction.

There are two principal ways to categorize muscle fibers: the type of myosin (fast or slow) present, and the degree of oxidative phosphorylation that the fiber undergoes. Skeletal muscle can thus be broken down into two broad categories: Type I and Type II. Type I fibers appear red due to the presence of the oxygen binding protein myoglobin. These fibers are suited for endurance and are slow to fatigue because they use oxidative metabolism to generate ATP. Type II fibers are white due to the absence of myoglobin and a reliance on glycolytic enzymes. These fibers are efficient for short bursts of speed and power and use both oxidative metabolism and anaerobic metabolism depending on the particular sub-type. These fibers are quicker to fatigue.

	Type I fibers	Type II a fibers	Type II x fibers	Type II b fibers
Contraction time	Slow	Moderately Fast	Fast	Very fast
Size of motor neuron	Small	Medium	Large	Very large
Resistance to fatigue	High	Fairly high	Intermediate	Low
Activity Used for	Aerobic	Long-term anaerobic	Short-term anaerobic	Short-term anaerobic
Maximum duration of use	Hours	<30 minutes	<5 minutes	<1 minute
Power produced	Low	Medium	High	Very high
Mitochondrial density	High	High	Medium	Low
Capillary density	High	Intermediate	Low	Low
Oxidative	High	High	Intermediate	Low

capacity				
Glycolytic capacity	Low	High	High	High
Major storage fuel	Triglycerides	Creatine phosphate, glycogen	Creatine phosphate, glycogen	Creatine phosphate, glycogen
Myosin heavy chain, human genes	MYH7	MYH2	MYH1	MYH4

Skeletal muscle fibers are not all the same. Traditionally, they were categorized depending on their varying color.

Red Fibers: Those containing high levels of myoglobin and oxygen storing proteins had a red appearance. Red muscle fibers tend to have more mitochondria and blood vessels than the white ones.

White Fibers: Those with a low content had a white appearance.

Skeletal muscle fibers are also classified, depending on their twitch capabilities, into fast and slow twitch.

Fast Twitch: Some authors define a fast twitch fiber as one in which the myosin can split ATP very quickly.

However, fast twitch fibers also demonstrate a higher capability for electrochemical transmission of action potentials and a rapid level of calcium release and uptake by the sarcoplasmic reticulum. The fast twitch fibers rely on a well developed, short term, glycolytic system for energy transfer and can contract and develop tension at 2-3 times the rate of slow twitch fibers.

Slow Twitch: The slow twitch fibers generate energy for ATP re-synthesis by means of a long term system of aerobic energy transfer. They tend to have a low activity level of ATPase, a slower speed of contraction with a less well developed glycolytic capacity. They contain large and numerous mitochondria and with the high levels of myoglobin that gives them a red pigmentation they have been demonstrated to have high concentration of mitochondrial enzymes, thus they are fatigue resistant.

The 2 main categories of muscle fibers become 3 when we split the white muscle fibers into 2 sections. So we expand further:

Type I Red fibers. Slow oxidative (also called slow twitch or fatigue resistant fibers). *Contain:*

- Large amounts of myoglobin.

- Many mitochondria.
- Many blood capillaries.
- Generate ATP by the aerobic system, hence the term oxidative fibers.
- Split ATP at a slow rate.
- Slow contraction velocity.
- Resistant to fatigue.
- Found in large numbers in postural muscles.
- Needed for aerobic activities like long distance running.

Type IIa Red fibers. Fast oxidative (also called fast twitch A or fatigue resistant fibers). *Contain:*

- Large amounts of myoglobin.
- Many mitochondria.
- Many blood capillaries.
- High capacity for generating ATP by oxidation. Split ATP at a very rapid rate and, hence, high contraction velocity
- Resistant to fatigue but not as much as slow oxidative fibers.
- Needed for sports such as middle distance running and swimming.

Type IIb White. Fast glycolytic (also called fast twitch B or fatigable fibers). *Contain:*

- Low myoglobin content.
- Few mitochondria.
- Few blood capillaries.
- Large amount of glycogen.
- Split ATP very quickly.
- Fatigue easily.
- Needed for sports like sprinting.

Individual muscles are a mixture of 3 types of muscle fibers (type 1, type 2a and type 2b), but their proportions vary depending on the action of that muscle. It must be remembered that skeletal muscles, although a mixture, can only have one type of muscle fiber within a motor unit. This is demonstrated if we look at contractions. E.g. If a weak contraction is needed only the type 1 motor units will be activated. These fibers are used mainly for endurance activities. If a stronger contraction is required the type 2a fibers will be activated or used to assist the type 1 fibers. Maximal contractions facilitate the use of type 2b fibers which are always activated last. These fibers are used during ballistic activities but tire easily. With advanced EMG techniques it is possible to look at which muscle fibers are recruited when performing an exercise/test. The total number of skeletal muscle fibers has traditionally been thought not to change. It is believed there are no sex or age differences in fiber distribution, however, relative fiber types vary considerably from muscle to muscle and person to person. Sedentary men and women (as well as young children) have 45% type 2 and 55% type 1 fibers. People at the higher end of any sport tend to demonstrate patterns of fiber distribution e.g. endurance athletes show a higher level of type 1 fibers. Sprint athletes, on the other hand, require large numbers of

type 2 b fibers. Middle distance event athletes show approximately equal distribution of the 2 types. This is also often the case for power athletes such as throwers and jumpers. It has been suggested that various types of exercise can induce changes in the fibers of a skeletal muscle. It is thought that if you perform endurance type events for a sustained period of time, some of the type 2b fibers transform into type 2a fibers. However, there is no consensus on the subject. It may well be that the type 2b fibers show enhancements of the oxidative capacity after high intensity endurance training which brings them to a level at which they are able to perform oxidative metabolism as effectively as slow twitch fibers of untrained subjects. This would be brought about by an increase in mitochondrial size and number and the associated related changes not a change in fiber type.

Structure of skeletal muscle fiber

Every organelle and macromolecule of a muscle fiber are arranged to ensure form meets function. The plasma membrane is called the sarcolemma with the cytoplasm known as the sarcoplasm. In the sarcoplasm are the myofibrils. The myofibrils are long protein bundles about 1 micrometer in diameter each containing myofilaments. Pressed against the inside of the sarcolemma are the unusual flattened nuclei. Between the myofibrils are the mitochondria. While the muscle fiber does not have a smooth endoplasmic reticulum it contains a sarcoplasmic reticulum. The sarcoplasmic reticulum surrounds the myofibrils and holds a reserve of the calcium ions needed to cause a muscle contraction. Periodically it has dilated end sacs known as terminal cisternae. These cross the muscle fiber from one side to the other. In between two terminal cisternae is a tubular infoldings called a transverse tubule (T tubule). The T tubule are the pathway for the action potential to signal the sarcoplasmic reticulum to release calcium causing a muscle contraction. Together two terminal cisternae and a transverse tubule form a triad.

Cellular physiology and contraction

In addition to the actin and myosin components that constitute the sarcomere, skeletal muscle fibers also contain two other important regulatory proteins, troponin and tropomyosin, that are necessary for muscle contraction to occur. These proteins are associated with actin and cooperate to prevent its interaction with myosin. Skeletal muscle cells are excitable and are subject to depolarization by the neurotransmitter acetylcholine, released at the neuromuscular junction by motor neurons.

Once a cell is sufficiently stimulated, the cell's sarcoplasmic reticulum releases ionic calcium (Ca^{2+}), which then interacts with the regulatory protein troponin. Calcium-bound troponin undergoes a conformational change that leads to the movement of tropomyosin, subsequently exposing the myosin-binding sites on actin. This allows for myosin and actin ATP-dependent cross-bridge cycling and shortening of the muscle.

Physics

Muscle force is proportional to physiologic cross-sectional area (PCSA), and muscle velocity is proportional to muscle fiber length. The strength of a joint, however, is

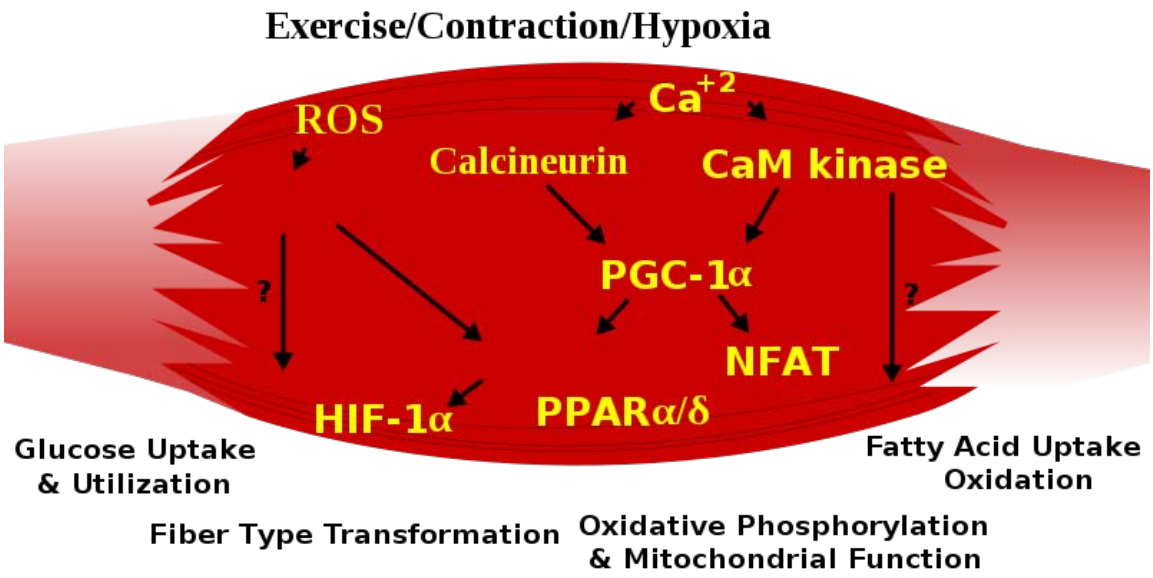
determined by a number of biomechanical parameters, including the distance between muscle insertions and pivot points and muscle size. Muscles are normally arranged in opposition so that as one group of muscles contract, another group relaxes or lengthens. Antagonism in the transmission of nerve impulses to the muscles means that it is impossible to stimulate the contraction of two antagonistic muscles at any one time. During ballistic motions such as throwing, the antagonist muscles act to 'brake' the agonist muscles throughout the contraction, particularly at the end of the motion. In the example of throwing, the chest and front of the shoulder (anterior Deltoid) contract to pull the arm forward, while the muscles in the back and rear of the shoulder (posterior Deltoid) also contract and undergo eccentric contraction to slow the motion down to avoid injury. Part of the training process is learning to relax the antagonist muscles to increase the force input of the chest and anterior shoulder.

Contracting muscles produce vibration and sound. Slow twitch fibers produce 10 to 30 contractions per second (10 to 30 Hz). Fast twitch fibers produce 30 to 70 contractions per second (30 to 70 Hz). The vibration can be witnessed and felt by highly tensing one's muscles, as when making a firm fist. The sound can be heard by pressing a highly tensed muscle against the ear, again a firm fist is a good example. The sound is usually described as a rumbling sound. Some individuals can voluntarily produce this rumbling sound by contracting the tensor tympani muscle of the middle ear. The rumbling sound can also be heard when the neck or jaw muscles are highly tensed.

Signal transduction pathways

Skeletal muscle fiber-type phenotype in adult animals, and probably people, is regulated by several independent signaling pathways. These include pathways involved with the Ras/mitogen-activated protein kinase (MAPK), calcineurin, calcium/calmodulin-dependent protein kinase IV, and the peroxisome proliferator γ coactivator 1 (PGC-1). The Ras/MAPK signaling pathway links the motor neurons and signaling systems, coupling excitation and transcription regulation to promote the nerve-dependent induction of the slow program in regenerating muscle. Calcineurin, a Ca2+/calmodulin-activated phosphatase implicated in nerve activity-dependent fiber-type specification in skeletal muscle, directly controls the phosphorylation state of the transcription factor NFAT, allowing for its translocation to the nucleus and leading to the activation of slow-type muscle proteins in cooperation with myocyte enhancer factor 2 (MEF2) proteins and other regulatory proteins. Calcium-dependent Ca2+/calmodulin kinase activity is also upregulated by slow motor neuron activity, possibly because it amplifies the slow-type calcineurin-generated responses by promoting MEF2 transactivator functions and enhancing oxidative capacity through stimulation of mitochondrial biogenesis.

Contraction-induced changes in intracellular calcium or reactive oxygen species provide signals to diverse pathways that include the MAPKs, calcineurin and calcium/calmodulin-dependent protein kinase IV to activate transcription factors that regulate gene expression and enzyme activity in skeletal muscle.



Exercise-Included Signaling Pathways in Skeletal Muscle That Determine Specialized Characteristics of ST and FT Muscle Fibers

PGC1-α (PPARGC1A), a transcriptional coactivator of nuclear receptors important to the regulation of a number of mitochondrial genes involved in oxidative metabolism, directly interacts with MEF2 to synergistically activate selective ST muscle genes and also serves as a target for calcineurin signaling. A peroxisome proliferator-activated receptor δ (PPARδ)-mediated transcriptional pathway is involved in the regulation of the skeletal musclefiber phenotype. Mice that harbor an activated form of PPARd display an "endurance" phenotype, with a coordinated increase in oxidative enzymes and mitochondrial biogenesis and an increased proportion of ST fibers. Thus—through functional genomics—calcineurin, calmodulin-dependent kinase, PGC-1α, and activated PPARδ form the basis of a signaling network that controls skeletal muscle fiber-type transformation and metabolic profiles that protect against insulin resistance and obesity.

The transition from aerobic to anaerobic metabolism during intense work requires that several systems are rapidly activated to ensure a constant supply of ATP for the working muscles. These include a switch from fat-based to carbohydrate-based fuels, a redistribution of blood flow from nonworking to exercising muscles, and the removal of several of the by-products of anaerobic metabolism, such as carbon dioxide and lactic acid. Some of these responses are governed by transcriptional control of the FT glycolytic phenotype. For example, skeletal muscle reprogramming from an ST glycolytic phenotype to an FT glycolytic phenotype involves the Six1/Eya1 complex, composed of members of the Six protein family. Moreover, the Hypoxia Inducible Factor-1α (HIF-1α) has been identified as a master regulator for the expression of genes involved in essential hypoxic responses that maintain ATP levels in cells. Ablation of HIF-1α in skeletal muscle was associated with an increase in the activity of bob-limiting enzymes of the mitochondria, indicating that the citric acid cycle and increased fatty acid oxidation may be compensating for decreased flow through the glycolytic pathway in these animals. However, hypoxia-mediated HIF-1α responses are also linked to the regulation of

mitochondrial dysfunction through the formation of excessive reactive oxygen species in mitochondria.

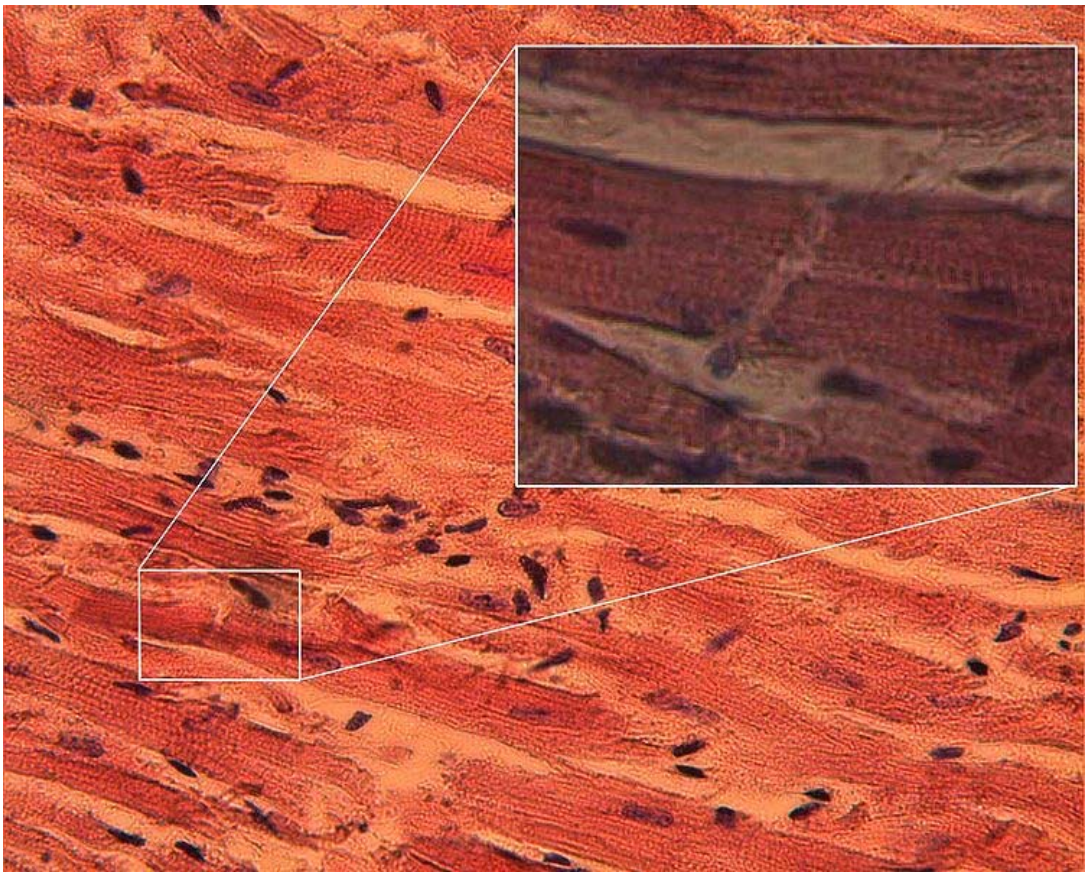
Other pathways also influence adult muscle character. For example, physical force inside a muscle fiber may release the transcription factor Serum Response Factor (SRF) from the structural protein titin, leading to altered muscle growth.

Research

Research on skeletal muscle properties uses many techniques. Electrical muscle stimulation is used to determine force and contraction speed at different stimulation frequencies, which are related to fiber-type composition and mix within an individual muscle group. In vitro muscle testing is used for more complete characterization of muscle properties.

Chapter 3

Cardiac Muscle



Cardiac muscle is a type of involuntary striated muscle found in the walls and histologic foundation of the heart, specifically the myocardium. Cardiac muscle is one of three major types of muscle, the others being skeletal and smooth muscle. The cells that comprise cardiac muscle, called myocardiocyteal muscle cells, are mononuclear, like smooth muscle cells.

Coordinated contractions of cardiac muscle cells in the heart propel blood out of the atria and ventricles to the blood vessels of the left/body/systemic and right/lungs/pulmonary circulatory systems. This complex of actions makes up the systole of the heart.

Cardiac muscle cells, like all tissues in the body, rely on an ample blood supply to deliver oxygen and nutrients and to remove waste products such as carbon dioxide. The coronary arteries fulfill this function.

Metabolism

Cardiac muscle is adapted to be highly resistant to fatigue: it has a large number of mitochondria, enabling continuous aerobic respiration via oxidative phosphorylation, numerous myoglobins (oxygen-storing pigment) and a good blood supply, which provides nutrients and oxygen. The heart is so tuned to aerobic metabolism that it is unable to pump sufficiently in ischaemic conditions. At basal metabolic rates, about 1% of energy is derived from anaerobic metabolism. This can increase to 10% under moderately hypoxic conditions, but, under more severe hypoxic conditions, not enough energy can be liberated by lactate production to sustain ventricular contractions.

Under basal aerobic conditions, 60% of energy comes from fat (free fatty acids and triglycerides), 35% from carbohydrates, and 5% from amino acids and ketone bodies. However, these proportions vary widely according to nutritional state. For example, during starvation, lactate can be recycled by the heart. This is very energy efficient, because one NAD^+ is reduced to NADH and H^+ (equal to 2.5 or 3 ATP) when lactate is oxidized to pyruvate, which can then be burned aerobically in the TCA cycle, liberating much more energy (ca 14 ATP per cycle).

In the condition of diabetes, more fat and less carbohydrate is used due to the reduced induction of GLUT4 glucose transporters to the cell surfaces. However, contraction itself plays a part in bringing GLUT4 transporters to the surface. This is true of skeletal muscle as well, but relevant in particular to cardiac muscle due to its continuous contractions.

Appearance

Striation

Cardiac muscle exhibits cross striations formed by alternating segments of thick and thin protein filaments. Like skeletal muscle, the primary structural proteins of cardiac muscle are actin and myosin. The actin filaments are thin causing the lighter appearance of the I bands in striated muscle, while the myosin filament is thicker lending a darker appearance to the alternating A bands as observed with electron microscopy. However, in contrast to skeletal muscle, cardiac muscle cells may be branched instead of linear and longitudinal.

T-Tubules

Another histological difference between cardiac muscle and skeletal muscle is that the T-tubules in the cardiac muscle are larger, broader and run along the Z-Discs. There are fewer T-tubules in comparison with skeletal muscle. Additionally, cardiac muscle forms diads instead of the triads formed between the T-tubules and the sarcoplasmic reticulum

in skeletal muscle. T-tubules play critical role in excitation-contraction coupling (ECC). Recently, the action potentials of T-tubules were recorded optically by Guixue Bu et al.

Intercalated discs

Intercalated discs (IDs) are complex adhering structures which connect single cardiac myocytes to an electrochemical syncytium (in contrast to the skeletal muscle, which becomes a multicellular syncytium during mammalian embryonic development) and are mainly responsible for force transmission during muscle contraction. Intercalated discs also support the rapid spread of action potentials and the synchronized contraction of the myocardium. IDs are described to consist of three different types of cell-cell junctions: the actin filament anchoring adherens junctions (fascia adherens), the intermediate filament anchoring desmosomes (macula adherens) and gap junctions. Gap junctions are responsible for electrochemical and metabolic coupling. They allow action potentials to spread between cardiac cells by permitting the passage of ions between cells, producing depolarization of the heart muscle. However, novel molecular biological and comprehensive studies unequivocally showed that IDs consist for the most part of mixed type adhering junctions named *area composita* (pl. *areae compositae*) representing an amalgamation of typical desmosomal and *fascia adhaerens* proteins (in contrast to various epithelia). The authors discuss the high importance of these findings for the understanding of inherited cardiomyopathies (such as Arrhythmogenic Right Ventricular Cardiomyopathy, ARVC).

Under light microscopy, intercalated discs appear as thin, typically dark-staining lines dividing adjacent cardiac muscle cells. The intercalated discs run perpendicular to the direction of muscle fibers. Under electron microscopy, an intercalated disc's path appears more complex. At low magnification, this may appear as a convoluted electron dense structure overlying the location of the obscured Z-line. At high magnification, the intercalated disc's path appears even more convoluted, with both longitudinal and transverse areas appearing in longitudinal section.

Role of calcium in contraction

In contrast to skeletal muscle, cardiac muscle requires *extracellular calcium ions* for contraction to occur. Like skeletal muscle,the initiation and upshoot of the action potential in ventricular muscle cells is derived from the entry of sodium ions across the sarcolemma in a regenerative process. However, an inward flux of extracellular calcium ions through L-type calcium channels sustains the depolarization of cardiac muscle cells for a longer duration. The reason for the calcium dependence is due to the mechanism of calcium-induced calcium release (CICR) from the sarcoplasmic reticulum that must occur under normal excitation-contraction (EC) coupling to cause contraction. Once the intracellular concentration of calcium increases, calcium ions bind to the protein troponin, which initiates contraction by allowing the contractile proteins, myosin and actin to associate through cross-bridge formation. Cardiac muscle is intermediate between smooth muscle, which has an unorganized sarcoplasmic reticulum and derives

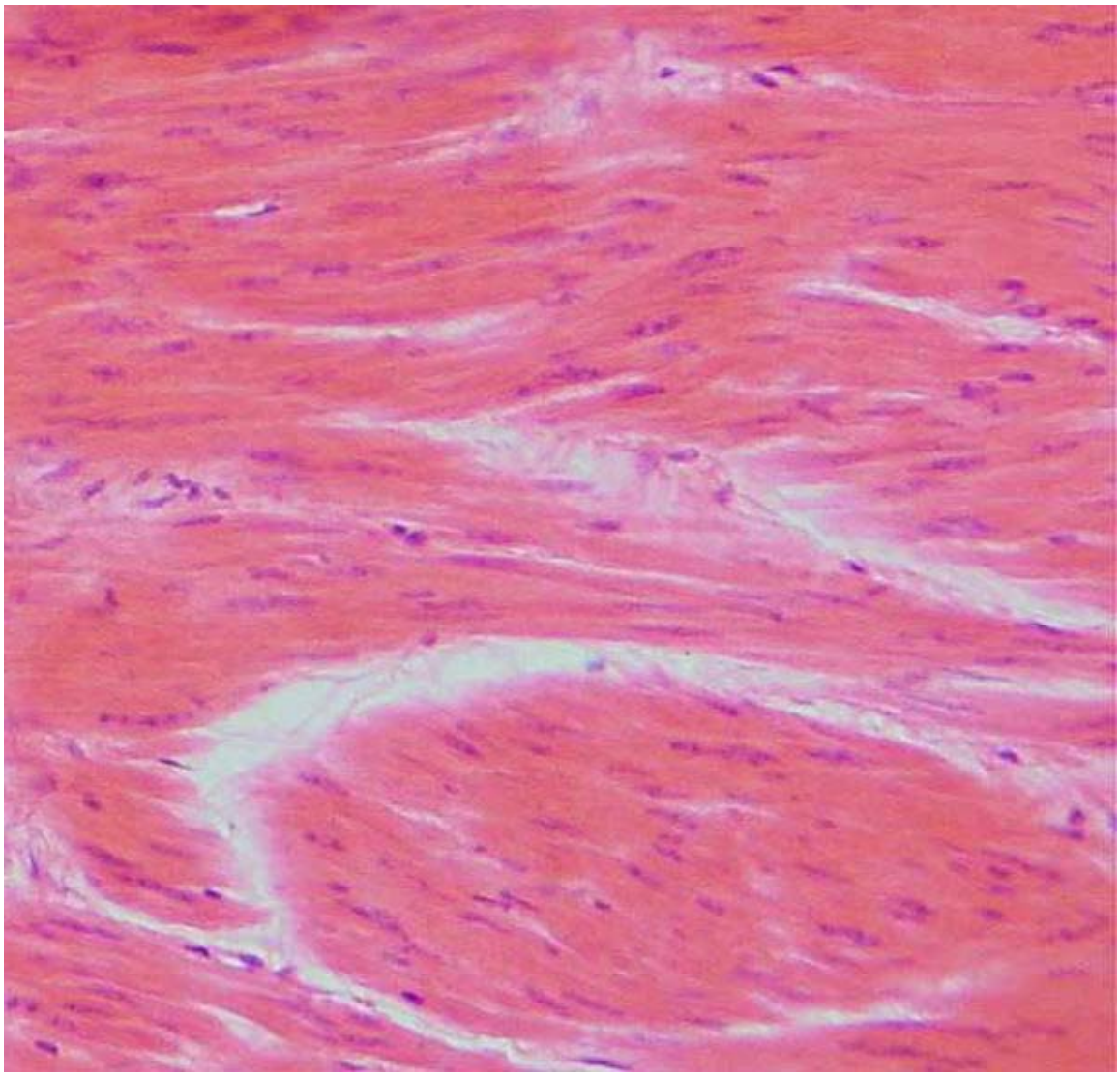
its calcium from both the extracellular fluid and intracellular stores, and skeletal muscle, which is only activated by calcium stored in the sarcoplasmic reticulum.

Regeneration of heart muscle cells

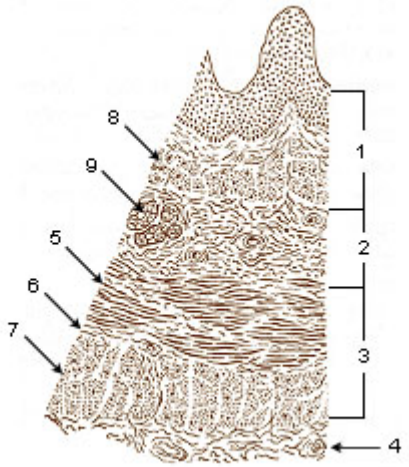
Until recently, it was commonly believed that cardiac muscle cells could not be regenerated. However, a study reported in the April 3, 2009 issue of *Science* contradicts that belief. Olaf Bergmann and his colleagues at the Karolinska Institute in Stockholm tested samples of heart muscle from people born before 1955 when nuclear bomb testing caused elevated levels of radioactive carbon 14 in the Earth's atmosphere. They found that samples from people born before 1955 did have elevated carbon 14 in their heart muscle cell DNA, indicating that the cells had divided after the person's birth. By using DNA samples from many hearts, the researchers estimated that a 20-year-old renews about 1% of heart muscle cells per year and about 45 percent of the heart muscle cells of a 50-year-old were generated after he or she was born.

Chapter 4

Smooth Muscle



Smooth muscle



Layers of Esophageal Wall:
1. Mucosa
2. Submucosa
3. Muscularis
4. Adventitia
5. Striated muscle
6. Striated and smooth
7. Smooth muscle
8. Lamina muscularis mucosae
9. Esophageal glands

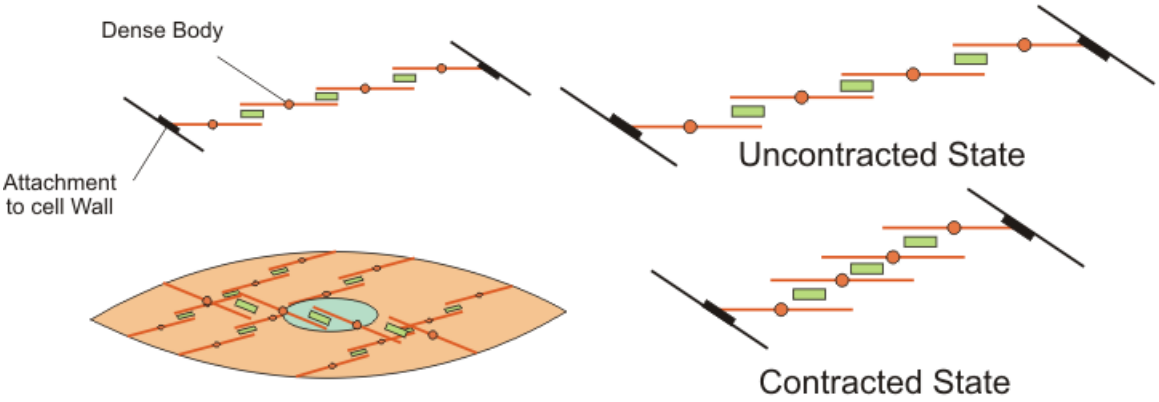
Smooth muscle is an involuntary non-striated muscle. It is divided into two sub-groups; the single-unit (unitary) and multiunit smooth muscle. Within single-unit smooth muscle tissues, the autonomic nervous system innervates a single cell within a sheet or bundle and the action potential is propagated by gap junctions to neighboring cells such that the whole bundle or sheet contracts as a syncytium (i.e., a multinucleate mass of cytoplasm that is not separated into cells). Multiunit smooth muscle tissues innervate individual cells; as such, they allow for fine control and gradual responses, much like motor unit recruitment in skeletal muscle.

Smooth muscle is found within the walls of blood vessels (such smooth muscle specifically being termed vascular smooth muscle) such as in the tunica media layer of large (aorta) and small arteries, arterioles and veins. Smooth muscle is also found in lymphatic vessels, the urinary bladder, uterus (termed uterine smooth muscle), male and female reproductive tracts, gastrointestinal tract, respiratory tract, arrector pili of skin, the ciliary muscle, and iris of the eye. The structure and function is basically the same in smooth muscle cells in different organs, but the inducing stimuli differ substantially, in order to perform individual effects in the body at individual times. In addition, the glomeruli of the kidneys contain a smooth muscle-like cells called mesangial cells.

Structure

Most smooth muscle is of the single-unit variety, that is, either the whole muscle contracts or the whole muscle relaxes, but there is multiunit smooth muscle in the trachea, the large elastic arteries, and the iris of the eye. Single unit smooth muscle, however, is most common and lines blood vessels (except large elastic arteries), the urinary tract, and the digestive tract.

Smooth muscle is fundamentally different from skeletal muscle and cardiac muscle in terms of structure, function, regulation of contraction, and excitation-contraction coupling.



Smooth muscle fibers have a fusiform shape and, like striated muscle, can tense and relax. However, smooth muscle containing tissue tend to demonstrate greater elasticity and function within a larger length-tension curve than striated muscle. This ability to stretch and still maintain contractility is important in organs like the intestines and urinary bladder. In the relaxed state, each cell is spindle-shaped, 20-500 micrometers in length.

Molecular structure

A substantial portion of the volume of the cytoplasm of smooth muscle cells are taken up by the molecules myosin and actin, which together have the capability to contract, and, through a chain of tensile structures, make the entire smooth muscle tissue contract with them.

Myosin

Myosin is primarily of class II in smooth muscle.

- Myosin II contains two *heavy chains* which constitute the head and tail domains. Each of these heavy chains contains the N-terminal head domain, while the C-terminal tails take on a coiled-coil morphology, holding the two heavy chains together (imagine two snakes wrapped around each other, such as in a caduceus). Thus, myosin II has two heads. In smooth muscle, there is a single gene (MYH11) that codes for the heavy chains myosin II, but there are splice variants of this gene that result in four distinct isoforms. Also, smooth muscle may contain MHC that is not involved in contraction, and that can arise from multiple genes.
- Myosin II also contains 4 *light chains*, resulting in 2 per head, weighing 20 (MLC_{20}) and 17 (MLC_{17}) kDa. These bind the heavy chains in the "neck" region between the head and tail.
    - The MLC_{20} is also known as the *regulatory light chain* and actively participates in muscle contraction. Two MLC_{20} isoforms are found in smooth muscle, and they are encoded by different genes, but only one isoform participates in contractility.
    - The MLC_{17} is also known as the *essential light chain*. Its exact function is unclear, but it's believed that it contributes to the structural stability of the myosin head along with MLC_{20}. Two variants of MLC_{17} ($MLC_{17a/b}$) exist as a result of alternate splicing at the MLC_{17} gene.

Different combinations of heavy and light chains allow for up to hundreds of different types of myosin structures, but it is unlikely that more than a few such combinations are actually used or permitted within a specific smooth muscle bed. In the uterus, a shift in myosin expression has been hypothesized to avail for changes in the directions of uterine contractions that are seen during the menstrual cycle.

Actin

The thin filaments that form part of the contractile machinery are predominantly composed of α- and γ-actin. Smooth muscle α-actin (alpha actin) is the predominate isoform within smooth muscle. There are also lots of actin (mainly β-actin) that does not take part in contraction, but that polymerizes just below the plasma membrane in the presence of a contractile stimulant and, and may thereby assist in mechanical tension.

The ratio of actin to myosin is between 2:1 and 10:1 in smooth muscle, compared to ~6:1 in skeletal muscle and 4:1 in cardiac muscle.

Smooth muscle does not contain the protein troponin; instead calmodulin (which takes on the regulatory role in smooth muscle), caldesmon and calponin are significant proteins expressed within smooth muscle. Tropomyosin is present in smooth but doesn't serve the same regulatory function as in striated muscle.

Other tensile structures

The myosin and actin form the contractile parts of continuous chains of tensile structures that stretch both across and between smooth muscle cells.

The actin filaments of contractile units are attached to *dense bodies*. Dense bodies are rich in α-actinin, and also attach intermediate filaments (consisting largely of vimentin and desmin), and thereby appear to serve as anchors from which the thin filaments can exert force. Dense bodies also are associated with β-actin, which is the type found in the cytoskeleton, suggesting that dense bodies may coordinate tensions from both the contractile machinery and the cytoskeleton.

The intermediate filaments are connected to other intermediate filaments via dense bodies, which eventually are attached to adherens junctions (also called focal adhesions) in the cell membrane of the smooth muscle cell, called the sarcolemma. The adherens junctions consist of large number of proteins including α-actinin, vinculin and cytoskeletal actin. The adherens junctions are scattered around *dense bands* that are circumfering the smooth muscle cell in a rib-like pattern. The dense band (or dense plaques)areas alternate with regions of membrane containing numerous caveolae. When actin and myosin interact, force is transduced to the sarcolemma. Smooth muscle cells have been observed contracting in a spiral corkscrew fashion, and contractile proteins have been observed organizing into zones of actin and myosin along the axis of the cell. The number of myosin filaments is dynamic between the relaxed and contracted state in some tissues as the ratio of actin to myosin changes, and the length and number of myosin filaments change.

Smooth muscle-containing tissue often must be stretched, so elasticity is an important attribute of smooth muscle. Smooth muscle cells may secrete a complex extracellular matrix containing collagen (predominantly types I and III), elastin, glycoproteins, and proteoglycans. These fibers with their extracellular matrices contribute to the viscoelasticity of these tissues. The great arteries are viscolelastic vessels that act like a Windkessel propagating ventricular contraction and smoothing out the pulsatile flow. Smooth muscle also has specific elastin and collagen receptors to interact with these proteins.

Caveolae

The sarcolemma also contains caveolae, which are microdomains of lipid rafts specialized to cell-signaling events and ion channels. These invaginations in the sarcoplasma contain a host of receptors (prostacyclin, endothelin, serotonin, muscarinic receptors, adrenergic receptors), second messenger generators (adenylate cyclase, Phospholipase C), G proteins (RhoA, G alpha), kinases (rho kinase-ROCK, Protein kinase C, Protein Kinase A), ion channels (L type Calcium channels, ATP sensitive Potassium channels, Calcium sensitive Potassium channels) in close proximity. The caveolae are often close to sarcoplasmic reticulum or mitochondria, and have been proposed to organize signaling molecules in the membrane.

Excitation-contraction coupling

A smooth muscle is excited by external stimuli, which causes contraction. Each step is further detailed below.

Inducing stimuli and factors

Smooth muscle may contract spontaneously (via ionic channel dynamics) or as in the gut special pacemakers cells interstitial cells of Cajal produce rhythmic contractions. Also, contraction, as well as relaxation, can be induced by a number of physiochemical agents (e.g., hormones, drugs, neurotransmitters - particularly from the autonomic nervous system.

Smooth muscle in various regions of the vascular tree, the airway and lungs, kidneys and vagina is different in their expression of ionic channels, hormone receptors, cell-signaling pathways, and other proteins that determine function.

External substances

For instance, most blood vessels respond to norepinephrine and epinephrine (from sympathetic stimulation or the adrenal medulla)by producing vasoconstriction (this response is mediated through alpha 1-adrenergic receptors). Blood vessels in skeletal muscle and cardiac muscle respond to these catecholamines producing vasodilation because the smooth muscle possess beta-adrenergic receptors.

Generally, arterial smooth muscle responds to carbon dioxide by producing vasodilation, and responds to oxygen by producing vasoconstriction. Plumonary blood vessels within the lung are unique as they vasodilate to high oxygen tension and vasoconstrict when it falls. Bronchiole smooth muscle that lines the airways of the lung respond to high carbon dioxide producing vasodilation and vasoconstrict when carbon dioxide is low. These responses to carbon dioxide and oxygen by pulmonary blood vessels and bronchiole airway smooth muscle aid in matching perfusion and ventilation within the lungs. Further different smooth muscle tissues display extremes of abundant to little sarcoplasmic reticulum so excitation-contraction coupling varies with its dependence on intracellular or extracellular calcium.

Stretch

Recent research indicates that sphingosine-1-phosphate (S1P) signaling is an important regulator of vascular smooth muscle contraction. When transmural pressure increases, sphingosine kinase 1 phosphorylates sphingosine to S1P, which binds to the S1P2 receptor in plasma membrane of cells. This leads to a transient increase in intracellular calcium, and activates Rac and Rhoa signaling pathways. Collectively, these serve to increase MLCK activity and decrease MLCP activity, promoting muscle contraction. This allows arterioles to increase resistance in response to increased blood pressure and thus maintain constant blood flow. The Rhoa and Rac portion of the signaling pathway provides a calcium-independent way to regulate resistance artery tone.

Spread of impulse

To maintain organ dimensions against force, cells are fastened to one another by adherens junctions. As a consequence, cells are mechanically coupled to one another such that contraction of one cell invokes some degree of contraction in an adjoining cell. Gap junctions couple adjacent cells chemically and electrically, facilitating the spread of chemicals (e.g., calcium) or action potentials between smooth muscle cells. Single unit smooth muscle displays numerous gap junctions and these tissues often organize into sheets or bundles which contract in bulk.

Contraction

Smooth muscle contraction is caused by the sliding of myosin and actin filaments (a sliding filament mechanism) over each other. The energy for this to happen is provided by the hydrolysis of ATP. Myosin functions as an ATPase utilizing ATP to produce a molecular conformational change of part of the myosin and produces movement. Movement of the filaments over each other happens when the globular heads protruding from myosin filaments attach and interact with actin filaments to form crossbridges. The myosin heads tilt and drag along the actin filament a small distance (10-12 nm). The heads then release the actin filament and then changes angle to relocate to another site on the actin filament a further distance (10-12 nm) away. They can then re-bind to the actin molecule and drag it along further. This process is called crossbridge cycling and is the same for all muscles. Unlike cardiac and skeletal muscle, smooth muscle does not contain the calcium-binding protein troponin. Contraction is initiated by a calcium-regulated phosphorylation of myosin, rather than a calcium-activated troponin system.

Crossbridge cycling causes contraction of myosin and actin complexes, in turn causing increased tension along the entire chains of tensile structures, ultimately resulting in contraction of entire smooth muscles.

Phasic or tonic

Smooth muscle may contract phasically with rapid contraction and relaxation, or tonically with slow and sustained contraction. The reproductive, digestive, respiratory, and urinary tracts, skin, eye, and vasculature all contain this tonic muscle type. This type of smooth muscle can maintain force for prolonged time with only little energy utilization. There are differences in the myosin heavy and light chains that also correlate with these differences in contractile patterns and kinetics of contraction between tonic and phasic smooth muscle.

Activation of myosin heads

Crossbridge cycling cannot occur until the myosin heads have been activated to allow crossbridges to form. When the light chains are phosphorylated, they become active and will allow contraction to occur. The enzyme that phosphorylates the light chains is called myosin light-chain kinase (MLCK), also called MLC_{20} kinase. In order to control

contraction, MLCK will work only when the muscle is stimulated to contract. Stimulation will increase the intracellular concentration of calcium ions. These bind to a molecule called calmodulin, and form a calcium-calmodulin complex. It is this complex that will bind to MLCK to activate it, allowing the chain of reactions for contraction to occur.

Activation consists of phosphorylation of a serine on position 19 (Ser19) on the MLC_{20} light chain, which causes a conformational change that increases the angle in the neck domain of the myosin heavy chain, which corresponds to the part of the cross-bridge cycle where the myosin head is unattached to the actin filament and relocates to another site on it. After attachment of the mysin head to the actin filament, this serine phosphorylation also activates the ATPase activity of the myosin head region to provide the energy to fuel the subsequent contraction. Phosphorylation of a threonine on position 18 (Thr18) on MLC20 is also possible and may further increase the ATPase activity of the myosin complex.

Sustained maintenance

Phosphorylation of the MLC_{20} myosin light chains correlates well with the shortening velocity of smooth muscle. During this period there is a rapid burst of energy utilization as measured by oxygen consumption. Within a few minutes of initiation the calcium level markedly decrease, MLC_{20} myosin light chains phosphorylation decreases, and energy utilization decreases and the muscle can relax. Still, smooth muscle has the ability of sustained maintenance of force in this situation as well. This sustained phase has been attributed to certain myosin crossbridges, termed latch-bridges, that are cycling very slowly, notably at the cycle stage where dephosphorylated myosin complexes detach from the actin, thereby maintaining the force at low energy costs. This phenomenon is of great value especially for tonically active smooth muscle.

Isolated preparations of vascular and visceral smooth muscle contract with depolarizing high potassium balanced saline generating a certain amount of contractile force. The same preparation stimulated in normal balanced saline with an agonist such as endothelin or serotonin will generate more contractile force. This increase in force is termed calcium sensitization. The myosin light chain phosphatase is inhibited to increase the gain or sensitivity of myosin light chain kinase to calcium. There are number of cell signalling pathways believed to regulate this decrease in myosin light chain phosphatase: a RhoA-Rock kinase pathway, a Protein kinase C-Protein kinase C potentiation inhibitor protein 17 (CPI-17) pathway, telokin, and a Zip kinase pathway. Further Rock kinase and Zip kinase have been implicated to directly phosphorylate the 20kd myosin light chains.

Other contractile mechanisms

Other cell signaling pathways and protein kinases (Protein kinase C, Rho kinase, Zip kinase, Focal adhesion kinases) have been implicated as well and actin polymerization dynamics plays a role in force maintenance. While myosin light chain phosphorylation correlates well with shortening velocity, other cell signaling pathways have been implicated in the development of force and maintenance of force. Notably the

phosphorylation of specific tyrosine residues on the focal adhesion adapter protein-paxillin by specific tyrosine kinases has been demonstrated to be essential to force development and maintenance. Cyclic nucleotides can relax arterial smooth muscle without reductions in crossbridge phosphorylation, a process termed force suppression. This process is mediated by the phosphorylation of the small heat shock protein, hsp20, and may prevent phosphorylated myosin heads from interacting with actin.

Relaxation

The phosphorylation of the light chains by MLCK is countered by a myosin light-chain phosphatase, which dephosphorylates the MLC_{20} myosin light chains and thereby inhibits contraction. Other signaling pathways have also been implicated in the regulation actin and myosin dynamics. In general, the relaxation of smooth muscle is by cell-signaling pathways that increase the myosin phosphatase activity, decrease the intracellular calcium levels, hyperpolarize the smooth muscle, and/or regulate actin and myosin dynamics.

Relaxation-inducing factors

The relaxation of smooth muscle can be mediated by the endothelium-derived relaxing factor-nitric oxide, endothelial derived hyperpolarizing factor (either an endogenous cannabinoid, cytochrome P450 metabolite, or hydrogen peroxide), or prostacyclin (PGI2). Nitric oxide and PGI2 stimulate soluble guanylate cyclase and membrane bound adenylate cyclase, respectively. The cyclic nucleotides (cGMP and cAMP) produced by these cyclases activate Protein Kinase G and Proten Kinase A and phosphorylate a number of proteins. The phosphorylation events lead to a decrease in intracelluar calcium (inhibit L type Calcium channels, inhibits IP3 receptor channels, stimulates sarcoplasmic reticulum Calcium pump ATPase), a decrease in the 20kd myosin light chain phosphorylation by altering calcium sensitization and increasing myosin light chain phosphatase activity, a stimulation of calcium sensitive potassium channels which hyperpolarize the cell, and the phosphorylation of amino acid residue serine 16 on the small heat shock protein (hsp20)by Protein Kinases A and G. The phosphorylation of hsp20 appears to alter actin and focal adhesion dynamics and actin-myosin interaction, and recent evidence indicates that hsp20 binding to 14-3-3 protein is involved in this process. An alternative hypothesis is that phosphorylated Hsp20 may also alter the affinity of phosphorylated myosin with actin and inhibit contractility by interfering with crossbridge formation. The endothelium derived hyperpolarizing factor stimulates calcium sensitive potassium channels and/or ATP sensitive potassium channels and stimulate potassium efflux which hyperpolarizes the cell and produces relaxation.

Invertebrate smooth muscle

In invertebrate smooth muscle, contraction is initiated with the binding of calcium directly to myosin and then rapidly cycling cross-bridges, generating force. Similar to the mechanism of vertebrate smooth muscle, there is a low calcium and low energy utilization catch phase. This sustained phase or catch phase has been attributed to a catch

protein that has similarities to myosin light-chain kinase and the elastic protein-titin called twitchin. Clams and other bivalve mollusks use this catch phase of smooth muscle to keep their shell closed for prolonged periods with little energy usage.

Specific effects

Although the structure and function is basically the same in smooth muscle cells in different organs, their specific effects or end-functions differ.

Smooth muscle forms precapillary sphincters in blood vessels in metarterioles which regulates the blood flow in capillary beds of various organs and tissues. The contractile function of vascular smooth muscle also regulates the lumenal diameter of the small arteries-arterioles called resistance vessels, thereby contributing significantly to setting the level of blood pressure. Smooth muscle contracts slowly and may maintain the contraction (tonically) for prolonged periods in blood vessels, bronchioles, and some sphincters. Activating arteriole smooth muscle can decrease the lumenal diameter 1/3 of resting so it drastically alters blood flow and resistance. Activation of aortic smooth muscle doesn't significantly alter the lumenal diameter but serves to increase the viscoelasticity of the vascular wall.

In the digestive tract, smooth muscle contracts in a rhythmic peristaltic fashion, rhythmically forcing foodstuffs through the digestive tract as the result of phasic contraction.

A non-contractile function is seen in specialized smooth muscle within the afferent arteriole of the juxtaglomerular apparatus, which secretes renin in response to osmotic and pressure changes, and also it is believed to secrete ATP in tubuloglomerular regulation of glomerular filtration rate. Renin in turn activates the renin-angiotensin system to regulate blood pressure.

Growth and rearrangement

The mechanism in which external factors stimulate growth and rearrangement is not yet fully understood. A number of growth factors and neurohumoral agents influence smooth muscle growth and differentiation. The Notch receptor and cell-signaling pathway have been demonstrated to be essential to vasculogenesis and the formation of arteries and veins.

The embryological origin of smooth muscle is usually of mesodermal origin. However, the smooth muscle within the Aorta and Pulmonary arteries (the Great Arteries of the heart) is derived from ectomesenchyme of neural crest origin, although coronary artery smooth muscle is of mesodermal origin.

Related diseases

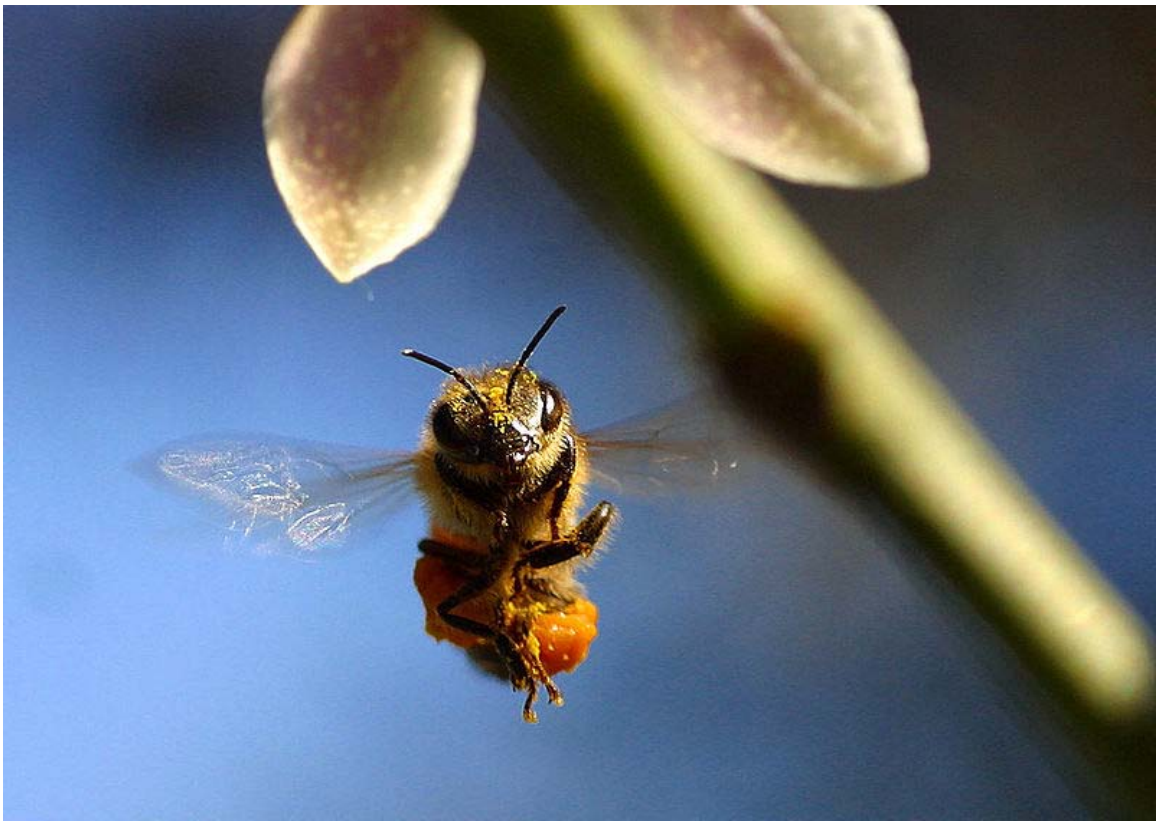
"Smooth muscle condition" is a condition in which the body of a developing embryo does not create enough smooth muscle for the gastrointestinal system. This condition is fatal.

Anti-smooth muscle antibodies (ASMA) can be a symptom of an auto-immune disorder, such as hepatitis, cirrhosis, or lupus.

Vascular smooth muscle tumors are very rare. They can be malignant or benign, and morbidity can be significant with either type. Intravascular leiomyomatosis is a benign neoplasm that extends through the veins; angioleiomyoma is a benign neoplasm of the extremities; vascular leiomyosarcomas is a malign neoplasm that can be found in the inferior vena cava, pulmonary arteries and veins, and other peripheral vessels.

Chapter 5

Animal Locomotion



A bee in flight

Animal locomotion, which is the act of self-propulsion by an animal, has many manifestations, including running, jumping and flying. Animals move for a variety of reasons, such as to find food, a mate, or a suitable microhabitat, and to escape predators. For many animals the ability to move is essential to survival and, as a result, selective pressures have shaped the locomotion methods and mechanisms employed by moving organisms. For example, migratory animals that travel vast distances (such as the Arctic Tern) typically have a locomotion mechanism that costs very little energy per unit distance, whereas non-migratory animals that must frequently move quickly to escape predators (such as frogs) are likely to have costly but very fast locomotion. The study of animal locomotion is typically considered to be a sub-field of biomechanics.

Locomotion requires energy to overcome friction, drag, inertia, and gravity, though in many circumstances some of these factors are negligible. In terrestrial environments gravity must be overcome, though the drag of air is much less of an issue. In aqueous environments however, friction (or drag) becomes the major challenge, with gravity being less of a concern. Although animals with natural buoyancy need not expend much energy maintaining vertical position, some will naturally sink and must expend energy to remain afloat. Drag may also present a problem in flight, and the aerodynamically efficient body shapes of birds highlight this point. Flight presents a different problem from movement in water however, as there is no way for a living organism to have lower density than air. Limbless organisms moving on land must often contend with surface friction, but do not usually need to expend significant energy to counteract gravity.

Newton's third law of motion is widely used in the study of animal locomotion: if at rest, to move forwards an animal must push something backwards. Terrestrial animals must push the solid ground, swimming and flying animals must push against a fluid or gas (either water or air). The effect of forces during locomotion on the design of the skeletal system is also important, as is the interaction between locomotion and muscle physiology, in determining how the structures and effectors of locomotion enable or limit animal movement.

Introduction

Animals move through a variety of fluids, such as water, air and mud. Some, for example seals and otters, move through more than one type of fluid. In some cases locomotion is facilitated by the substrate on which they move. Forms of locomotion include:

Through a fluid medium

Swimming

In the water staying afloat is possible through buoyancy. Provided an aquatic animal's body is no denser than its aqueous environment, it should be able to stay afloat well enough. Though this means little energy need be expended maintaining vertical position, it makes movement in the horizontal plane much more difficult. The drag encountered in water is much higher than that of air, which is almost negligible at low speeds. Body shape is therefore important for efficient movement, which is essential for basic functions like catching prey. A fusiform, torpedo-like body form is seen in many marine animals, though the mechanisms they employ for movement are diverse. Movement of the body may be from side to side, as in sharks and many fishes, or up and down, as in marine mammals. Other animals, such as those from the class *Cephalopoda*, use jet-propulsion, taking in water then squirting it back out in an explosive burst. Others may rely predominantly on their limbs, much as humans do when swimming. Though life on land originated from the seas, terrestrial animals have returned to an aquatic lifestyle on several occasions, such as the fully aquatic cetaceans, now far removed from their terrestrial ancestors.

Flight

Gravity is a major problem for flight through the air. Because it is impossible for any organism to approach the density of air, flying animals must generate enough lift to ascend and remain airborne. Wing shape is crucial in achieving this, generating a pressure gradient that results in an upward force on the animal' body. The same principle applies to airplanes, the wings of which are also airfoils. Unlike aircraft however, flying animals must be very light to achieve flight, the largest living flying animals being birds of around 20 kilograms. Other structural modifications of flying animals include reduced and redistributed body weight, fusiform shape and powerful flight muscles.

Rather than fly, some animals simply reduce their rate of falling by gliding. Flight has independently evolved at least four times, in the insects, pterosaurs, birds, and bats. Gliding has evolved on many more occasions. The advantage gliding provides to arboreal animals provides a bridge for the evolution of flight.

On a substrate

Terrestrial

Forms of locomotion on land include walking, running, hopping or jumping, and crawling or slithering. Here friction and buoyancy are no longer an issue, but a strong skeletal and muscular framework are required in most terrestrial animals for structural support. Each step also requires much energy to overcome inertia, and animals can store elastic potential energy in their tendons to help overcome this. Balance is also required for movement on land. Human infants learn to crawl first before they are able to stand on two feet, which requires good coordination as well as physical development. Humans are bipedal animals, standing on two feet and keeping one on the ground at all times while walking. When running, only one foot is on the ground at any one time at most, and both leave the ground briefly. At higher speeds momentum helps keep the body upright, so more energy can be used in movement. The number of legs an animal has varies greatly, resulting in differences in locomotion. Many familiar mammals have four legs; insects have six, while arachnids have eight. Centipedes and millipedes have many sets of legs that move in metachronal rhythm. Some have none at all, relying on other modes of locomotion.

Other animals move in terrestrial habitats without the aid of legs. Earthworms crawl by a peristalsis, the same rhythmic contractions that propel food through the digestive tract. Snakes move using several different modes of locomotion, depending upon substrate type and desired speed. Some animals even roll, though typically not as a primary means of locomotion.

Some animals are specialized for moving on non-horizontal surfaces. One common habitat for such climbing animals is in trees, for example the gibbon is specialized for arboreal movement, traveling rapidly by brachiation. Another case is animals like the snow leopard living on steep rock faces such as are found in mountains. Some light

animals are able to climb up smooth sheer surfaces or hang upside down by adhesion. Many insects can do this, though much larger animals such as geckos can also perform similar feats.

On water

While animals like ducks can swim in water by floating, some small animals move across it without breaking through the surface. This surface locomotion takes advantage of the surface tension of water. Animals that move in such a way include the water strider. Water striders have legs that are hydrophobic, preventing them from interfering with the structure of water. Another form of locomotion (in which the surface layer is broken) is used by the Basilisk lizard.

Through a solid medium

Some animals move through solids such as soil by burrowing using claws, teeth, or other methods. A burrow is a hole or tunnel dug into the ground by an animal to create a space suitable for habitation, temporary refuge, or as a byproduct of locomotion. In loose solids such a sand some animals, such as the golden mole, marsupial mole, and the pink fairy armadillo, are able to move more rapidly, 'swimming' through the loose substrate. Burrowing animals include moles, ground squirrels, naked mole rats, tilefish, mole crickets, and earthworms.

Energetics

The energetics of locomotion involves the energy expenditure by animals in moving. Energy consumed in locomotion is not available for other efforts, so animals typically have evolved to use the minimum energy possible during movement. However, in the case of certain behaviors, such as locomotion to escape a predator, performance (such as speed or maneuverability) is more crucial, and such movements may be energetically expensive. Furthermore, animals may use energetically expensive methods of locomotion when environmental conditions (such as being within a tunnel) preclude other modes.

The most common metric of energy use during locomotion is net cost of transport, defined as the calories needed above baseline metabolism to move a given distance, per unit body mass. For aerobic locomotion, most animals have a nearly constant cost of transport - moving a given distance requires the same caloric expenditure, regardless of speed. This constancy is usually accomplished by changes in gait. The net cost of transport of swimming is lowest, followed by flight, with terrestrial limbed locomotion being the most expensive per unit distance. However, because of the speeds involved, flight requires the most energy per unit time. This does not mean that an animal that normally moves by running would be a more efficient swimmer, however; these comparisons assume an animal is specialized for that form of motion. Another consideration here is body mass—heavier animals, though using more total energy, require less energy *per unit mass* to move. Physiologists generally measure energy use by

the amount of oxygen consumed, or the amount of carbon dioxide produced, in an animal's respiration.

Methods of study

A variety of methods and equipment are used to study animal locomotion:

- **Kinematics** is the study of the motion of an entire animal or parts of its body. It is typically accomplished by placing visual markers at particular anatomical locations on the animal and then recording video of its movement. The video is often captured from multiple angles, with frame rates exceeding 2000 frames per second when capturing high speed movement. The location of each marker is determined for each video frame, and data from multiple views is integrated to give positions of each point through time. Computers are sometimes used to track the markers, although this task must often be performed manually. The kinematic data can be used to determine fundamental motion attributes such as velocity, acceleration, joint angles, and the sequencing and timing of kinematic events. These fundamental attributes can be used to quantify various higher level attributes, such as the physical abilities of the animal (e.g., its maximum running speed, how steep a slope it can climb), neural control of locomotion, gait, and responses to environmental variation. These, in turn, can aid in formulation of hypotheses about the animal or locomotion in general.

- **Force plates** are platforms, usually part of a trackway, that can be used to measure the magnitude and direction of forces of an animal's step. When used with kinematics and a sufficiently detailed model of anatomy, inverse dynamics solutions can determine the forces not just at the contact with the ground, but at each joint in the limb.

- **Electromyography** (EMG) is a method of detecting the electrical activity that occurs when muscles are activated, thus determining which muscles are used when in a given movement. This can be accomplished either by surface electrodes (usually in large animals) or implanted electrodes (often wires thinner than a human hair). Furthermore, the intensity of electrical activity can correlate to the level of muscle activity, with greater activity implying (though not definitively showing) greater force.

- **Sonomicrometry** employs a pair of piezoelectric crystals implanted in a muscle or tendon to continuously measure the length of a muscle or tendon. This is useful because surface kinematics may be inaccurate due to skin movement. Similarly, if an elastic tendon is in series with the muscle, the muscle length may not be accurately reflected by the joint angle.

- **Tendon force buckles** measure the force produced by a single muscle by measuring the strain of a tendon. After the experiment, the tendon's elastic

modulus is determined and used to compute the exact force produced by the muscle. However, this can only be used on muscles with long tendons.

- **Particle image velocimetry** is used in aquatic systems to measure the flow of fluid around and past a moving aquatic organism, allowing fluid dynamics calculations to determine pressure gradients, speeds, etc.

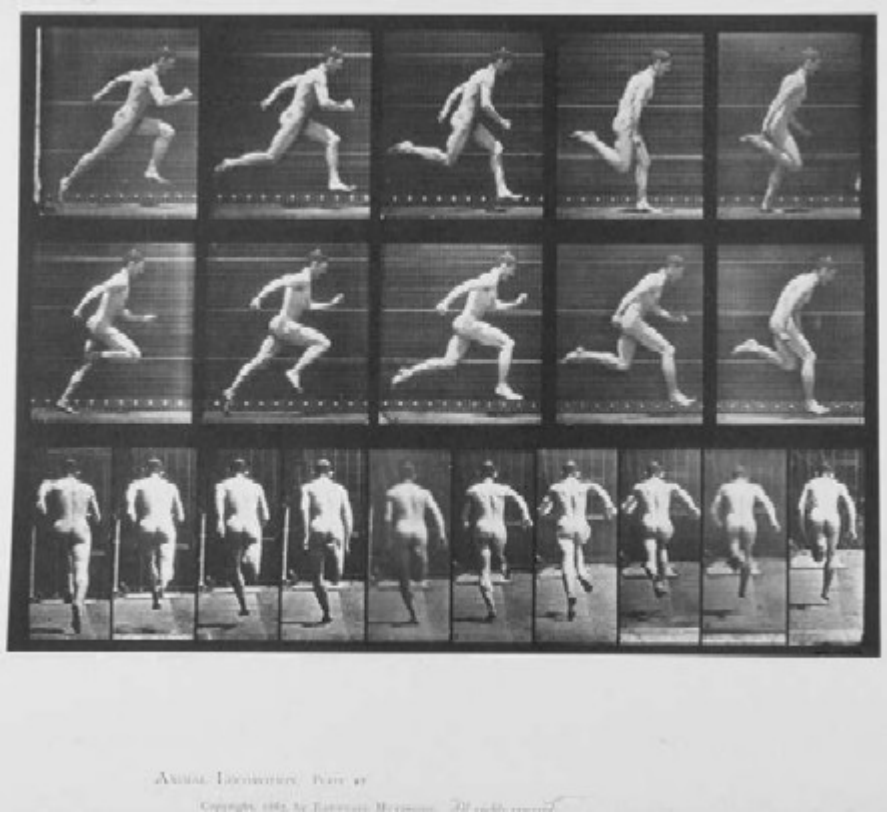
- **Fluoroscopy** allows real-time X-ray video, for precise kinematics of moving bones. Markers which are opaque to X-rays can allow simultaneous tracking of muscle length.

All of the methods can be combined. For example, studies frequently combine EMG and kinematics to determine "motor pattern", the series of electrical and kinematic events which produce a given movement.

Chapter 6

Bipedalism

An ostrich, one of the fastest of living bipeds



A Man Running - Eadweard Muybridge

Bipedalism is a form of terrestrial locomotion where an organism moves by means of its two rear limbs, or legs. An animal or machine that usually moves in a **bipedal** manner is known as a **biped**, meaning "two feet" (from the Latin *bi* for "two" and *ped* for "foot"). Types of bipedal movement include walking, running, or hopping, on two appendages (typically legs).

Relatively few modern species are habitual bipeds whose normal method of locomotion is two-legged. Within mammals, habitual bipedalism has evolved four times, with the macropods, kangaroo mice, springhare and homininan apes. In the Triassic period some groups of archosaurs (a group that includes the ancestors of crocodiles) developed bipedalism; among their descendants the dinosaurs all the early forms and many later groups were habitual or exclusive bipeds; the birds descended from one group of exclusively bipedal dinosaurs.

A larger number of modern species are capable of bipedal movement for a short time in exceptional circumstances. Several non-archosaurian lizard species move bipedally when running, usually to escape from threats. Many animals rear up on their hind legs whilst fighting or copulating. A few animals commonly stand on their hind legs, in order to reach food, to keep watch, to threaten a competitor or predator, or to pose in courtship, but do not move bipedally.

There are two main types of bipedal locomotion: macropods, some smaller birds, and heteromyid rodents move by hopping on both legs simultaneously; other groups, including apes and larger birds, walk or run by moving one leg at a time.

Definition

The word is derived from the Latin words *bi(s)* 'two (2)' and *ped-* 'foot', as contrasted with quadruped 'four feet'.

Facultative and obligate bipedalism

Zoologists often label behaviors, including bipedalism, as "facultative" (i.e. optional) or "obligate" (the animal has no reasonable alternative). Even this distinction is not completely clear-cut - for example humans normally walk and run in biped fashion, but almost all can crawl on hands and knees when necessary. There are even reports of humans who normally walk on all fours with their feet but not their knees on the ground, but these cases are a result of conditions such as Uner Tan syndrome - very rare genetic neurological disorders rather than normal behavior. Even if one ignores exceptions caused by some kind of injury or illness, there are many unclear cases, including the fact that "normal" humans can crawl on hands and knees. Here we, therefore avoids the terms "facultative" and "obligate", and focuses on the range of styles of locomotion *normally* used by various groups of animals.

Movement

There are a number of states of movement commonly associated with bipedalism.

1. Standing. Staying still on both legs. In most bipeds this is an active process, requiring constant adjustment of balance.
2. Walking. One foot in front of another, with at least one foot on the ground at any time.
3. Running. One foot in front of another, with periods where both feet are off the ground.
4. Jumping/Hopping. Moving by a series of jumps with both feet moving together.

Bipedal animals

The great majority of living terrestrial vertebrates are quadrupeds. Among mammals, bipedalism is a normal method of ground locomotion in various groups of primates (e.g. lemurs, gibbons and Hominina), in the macropods (kangaroos, wallabies, etc.), and in a few groups of rodents, including kangaroo rats and kangaroo mice in the family Heteromyidae, as well as gerbils and spring hares). All birds are bipeds when on the ground, a feature inherited from their dinosaur ancestors. Bipedalism evolved more than once in archosaurs, the group that includes both dinosaurs and crocodilians. Many species of lizards become bipedal during high-speed, sprint locomotion, including the world's

fastest lizard, the spiny-tailed iguana (genus *Ctenosaura*). There are no known living or fossil bipedal amphibians.

Most bipedal animals move with their backs close to horizontal, using a long tail to balance the weight of their bodies. The primate version of bipedalism is unusual because the back is close to upright (completely upright in humans) and, among primates that move bipedally, only the lemurs have tails.

Humans and large birds walk by raising one foot at a time. On the other hand most macropods, smaller birds and bipedal rodents move by hopping on both legs simultaneously. Tree kangaroos are able to utilize either form of locomotion, most commonly alternating feet when moving arboreally and hopping on both feet simultaneously when on the ground.

Dinosaurs and other archosaurs

All dinosaurs are believed to be descended from a fully bipedal ancestor, perhaps similar to *Eoraptor*. Bipedal movement also re-evolved in a number of other dinosaur lineages such as the iguanodons. Some extinct members of the crocodilian line, a sister group to the dinosaurs and birds, also evolved bipedal forms - a crocodile relative from the triassic, *Effigia okeeffeae*, was believed to be bipedal. Pterosaurs were previously thought to have been bipedal, but recent trackways have all shown quadrupedal locomotion.

Mammals

Bipedal movement is less common among mammals, most of which are quadrupedal. All primates possess some bipedal ability, though non-human primates primarily use quadrupedal locomotion on land. Primates aside, the largest mammalian group using exclusive bipedal movement are the macropods (kangaroos, wallabies and their relatives), which move via hopping. Other mammals also move via hopping, such as the kangaroo rat, springhare and certain primates such as the sifaka and sportive lemur. Possibly the only mammals other than primates that commonly move bipedally by an alternating gait rather than hopping is the ground pangolin.

Primates

Primates can be distinguished from other quadrupedal mammals as they exhibit a greater diversity in locomotor behaviors. These include arm swinging (brachiation), quadrumanous climbing, knuckle walking, and regular short bouts of bipedalism. In addition, quadrupedal locomotion in primates also exhibits significant differences from other mammals. These differences in gait characteristics are primarily adaptations to an arboreal environment. All primates can sit upright. Many primates can stand upright on their hind legs without any support. Chimpanzees, bonobos, gibbons and baboons exhibit relatively advanced forms of bipedalism. Injured chimpanzees and bonobos have been capable of sustained bipedalism.

Primates that live in tropical areas often wade through water in a bipedal stance. Gorillas, bonobos, proboscis monkeys and baboons have been observed wading bipedally.Three captive primates, one macaque Natasha and two chimps, Oliver and Poko (chimpanzee), were found to move bipedally. Natasha switched to exclusive bipedalism after an illness, while Poko was discovered in captivity in a tall, narrow cage. Oliver reverted to knuckle-walking after developing arthritis.

In addition, non-human primates often use bipedal locomotion when carrying food. One hypothesis for human bipedalism is thus that it evolved as a result of differentially successful survival from carrying food to share with group members, although there are other hypotheses, as below.

Limited bipedalism in mammals

Other mammals engage in limited, non-locomotory, bipedalism. A number of other animals, such as rats, racoons, and beavers will squat on their hindlegs to manipulate some objects but revert to four limbs when moving (the beaver may also move bipedally if transporting wood for their dams). Bears will fight in a bipedal stance to use their forelegs as weapons. Ground squirrels and meerkats will stand on hind legs to survey their surroundings, but will not walk bipedally. Dogs can stand or move on two legs if trained, or if birth defect or injury precludes quadrupedalism. The gerenuk antelope stands on its hind legs while eating from trees, as did the extinct giant ground sloth and chalicotheres. The spotted skunk will also use limited bipedalism when threatened, rearing up on its forelimbs while facing the attacker so its anal glands, capable of spraying an offensive oil, face its attacker.

Limited bipedalism in non-mammals

Bipedalism is unknown among the amphibians. Among the non-archosaur reptiles bipedalism is rare, but it is found in the 'reared-up' running of lizards such as agamids and monitor lizards. Many reptile species will also temporarily adopt bipedalism while fighting. One genus of basilisk lizard can run bipedally across the surface of water for some distance. Birds are also bipedal when not flying. Among arthropods, cockroaches are known to move bipedally at high speeds. Bipedalism is virtually solely found in terrestrial animals, though at least two types of octopus walk bipedally on the sea floor using two of their arms, allowing the remaining arms to be used to camouflage the octopus as a mat of algae or a floating coconut.

Advantages

Limited and exclusive bipedalism can offer a species several advantages. Bipedalism raises the head; this allows a greater field of vision with improved detection of distant dangers or resources, access to deeper water for wading animals and allows the animals to reach higher food sources with their mouths. While upright, non-locomotory limbs become free for other uses, including manipulation (in primates and rodents), flight (in birds), digging (in giant pangolin), combat (in bears and the large monitor lizard) or

camouflage (in certain species of octopus). Running speeds can be increased when an animal lacks a flexible backbone, though the maximum bipedal speed appears less fast than the maximum speed of quadrapedal movement with a flexible backbone - the ostrich reaches speeds of 65 km/h (40 mph) and the red kangaroo 70 km/h (43 mph), while the cheetah can exceed 100 km/h (62 mph). Bipedality in kangaroo rats has been hypothesized to improve locomotor performance, which could aid in escaping from predators.

Evolution

Recent evidence regarding modern human sexual dimorphism (physical differences between men and women) in the lumbar spine has been seen in pre-modern primates such as *Australopithecus africanus*. This dimorphism has been seen as an evolutionary adaptation of females to bear lumbar load better during pregnancy, an adaptation that non-bipedal primates would not need to make.

Bipedalism has a number of adaptive advantages, and has evolved independently in a number of lineages.

Early reptiles and lizards

The first known biped is the bolosaurid *Eudibamus* whose fossils date from 290 million years ago. Its long hindlegs, short forelegs, and distinctive joints all suggest bipedalism. This may have given increased speed. The species was extinct before the dinosaurs appeared.

Independent of *Eudibamus*, some modern lizard species have developed the capacity to run on their hind legs for added speed.

Dinosaurs and birds

Bipedalism also evolved independently among the dinosaurs. Dinosaurs diverged from their archosaur ancestors approximately 230 million years ago during the Middle to Late Triassic period, roughly 20 million years after the Permian-Triassic extinction event wiped out an estimated 95% of all [life on Earth]. Radiometric dating of fossils from the early dinosaur genus *Eoraptor* establishes its presence in the fossil record at this time. Paleontologists believe *Eoraptor* resembles the common ancestor of all dinosaurs; if this is true, its traits suggest that the first dinosaurs were small, bipedal predators. The discovery of primitive, dinosaur-like ornithodirans such as *Marasuchus* and *Lagerpeton* in Argentinian Middle Triassic strata supports this view; analysis of recovered fossils suggests that these animals were indeed small, bipedal predators.

Mammals (excluding humans)

A number of mammals will adopt a bipedal stance in specific situations such as for feeding or fighting. A number of groups of extant mammals have independently evolved

bipedalism as their main form of locomotion - for example humans, giant pangolins, the extinct giant ground sloths, numerous species of jumping rodents and macropods. Humans, as their bipedalism has been extensively studied are documented in the next section. Macropods are believed to have evolved bipedal hopping only once in their evolution, at some time no later than 45 million years ago.

Humans

There are at least twelve distinct hypotheses as to how and why bipedalism evolved in humans, and also some debate as to when. Bipedalism evolved well before the large human brain or the development of stone tools. Bipedal specializations are found in *Australopithecus* fossils from 4.2-3.9 million years ago The different hypotheses are not necessarily mutually exclusive and a number of selective forces may have acted together to lead to human bipedalism. It is important to distinguish between adaptations for bipedalism and adaptations for running, which came later still.

Possible reasons for the evolution of human bipedalism include freeing the hands for tool use and carrying, sexual dimorphism in food gathering, changes in climate and habitat (from jungle to savanna) and to reduce the amount of skin exposed to the tropical sun. The first two explanations have been criticized for projecting modern social concerns and prejudices onto ancestral species. The latter two have been criticized for not making sense in the context of the forest and woodland biomes occupied by human ancestors.

Traveling efficiency hypothesis

An alternative explanation is the mixture of savanna and scattered forests increased terrestrial travel by proto-humans between clusters of trees, and bipedalism offered greater efficiency for long-distance travel between these clusters than quadrupedalism.

Postural feeding hypothesis

The postural feeding hypothesis has been recently supported by Dr. Kevin Hunt, a professor at Indiana University. This hypothesis asserts that chimpanzees were only bipedal when they ate. While on the ground, they would reach up for fruit hanging from small trees and while in trees, bipedalism was utilized by grabbing for an overhead branch. These bipedal movements may have evolved into regular habits because they were so convenient in obtaining food. Also, Hunt hypothesises that these movements coevolved with chimpanzee arm-hanging, as this movement was very effective and efficient in harvesting food. When analyzing fossil anatomy, *Australopithecus afarensis* has very similar features of the hand and shoulder to the chimpanzee, which indicates hanging arms. Also, the *Australopithecus* hip and hind limb very clearly indicate bipedalism, but these fossils also indicate very inefficient locomotive movement when compared to humans. For this reason, Hunt argues that bipedalism evolved more as a terrestrial feeding posture than as a walking posture. As Hunt says, “A bipedal postural feeding adaptation may have been a preadaptation for the fully realized locomotor bipedalism apparent in Homo erectus.”

Provisioning model

One theory on the origin of bipedalism is the behavioral model presented by C. Owen Lovejoy, known as "male provisioning". Lovejoy theorizes that the evolution of bipedalism was a product of monogamy. As hominid males became monogamous, Lovejoy contends, they would leave their mates and offspring for the day to search for food. Once they found food for their family, the male hominids would return carrying the food in their arms and walking on their hind legs.

There is no particular evidence, however, that early hominids were monogamous. And some evidence indicates that early bipedal hominids were in fact polygynous. Among all monogamous primates, males and females are about the same size. That is sexual dimorphism is minimal. In Australopithecus afarensis, males were thought to be nearly twice the weight of females (as well as a great deal taller), which suggests that they were polygynous. Modern monogamous primates are also highly territorial, but fossil evidence indicates that *Australopithecus afarensis* lived in large groups. There is likewise no evidence that female hominids did not forage themselves. Early hominids did not have the large brains that require that infants be born premature and helpless. Females in ape species similar to early hominids do not wait for food to be brought to them. In short, there is no direct evidence to support either monogamy or polygamy in early hominids and indirect evidence points to polygamy.

Other behavioural models

There are a variety of ideas which promote a specific change in behaviour as the key driver for the evolution of hominid bipedalism. For example, Wescott (1967) and later Jablonski & Chaplin (1993) suggest that bipedal threat displays could have been the transitional behaviour which led to some groups of apes beginning to adopt bipedal postures more often. Others (*e.g.* Dart 1925) have offered the idea that the need for more vigilance against predators could have provided the initial motivation. Dawkins (*e.g.* 2004) has argued that it could have begun as a kind of fashion that just caught on and then escalated through sexual selection. And it has even been suggested (*e.g.* Tanner 1981:165) that male phallic display could have been the initial incentive.

Thermoregulatory model

The thermoregulatory model explaining the origin of bipedalism is one of the simplest theories so far advanced, but it is a viable explanation. Dr. Peter Wheeler, a professor of evolutionary biology, proposes that bipedalism raises the amount of body surface area higher above the ground which results in a reduction in heat gain and helps heat dissipation. When a hominid is higher above the ground, the organism accesses more favorable wind speeds and temperatures. During heat seasons, greater wind flow results in a higher heat loss, which makes the organism more comfortable. Also, Wheeler explains that a vertical posture minimizes the direct exposure to the sun whereas quadrupedalism exposes more of the body to direct exposure. Analysis and interpretations of Ardipithecus reveal that this hypothesis needs modification to consider

that the forest and woodland environmental preadaptation of early-stage hominid bipedalism preceded further refinement of bipedalism by the pressure of natural selection. This then allowed for the more efficient exploitation of the hotter conditions ecological niche, rather than the hotter conditions being hypothetically bipedalism's initial stimulus.

Carrying models

Charles Darwin wrote that "Man could not have attained his present dominant position in the world without the use of his hands, which are so admirably adapted to the act of obedience of his will" Darwin (1871:52) and many models on bipedal origins are based on this line of thought. Gordon Hewes (1961) suggested that the carrying of meat "over considerable distances" (Hewes 1961:689) was the key factor. Isaac (1978) and Sinclair et al. (1986) offered modifications of this idea as indeed did Lovejoy (1981) with his 'provisioning model' described above. Others, such as Nancy Tanner (1981) have suggested that infant carrying was key, whilst others have suggested stone tools and weapons drove the change.

Wading models

Several theories have been proposed regarding the influence of water on human bipedalism. The aquatic ape hypothesis, promoted for several decades by Elaine Morgan, proposed that swimming, diving and aquatic food sources exerted a strong influence on many aspects of human evolution, including bipedalism. It is not accepted by or considered a serious theory within anthropological scholarly community.

Other scholarly theories have been proposed that suggest wading and the exploitation of aquatic food sources (providing essential nutrients for human brain evolution or critical fallback foods) may have exerted evolutionary pressures on human ancestors leading to bipedalism.

Physiology

Bipedal movement occurs in a number of ways, and requires many mechanical and neurological adaptations. Some of these are described below.

Biomechanics

Standing

Energy-efficient means of standing bipedally involve constant adjustment of balance, and of course these must avoid overcorrection. The difficulties associated with simple standing in upright humans are highlighted by the greatly increased risk of falling present in the elderly, even with minimal reductions in control system effectiveness.

Walking

Walking is characterized by an "inverted pendulum" movement in which the body vaults over a stiff leg with each step. Force plates can be used to quantify the whole-body kinetic & potential energy, with walking displaying an out-of-phase relationship indicating exchange between the two. Interestingly, this model applies to all walking organisms regardless of the number of legs, and thus bipedal locomotion is does not differ in terms of whole-body kinetics.

In humans, walking is composed of several separate processes:

- Vaulting over a stiff stance leg
- Passive ballistic movement of the swing leg
- A short 'push' from the ankle prior to toe-off, propelling the swing leg
- Rotation of the hips about the axis of the spine, to increase stride length
- rotation of the hips about the horizontal axis to improve balance during stance

Running

Running is characterized by a spring-mass movement. Kinetic and potential energy are in phase, and the energy is stored & released from a spring-like limb during foot contact. Again, the whole-body kinetics are similar to animals with more limbs.

Musculature

Bipedalism requires strong leg muscles, particularly in the thighs. Contrast in domesticated poultry the well muscled legs, against the small and bony wings. Likewise in humans, the quadriceps and hamstring muscles of the thigh are both so crucial to bipedal activities that each alone is much larger than even the well-developed biceps of the arms.

Nervous system

The famous knee jerk (or patellar reflex) emphasizes the necessary bipedal control system: the only function served by the nerves involved being connected as they are is to ensure quick response to imminent disturbance of erect posture; it not only occurs without conscious mental activity, but also involves none of the nerves which lead from the leg to the brain.

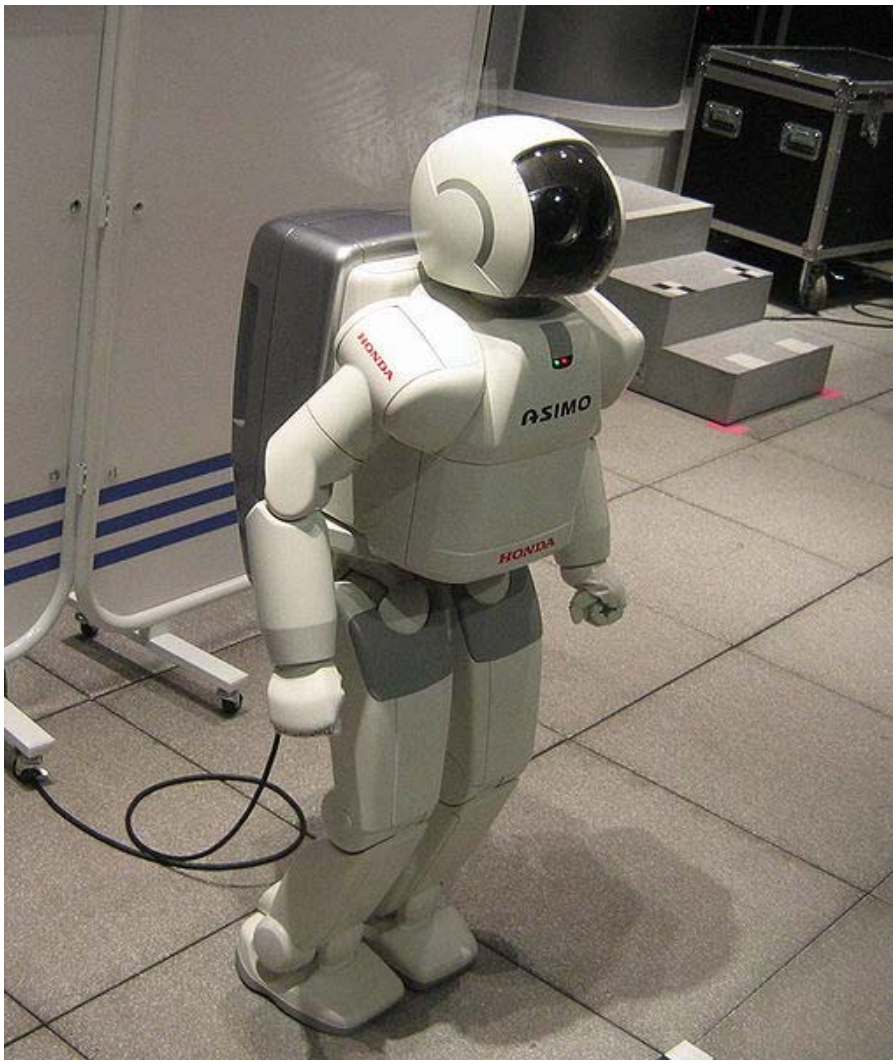
A less well-known aspect of bipedal neuroanatomy can be demonstrated in human infants who have not yet developed toward the ability to stand up. They can nevertheless run with great dexterity, provided they are supported in a vertical position and offered the stimulus of a moving treadmill beneath their feet.

Respiration

A biped also has the ability to breathe whilst it runs. Humans usually take a breath every other stride when their aerobic system is functioning. During a sprint, at which point the anaerobic system kicks in, breathing slows until the anaerobic system can no longer sustain a sprint.

This is not necessarily an advantage over quadrupeds, as not only can many quadrupeds breathe while running, but in mammals such as dogs, the act of running helps to expand and contract the lungs. The muscles of the trunk thus perform locomotive and respiratory tasks at the same time, making breathing while running more efficient in these animals than in bipeds.

Bipedal robots



ASIMO - a bipedal robot

For nearly the whole of the 20th century, bipedal robots were very difficult to construct and robot locomotion involved only wheels, treads, or multiple legs. Recent cheap and compact computing power has made two-legged robots more feasible. Some notable biped robots are ASIMO, HUBO and QRIO. Recently, spurred by the success of creating a fully passive, un-powered bipedal walking robot, those working on such machines have begun using principles gleaned from the study of human and animal locomotion, which often relies on passive mechanisms to minimize power consumption.

Bipedal molecule

In 2005, chemists at the University of California, Riverside developed the first bipedal molecule, 9,10-Dithioanthracene, which propels itself in a straight line when heated on a flat copper surface. Researchers believe the molecule has potential for use in molecular computers.

Chapter 7

Human Musculoskeletal System

A **musculoskeletal system** (also known as the **locomotor system**) is an organ system that gives animals (including humans) the ability to move using the muscular and skeletal systems. The musculoskeletal system provides form, support, stability, and movement to the body.

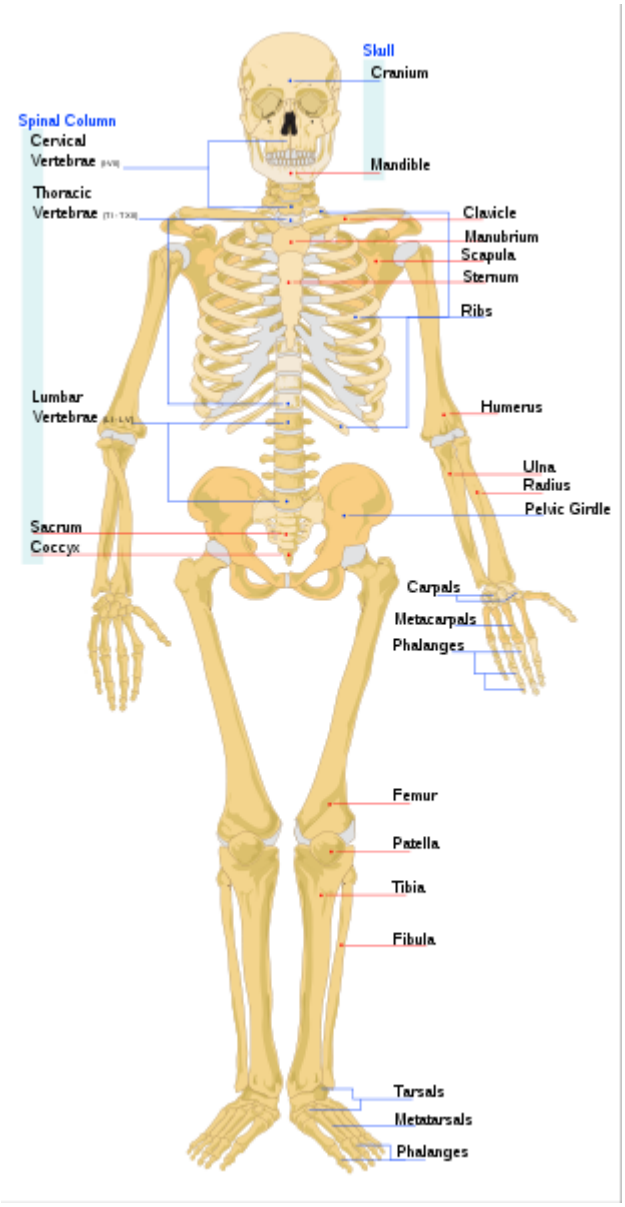
It is made up of the body's bones (the skeleton), muscles, cartilage, tendons, ligaments, joints, and other connective tissue that supports and binds tissues and organs together. The musculoskeletal system's primary functions include supporting the body, allowing motion, and protecting vital organs. The skeletal portion of the system serves as the main storage system for calcium and phosphorus and contains critical components of the hematopoietic system.

This system describes how bones are connected to other bones and muscle fibers via connective tissue such as tendons and ligaments. The bones provide the stability to a body in analogy to iron rods in concrete construction. Muscles keep bones in place and also play a role in movement of the bones. To allow motion, different bones are connected by joints. Cartilage prevents the bone ends from rubbing directly on to each other. Muscles contract (bunch up) to move the bone attached at the joint.

There are, however, diseases and disorders that may adversely affect the function and overall effectiveness of the system. These diseases can be difficult to diagnose due to the close relation of the musculoskeletal system to other internal systems. The musculoskeletal system refers to the system having its muscles attached to an internal skeletal system and is necessary for humans to move to a more favorable position. Complex issues and injuries involving the musculoskeletal system are usually handled by a physiatrist (specialist in Physical Medicine and Rehabilitation) or an orthopaedic surgeon.

Subsystems

Skeletal



Front view of a skeleton of an adult human

The Skeletal System serves many important functions; it provides the shape and form for our bodies in addition to supporting, protecting, allowing bodily movement, producing blood for the body, and storing minerals. The number of bones in the human skeletal system is a controversial topic. Humans are born with about 300 to 350 bones, however, many bones fuse together between birth and maturity. As a result an average adult skeleton consists of 206 bones. The number of bones varies according to the method used to derive the count. While some consider certain structures to be a single bone with multiple parts, others may see it as a single part with multiple bones. There are five

general classifications of bones. These are Long bones, Short bones, Flat bones, Irregular bones, and Sesamoid bones. The human skeleton is composed of both fused and individual bones supported by ligaments, tendons, muscles and cartilage. It is a complex structure with two distinct divisions. These are the axial skeleton and the appendicular skeleton.

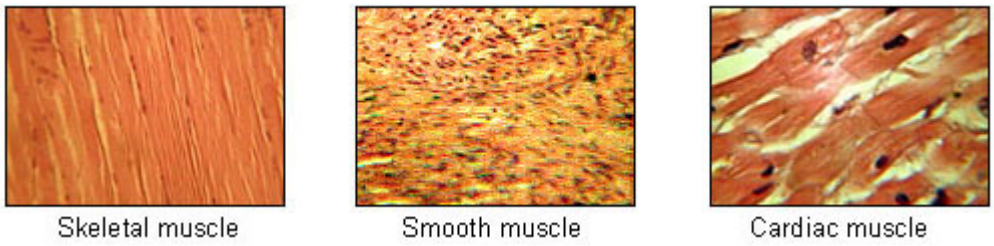
Function

The Skeletal System serves as a framework for tissues and organs to attach themselves to. This system acts as a protective structure for vital organs. Major examples of this are the brain being protected by the skull and the lungs being protected by the rib cage.

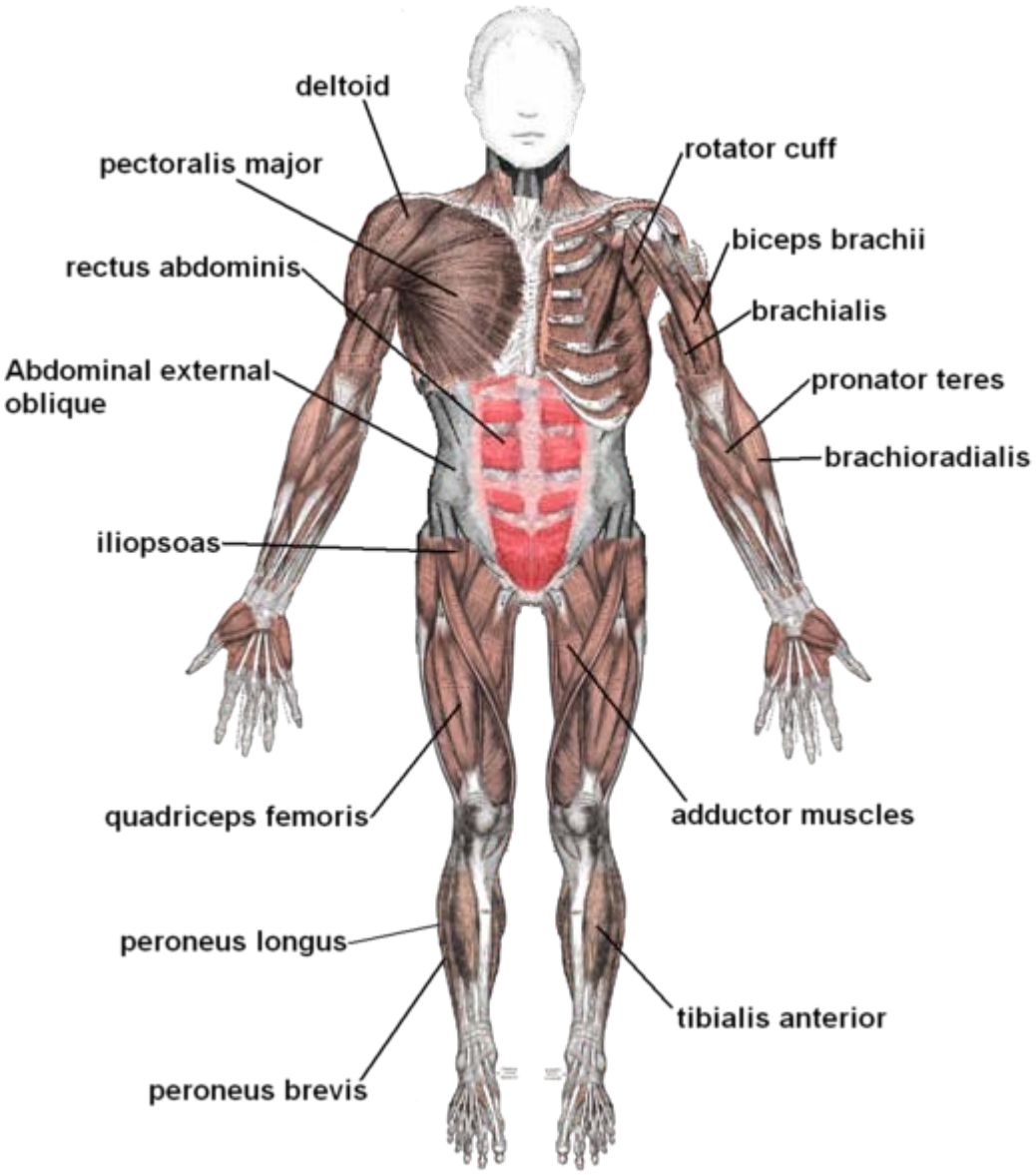
Located in long bones are two distinctions of bone marrow (yellow and red). The yellow marrow has fatty connective tissue and is found in the marrow cavity. During starvation, the body uses the fat in yellow marrow for energy. The red marrow of some bones is an important site for blood cell production, approximately 2.6 million red blood cells per second in order to replace existing cells that have been destroyed by the liver. Here all erythrocytes, platelets, and most leukocytes form in adults. From the red marrow, erythrocytes, platelets, and leukocytes migrate to the blood to do their special tasks.

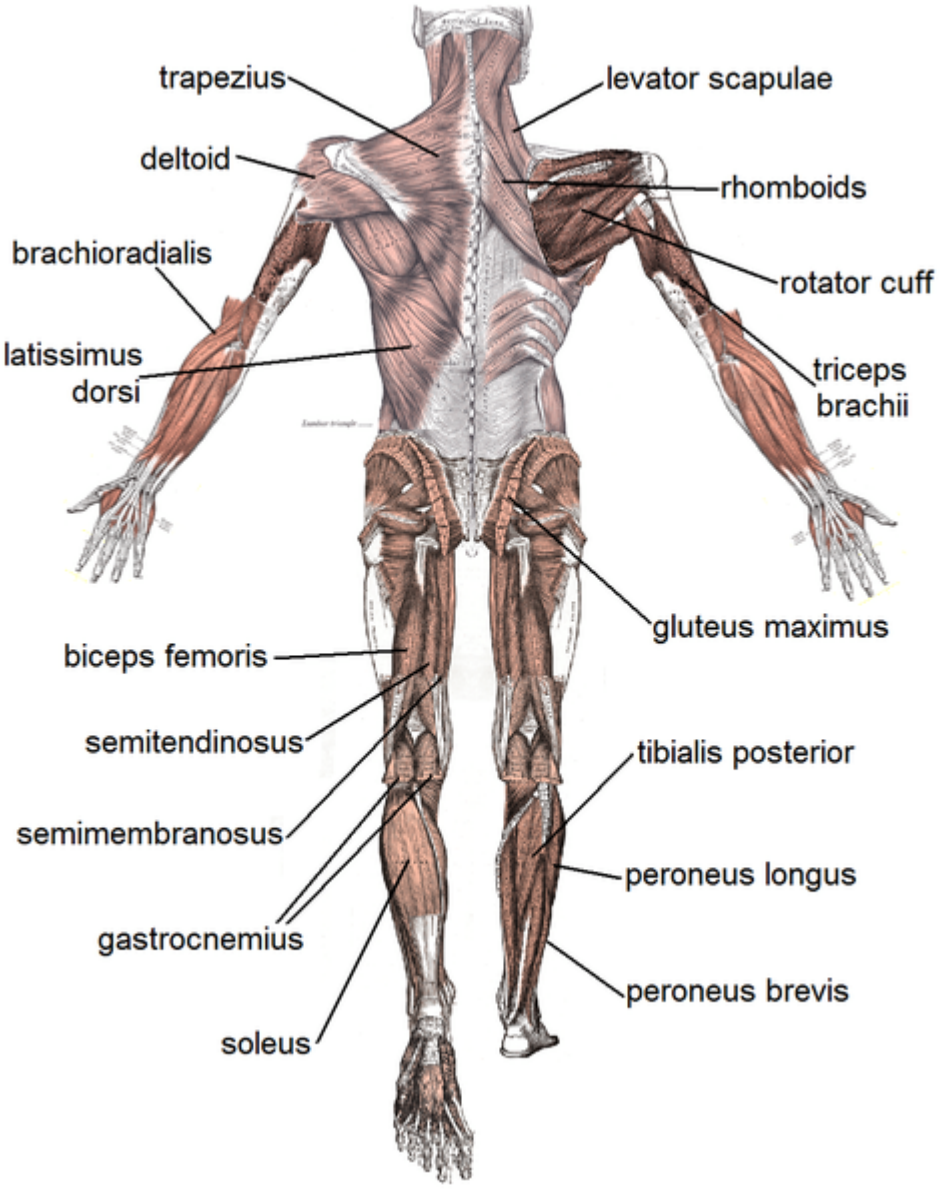
Another function of bones is the storage of certain minerals. Calcium and phosphorus are among the main minerals being stored. The importance of this storage "device" helps to regulate mineral balance in the bloodstream. When the fluctuation of minerals is high, these minerals are stored in bone; when it is low it will be withdrawn from the bone.

Muscular



Types of muscle and their appearance


deltoid
pectoralis major
rectus abdominis
Abdominal external oblique
iliopsoas
quadriceps femoris
peroneus longus
peroneus brevis
rotator cuff
biceps brachii
brachialis
pronator teres
brachioradialis
adductor muscles
tibialis anterior



There are three types of muscles—cardiac, skeletal, and smooth. Smooth muscles are used to control the flow of substances within the lumens of hollow organs, and are not consciously controlled. Skeletal and cardiac muscles have striations that are visible under a microscope due to the components within their cells. Only skeletal and smooth muscles are part of the musculoskeletal system and only the skeletal muscles can move the body. Cardiac muscles are found in the heart and are used only to circulate blood; like the smooth muscles, these muscles are not under conscious control. Skeletal muscles are attached to bones and arranged in opposing groups around joints. Muscles are innervated, to communicate nervous energy to, by nerves, which conduct electrical currents from the central nervous system and cause the muscles to contract.

Contraction initiation

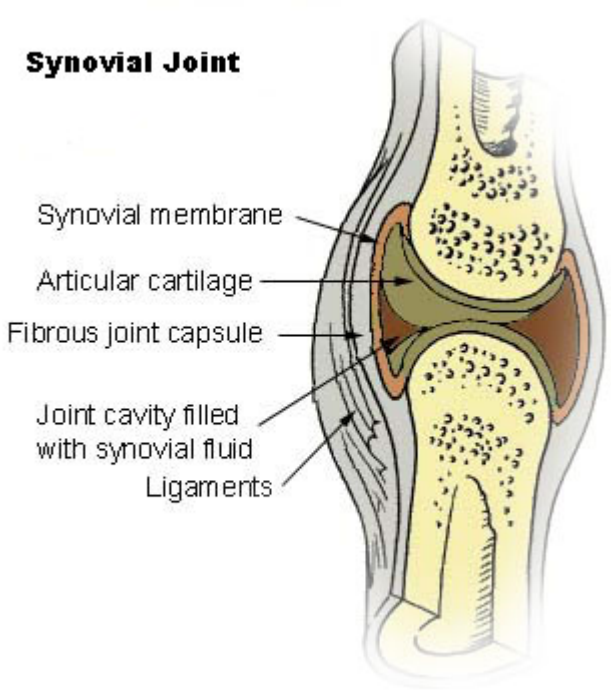
In mammals, when a muscle contracts, a series of reactions occur. Muscle contraction is stimulated by the motor neuron sending a message to the muscles from the somatic nervous system. Depolarization of the motor neuron results in neurotransmitters being released from the nerve terminal. The space between the nerve terminal and the muscle cell is called the neuromuscular junction. These neurotransmitters diffuse across the synapse and bind to specific receptor sites on the cell membrane of the muscle fiber. When enough receptors are stimulated, an action potential is generated and the permeability of the sarcolemma is altered. This process is known as initiation.

Tendons

A tendon is a tough, flexible band of fibrous connective tissue that connects muscles to bones. The extra-cellular connective tissue between muscle fibers binds to tendons at the distal & proximal ends, and the tendon binds to the periosteum of individual bones at the muscle's origin & insertion. As muscles contract, tendons transmit the forces to the rigid bones, pulling on them and causing movement. Tendons can stretch substantially, allowing them to function as springs during locomotion, thereby saving energy.

Joints, ligaments, and bursae

Joints



Human synovial joint composition

Joints are structures that connect individual bones and may allow bones to move against each other to cause movement. There are two divisions of joints, diarthroses which allow extensive mobility between two or more articular heads, and false joints or synarthroses, joints that are immovable, that allow little or no movement and are predominantly fibrous. Synovial joints, joints that are not directly joined, are lubricated by a solution called synovial Fluid that is produced by the synovial membranes. This fluid lowers the friction between the articular surfaces and is kept within an articular capsule, binding the joint with its taut tissue.

Ligaments

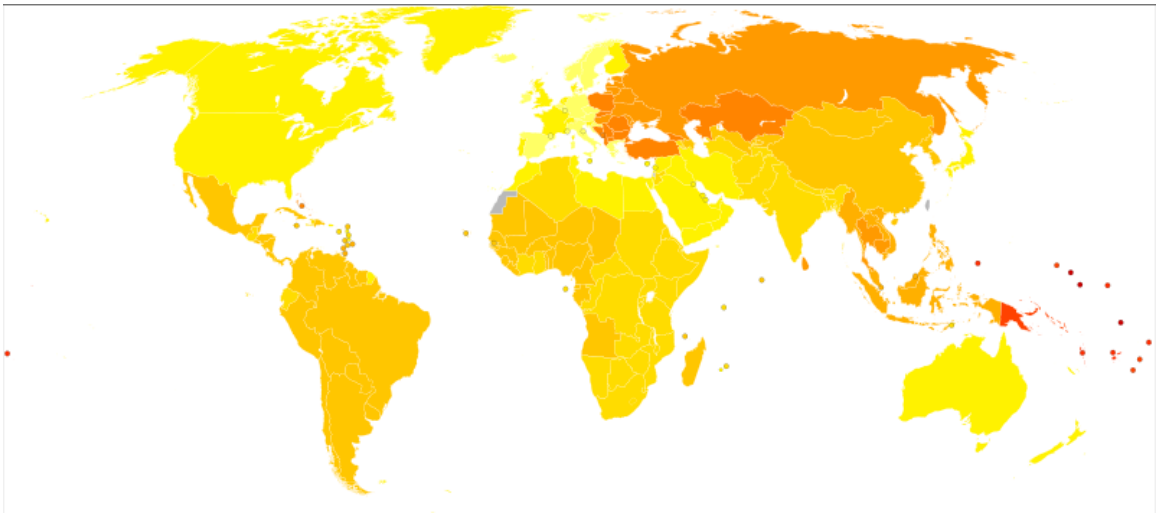
A ligament is a small band of dense, white, fibrous elastic tissue. Ligaments connect the ends of bones together in order to form a joint. Most ligaments limit dislocation, or prevent certain movements that may cause breaks. Since they are only elastic they increasingly lengthen when under pressure. When this occurs the ligament may be susceptible to break resulting in an unstable joint.

Ligaments may also restrict some actions: movements such as hyper extension and hyper flexion are restricted by ligaments to an extent. Also ligaments prevent certain directional movement.

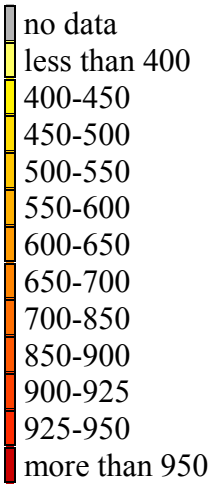
Bursa

A bursa is a small fluid-filled sac made of white fibrous tissue and lined with synovial membrane. Bursa may also be formed by a synovial membrane that extends outside of the joint capsule. It provides a cushion between bones and tendons and/or muscles around a joint; bursa are filled with synovial fluid and are found around almost every major joint of the body.

Diseases and disorders



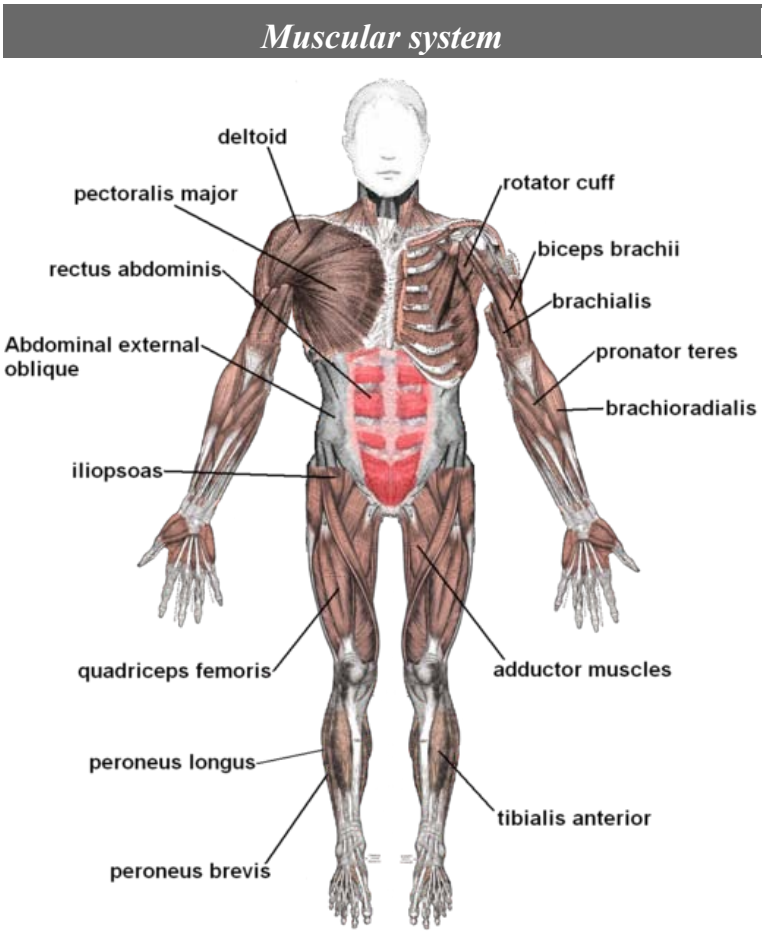
Disability-adjusted life year for musculoskeletal diseases per 100,000 inhabitants in 2004



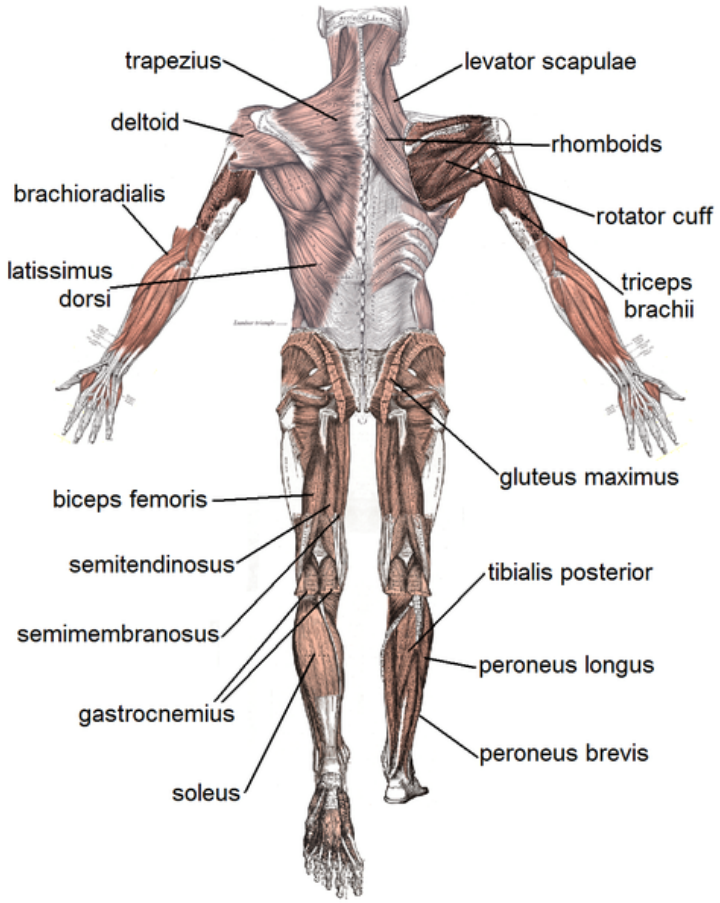
Because many other body systems, including the vascular, nervous, and integumentary systems, are interrelated, disorders of one of these systems may also affect the musculoskeletal system and complicate the diagnosis of the disorder's origin. Diseases of the musculoskeletal system mostly encompass functional disorders or motion discrepancies; the level of impairment depends specifically on the problem and its severity. Articular (of or pertaining to the joints) disorders are the most common. However, also among the diagnoses are: primary muscular diseases, neurologic (related to the medical science that deals with the nervous system and disorders affecting it) deficits, toxins, endocrine abnormalities, metabolic disorders, infectious diseases, blood and vascular disorders, and nutritional imbalances. Disorders of muscles from another body system can bring about irregularities such as: impairment of ocular motion and control, respiratory dysfunction, and bladder malfunction. Complete paralysis, paresis, or ataxia may be caused by primary muscular dysfunctions of infectious or toxic origin; however, the primary disorder is usually related to the nervous system, with the muscular system acting as the effector organ, an organ capable of responding to a stimulus, especially a nerve impulse. One understated disorder that begins during pregnancy is Pelvic girdle pain), it is complex and multi-factorial and likely to be also represented by a series of sub-groups driven by pain varying from peripheral or central nervous system, altered laxity/stiffness of muscles, laxity to injury of tendinous/ligamentous structures to ‘mal-adaptive’ body mechanics.

Chapter 8

Muscular System



Muscles anterior labeled



Muscle posterior labeled

Latin *systema musculare*

The **muscular system** is the anatomical system of a species that allows it to move. The muscular system in vertebrates is controlled through the nervous system, although some muscles (such as the cardiac muscle) can be completely autonomous.

Muscles

There are three distinct types of muscles: skeletal muscles, cardiac or heart muscles, and smooth (non-striated) muscles. Muscles provide strength, balance, posture, movement and heat for the body to keep warm.

Upon stimulation by an action potential, skeletal muscles perform a coordinated contraction by shortening each sarcomere. The best proposed model for understanding contraction is the sliding filament model of muscle contraction. Actin and myosin fibers overlap in a contractile motion towards each other. Myosin filaments have club-shaped heads that project toward the actin filaments.

Larger structures along the myosin filament called myosin heads are used to provide attachment points on binding sites for the actin filaments. The myosin heads move in a coordinated style, they swivel toward the center of the sarcomere, detach and then reattach to the nearest active site of the actin filament. This is called a rachet type drive system. This process consumes large amounts of adenosine triphosphate (ATP).

Energy for this comes from **ATP**, the energy source of the cell. ATP binds to the cross bridges between myosin heads and actin filaments. The release of energy powers the swiveling of the myosin head. Muscles store little ATP and so must continuously recycle the discharged adenosine diphosphate molecule (ADP) into ATP rapidly. Muscle tissue also contains a stored supply of a fast acting recharge chemical, creatine phosphate which can assist initially producing the rapid regeneration of ADP into ATP.

Calcium ions are required for each cycle of the sarcomere. Calcium is released from the sarcoplasmic reticulum into the sarcomere when a muscle is stimulated to contract. This calcium uncovers the actin binding sites. When the muscle no longer needs to contract, the calcium ions are pumped from the sarcomere and back into storage in the sarcoplasmic reticulum.

Anatomy

There are approximately 639 skeletal muscles in the human body.

The following are some major muscles and their basic features:

Muscle	Origin	Insertion	Artery	Nerve	Action	Antagonist
gastrocnemius	femur	calcaneus	sural arteries	tibial nerve	plantarflexion, flexion of knee (minor)key	Tibialis anterior muscle
tibialis posterior	tibia, fibula	Foot	posterior tibial artery	tibial nerve	inversion of the foot, plantar flexion of the foot at the ankle	Tibialis anterior muscle
soleus	fibula, medial border of tibia	calcaneus	sural arteries	tibial nerve	plantarflexion	Tibialis anterior muscle
tibialis anterior	tibia	foot	anterior tibial artery	Fibular nerve	dorsiflex and invert the foot	Fibularis longus, Gastrocnemius, Soleus, Plantaris, Tibialis posterior
longus	fibula	Foot	fibular artery	Superficial	plantarflexio	Tibialis

				fibular nerve	n, eversion	anterior muscle
brevis	fibula	Foot, eversion	peroneal artery	superficial peroneal nerve		
gluteus maximus muscle	ilium, sacrum, sacrotuberous ligament	Gluteal tuberosity of the femur	gluteal arteries	inferior gluteal nerve	external rotation and extension of the hip joint	Iliacus, Psoas major, Psoas minor
biceps femoris	ischium, femur	fibula	inferior gluteal artery, popliteal artery	tibial nerve, common peroneal nerve	flexes and laterally rotates knee joint, extends hip joint	Quadriceps muscle
semitendinosus	ischium	tibia	inferior gluteal artery	sciatic	flex knee, extend hip joint	Quadriceps muscle
semimembranosus	ischium	tibia	profunda femoris, gluteal artery	sciatic nerve	Hip extension, Knee flexion	Quadriceps muscle
iliopsoas	ilium	femur	medial femoral circumflex artery, iliolumbar artery	femoral nerve, lumbar nerves	flexion of hip	Gluteus maximus, posterior compartment of thigh
quadriceps femoris	combined rectus femoris and vastus muscles		femoral artery	Femoral nerve	Knee extension; Hip flexion	Hamstring
adductor muscles of the hip	pubis	femur, tibia		obturator nerve	adduction of hip	
levator scapulae	vertebral column	scapula	dorsal scapular artery	cervical nerve, dorsal scapular nerve	Elevates scapula, tilts its glenoid cavity inferiorly	
trapezius	the rear of the skull, vertebral column	clavicle, scapula		cranial nerve XI, cervical nerves	retraction of scapula	Serratus anterior muscle
rectus abdominis	pubis	Costal cartilage of ribs 5-7, sternum	inferior epigastric artery	segmentally by thoraco-abdominal nerves	flexion of trunk/lumbar vertebrae	Erector spinae

transversus abdominis	ribs, ilium	pubic tubercle		lower intercostal nerves, iliohypogastric nerve and the ilioinguinal nerve	compress the ribs and viscera, thoracic and pelvic stability	
Abdominal external oblique muscle	Lower 8 costae	Crista iliaca, ligamentum inguinale		lower 6 intercostal nerve, subcostal nerve	Rotates torso	
Abdominal internal oblique muscle	Inguinal ligament, Iliac crest and the Lumbodorsal fascia	Linea alba, sternum and the inferior ribs.			Compresses abdomen and rotates vertebral column.	
erector spinae	on the spines of the last four thoracic vertebræ	both the spines of the most cranial thoracic vertebrae and the cervical vertebrae	lateral sacral artery	posterior branch of spinal nerve	extends the vertebral column	Rectus abdominis muscle
pectoralis major	clavicle, sternum, costal cartilages	humerus	thoracoacromial trunk	lateral pectoral nerve and medial pectoral nerve	**Clavicular head**: flexes the humerus **Sternocostal head**: extends the humerus As a whole, adducts and medially rotates the humerus. It also draws the scapula anteriorly and inferiorly.	
biceps brachii	scapula	radius	brachial artery	Musculocutaneous nerve	flexes elbow and supinates forearm	Triceps brachii muscle
triceps brachii	scapula and humerus	ulna	deep brachial artery	radial nerve	extends forearm, caput longum adducts	Biceps brachii muscle

					shoulder	
brachialis	humerus	ulna	radial recurrent artery	musculocutaneous nerve	flexion at elbow joint	
pronator teres	humerus, ulna	radius	ulnar artery and radial artery	median nerve	pronation of forearm, flexes elbow	Supinator muscle
brachioradialis	humerus	radius	radial recurrent artery	radial nerve	Flexion of forearm	
rhomboids	nuchal ligaments, spinous processes of the C7 to T5 vertebrae	scapula	dorsal scapular artery	dorsal scapular nerve	Retracts the scapula and rotates it to depress the glenoid cavity. fixes the scapula to the thoracic wall.	Serratus anterior muscle
deltoid	clavicle, acromion, scapula	deltoid tuberosity of humerus	primarily posterior circumflex humeral artery	Axillary nerve	shoulder abduction, flexion and extension	Latissimus dorsi
latissimus dorsi	vertebral column, ilium and inferior 3 or 4 ribs	humerus	subscapular artery, dorsal scapular artery	thoracodorsal nerve	pulls the forelimb dorsally and caudally	deltoid, trapezius

Aerobic and anaerobic muscle activity

At rest, the body produces the majority of its ATP aerobically in the mitochondria without producing lactic acid or other fatiguing byproducts. During exercise, the method of ATP production varies depending on the fitness of the individual as well as the duration, and intensity of exercise. At lower activity levels, when exercise continues for a long duration (several minutes or longer), energy is produced aerobically by combining oxygen with carbohydrates and fats stored in the body. Activity that is higher in intensity, with possible duration decreasing as intensity increases, ATP production can switch to anaerobic pathways, such as the use of the creatine phosphate and the phosphagen system or anaerobic glycolysis. Aerobic ATP production is biochemically much slower and can only be used for long-duration, low intensity exercise, but produces no fatiguing waste products that can not be removed immediately from sarcomere and body and results in a much greater number of ATP molecules per fat or carbohydrate molecule. Aerobic training allows the oxygen delivery system to be more efficient, allowing aerobic metabolism to begin quicker. Anaerobic ATP production produces ATP much faster and allows near-maximal intensity exercise, but also produces significant amounts of lactic acid which render high intensity exercise unsustainable for greater than several minutes. The phosphagen system is also anaerobic, allows for the highest levels of exercise

intensity, but intramuscular stores of phosphocreatine are very limited and can only provide energy for exercises lasting up to ten seconds. Recovery is very quick, with full creatine stores regenerated within five minutes.

Cardiac muscle

Heart muscles are distinct from skeletal muscles because the muscle fibers are laterally connected to each other. Furthermore, just as with smooth muscles, they are not controlling themselves. Heart muscles are controlled by the sinus node influenced by the autonomic nervous system.

Smooth muscle

Smooth muscles are controlled directly by the autonomic nervous system and are involuntary, meaning that they are incapable of being moved by conscious thought. Functions such as heart beat and lungs (which are capable of being willingly controlled, be it to a limited extent) are involuntary muscles but are not smooth muscles.

Control of muscle contraction

Neuromuscular junctions are the focal point where a motor neuron attaches to a muscle. Acetylcholine, (a neurotransmitter used in skeletal muscle contraction) is released from the axon terminal of the nerve cell when an action potential reaches the microscopic junction, called a synapse. A group of chemical messengers cross the synapse and stimulate the formation of electrical changes, which are produced in the muscle cell when the acetylcholine binds to receptors on its surface. Calcium is released from its storage area in the cell's sarcoplasmic reticulum. An impulse from a nerve cell causes calcium release and brings about a single, short muscle contraction called a muscle twitch. If there is a problem at the neuromuscular junction, a very prolonged contraction may occur, tetanus. Also, a loss of function at the junction can produce paralysis.

Skeletal muscles are organized into hundreds of motor units, each of which involves a motor neuron, attached by a series of thin finger-like structures called axon terminals. These attach to and control discrete bundles of muscle fibers. A coordinated and fine tuned response to a specific circumstance will involve controlling the precise number of motor units used. While individual muscle units contract as a unit, the entire muscle can contract on a predetermined basis due to the structure of the motor unit. Motor unit coordination, balance, and control frequently come under the direction of the cerebellum of the brain. This allows for complex muscular coordination with little conscious effort, such as when one drives a car without thinking about the process.

Chapter 9

Rotating Locomotion in Living Systems

A passive wheel

Rotating locomotion encompasses two distinct modes of locomotion: simple rolling, and spinning relative to a fixed axle or body, in the manner of a wheel or propeller. Many living systems move by means of rolling; however, despite the integral role that the wheel has played in locomotion of vehicles designed by humans, true wheels do not appear to play any role in the locomotion of biological systems. The reason for this apparent lack of biological "wheels" has been a frequent topic of semi-serious debate among biologists, including noted evolutionary biologist Stephen Jay Gould.

Given the apparent utility of the wheel in human technology, and the existence of other technologies with biological analogues (such as wings and lenses), it might seem odd that nothing like a wheel has evolved naturally, but there are several likely explanations for this phenomenon. Firstly, there are several potential stumbling blocks to the evolution of a wheel by natural selection, and secondly, wheels do not necessarily carry a competitive advantage over other means of surface propulsion (such as walking, running, or slithering) for the environments in which ambulatory species have evolved. This latter fact also explains why wheels have not found use in some human civilizations, despite those civilizations being aware of the wheel.

Rotation in nature

Rolling

The pangolin *Manis temminckii* in a defensive position

Some organisms use rolling as a means of locomotion. These examples do not constitute the use of a wheel, as the entire organism rotates itself, with no fixed axle.

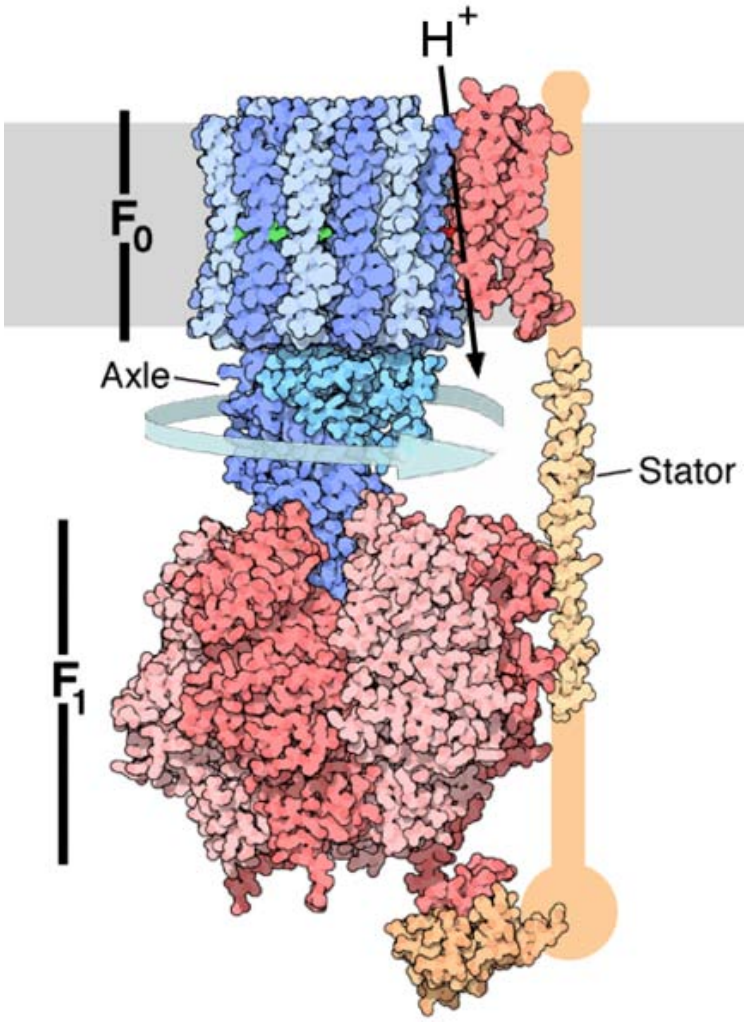
A species of caterpillar known as *Pleuroptya ruralis*, the Mother-Of-Pearl Moth, curls into a ring and rolls away when threatened. A species of mantis shrimp, *Nannosquilla decemspinosa*, performs partially rolling somersaults. The golden wheel spider *Carparachne aureoflava*, of the Namib Desert, escapes parasitic wasps by flipping onto its side and cartwheeling down sand dunes. A phylum of microscopic and near-microscopic pseudocoelomate animals called rotifers use cilia to sweep food into their

mouths, and to propel themselves through the water. The name of the phylum is derived from Latin, and means "wheel-bearer", for the wheel-like appearance of these cilia, although their motion is in fact reciprocal.

Other animals which roll their bodies, either actively or passively, include hedgehogs, armadillos, lizards such as *Cordylus cataphractus*, amphibians such as *Taricha granulosa* and *Echinotriton chinhaiensis*, isopods, myriapods, and fossilized trilobites.The salamander *Hydromantes platycephalus* and the pangolin also use rolling locomotion. The tumbleweed, *Corispermum hyssopifolium* uses passive rolling, powered by wind, to distribute its seeds. The dung beetle uses rolling to transport the feces on which it feeds.

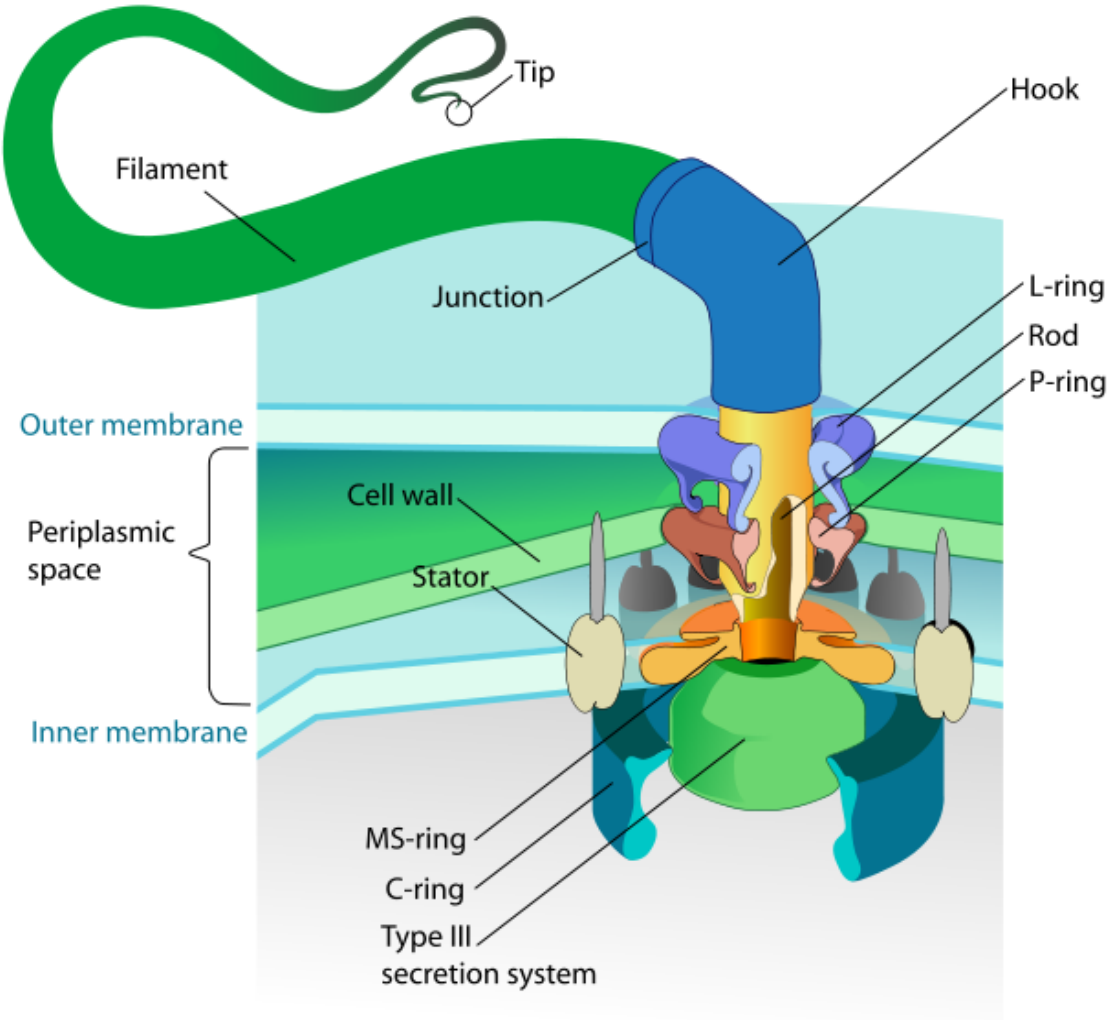
Keratinocytes (a type of skin cells) migrate with a rolling motion during the process of wound healing.

Wheel-like rotation



ATP synthase

While no known multi-cellular organism is able to spin part of its body freely relative to another part of its body, there are two clear examples of rotating molecular structures used by living cells. ATP synthase is an enzyme used in the process of energy storage and transfer, notably in photosynthesis and oxidative phosphorylation. It bears some similarity to flagellar motors. The evolution of ATP synthase is thought to be an example of modular evolution, where two subunits with their own functions have become associated and gained new functionality.



Bacterial flagellum

The only known example of a biological "wheel", a system capable of providing continuous propulsive torque about a fixed body, is the flagellum, a propeller-like tail used by single-celled prokaryotes for propulsion. The bacterial flagellum is the best known example. About half of all known bacteria have at least one flagellum, indicating that rotation may in fact be the most common form of locomotion in living systems.

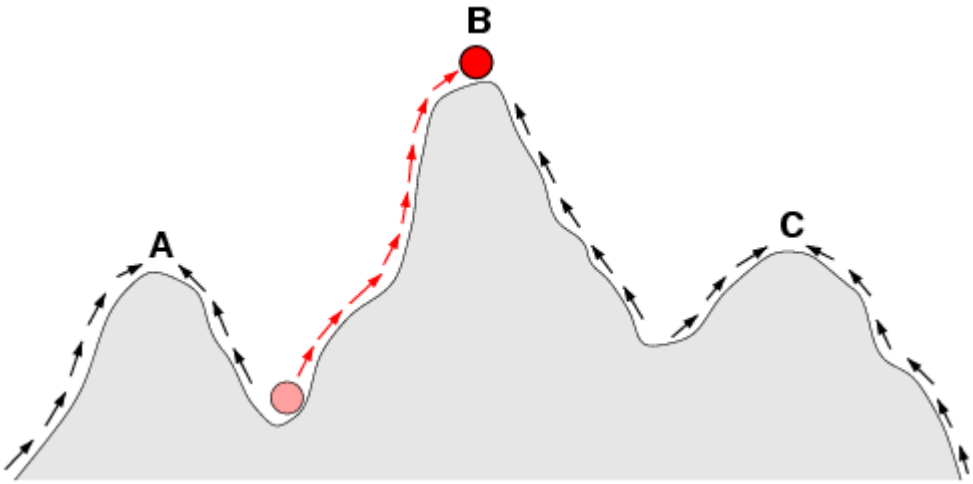
At the base of the bacterial flagellum, where it enters the cell membrane, a motor protein acts as a rotary engine. The engine is powered by proton motive force, i.e., by the flow of

protons (hydrogen ions) across the bacterial cell membrane due to a concentration gradient set up by the cell's metabolism. (In species of the genus *Vibrio*, there are two kinds of flagella, lateral and polar, and some are driven by a sodium ion pump rather than a proton pump.) Flagella are quite efficient, allowing bacteria to move at speeds up to 60 cell lengths per second. The rotary motor at the base of the flagellum is similar in structure to that of ATP synthase.

Archaea, a group of prokaryotes distinct from bacteria, also feature flagella driven by rotary motor proteins, though they are structurally and evolutionarily distinct from bacterial flagella. While bacterial flagella evolved from the bacterial Type III secretion system, archaeal flagella appear to have evolved from Type IV pili. Some eukaryotic cells, such as the protist *Euglena*, also have a flagellum, but eukaryotic flagella do not rotate at the base; rather, they bend in such a way that the tip of the flagellum whips in a circle. The eukaryotic flagellum, also called a cilium or undulipodium, is structurally and evolutionarily distinct from prokaryotic flagella.

Biological limitations on rotating locomotion

Evolutionary constraints



Sketch of a fitness landscape. The arrows indicate the preferred flow of a population on the landscape, and the points A and C are local optima. The red ball indicates a population that moves from a low fitness value to the top of a peak.

The processes of evolution, as they are presently understood, can help explain why wheeled locomotion has not evolved in multi-cellular organisms; simply put, a complex structure or system will not evolve if its incomplete form provides no benefit to an organism.

According to the modern evolutionary synthesis, adaptations are produced incrementally through natural selection, so major genetic changes will only usually spread within populations if they do not decrease the fitness of individuals. Although neutral changes that provide no benefit can spread through genetic drift, and detrimental changes can

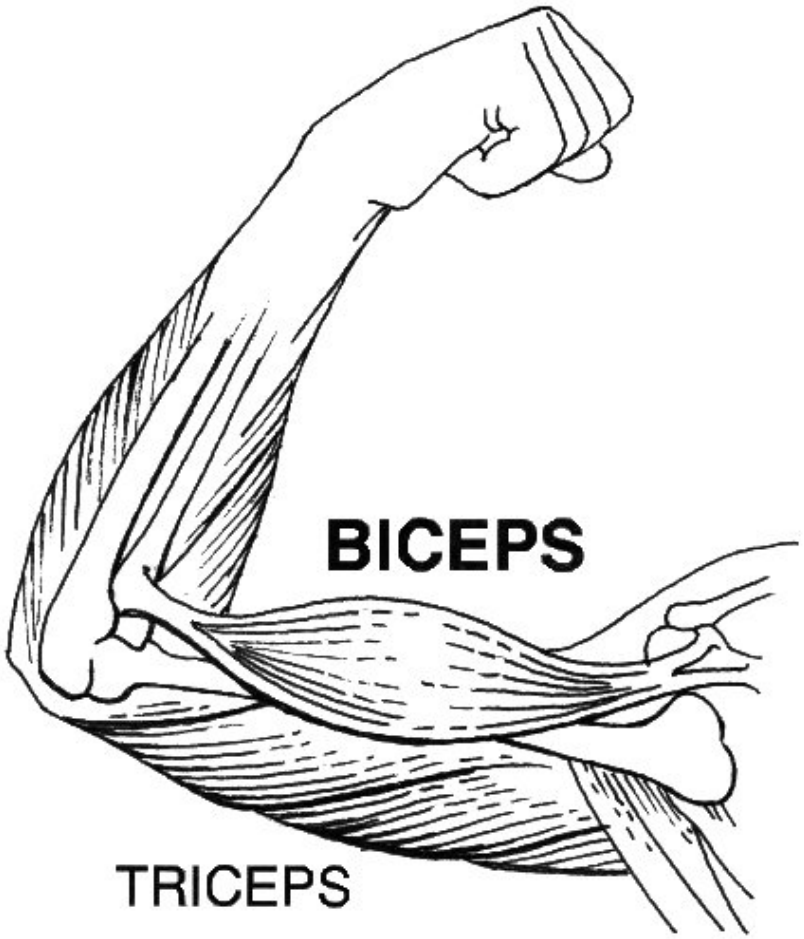
spread under some circumstances, large changes that require multiple steps will only occur if the intermediate stages increase fitness. Richard Dawkins describes this situation as follows: "The wheel may be one of those cases where the engineering solution can be seen in plain view, yet be unattainable in evolution because it lies [on] the other side of a deep valley, cutting unbridgeably across the massif of Mount Improbable." In such a fitness landscape, wheels might be a highly-beneficial "peak", but the valley around such a peak is too low or wide for the gene pool to move across by genetic drift or natural selection. As Gould explains, biological adaptation is limited to working with available components, saying "wheels work well, but animals are debarred from building them by structural constraints inherited as an evolutionary legacy".

Natural selection therefore explains why wheels have not appeared, as a wheel missing one or more of its key components would probably not impart an advantage to an organism. The same cannot, however, be said of the flagellum, the one known example of a freely rotating propulsive system in biology. In the evolution of flagella, individual components were recruited from other structures, where they performed tasks unrelated to propulsion. The basal body that is now the rotary motor may have evolved from a structure used by the bacterium to inject toxins into other cells. The recruitment of existing structures to serve a new purpose in evolution is called exaptation.

Anatomical constraints

The limitations of anatomical structures, both microscopic and macroscopic, present several impediments to the feasibility of a biological wheel. The major potential problem with multi-cellular organisms having wheels is the interface between the static and rotating components of the wheel. In either a passive or driven case, the wheel, or wheel and axle, must be able to rotate freely relative to the rest of the machine or organism. Unlike animal joints, which have a limited range of motion, a wheel must be able to rotate through an arbitrary angle without ever having to be "unwound". As such, a wheel cannot be permanently attached to the axle or shaft about which it rotates (or if the axle and wheel are fixed together, the axle cannot be affixed to the rest of the machine or organism). No true multi-cellular organism is known to grow tissue or organ structures which are not attached in some way to the rest of the organism.

Power transmission



Skeletal muscle

In the case of a driven wheel, some type of torque must be applied to the axle to generate the locomotive force. For human-made technology, this torque is generally provided by an engine, which may be electric, turbine-driven, combustion-driven, pneumatic, hydraulic, or of some other type. Torque may also be provided by human power, as in the case of a bicycle. In animals, motion is achieved by the use of skeletal muscles, which derive their energy from the metabolism of nutrients from food. Because these muscles are attached with connective tissue to both of the components which must move relative to each other, they would not be an effective means of directly driving a biological wheel. In addition, animals suffer degraded energy efficiency because their propulsive cycles employ only periodic accelerations (repeated flexion and extension of joints). Large animals can not produce high rates of acceleration, because as animal size increases, it becomes more difficult for muscles to quickly generate high enough stress to overcome relative inertia.

Friction

In typical mechanical systems, some sort of bearing must be used to reduce friction at the interface between two components. Reducing friction is vital for minimizing wear on components, and preventing overheating. As the relative speed of the components increases, and as the force of contact between the components increases, the importance of friction reduction increases as well. In biological joints such as the human knee, friction is reduced by means of cartilage with a very low friction coefficient, as well as a lubricant called synovial fluid, which has very low viscosity. Gerhard Scholtz, professor at the *Institut für Biologie Vergleichende Zoologie* ("Institute for Biology and Comparative Zoology") at Humboldt University of Berlin, asserts that a similar excreted lubricant or dead cellular material could allow a biological wheel to rotate freely, though such a mechanism is not found in nature.

Material transfer

One other potential problem at the interface is the ability to transfer materials across it. If the tissues which make up a wheel are living, they will need to be supplied with oxygen and nutrients and have wastes removed. A typical animal circulatory system, composed of blood vessels, would not be able to provide transportation across the interface. Lacking circulation, oxygen and nutrients would have to be able to diffuse across the interface, a process which would be greatly limited by the available partial pressure and surface area, in accordance with Fick's law of diffusion. For large multi-cellular animals, diffusion would be insufficient. Alternately, a wheel could be composed of excreted, non-living material, such as keratin, of which hair and nails are composed. However, a material excreted along the inner surface of a wheel would presumably need to stretch out as it moved toward the outer edge of the wheel, to account for the increasing circumference.

Mechanical disadvantages of rotating locomotion

Rotating locomotion incurs mechanical disadvantages in certain environments and situations, which would represent a decreased fitness when compared with limbed locomotion, and may help to explain why multi-cellular life has not evolved wheels for locomotion.

Wheels can be considered to fall into two types: passive and driven. A passive wheel simply rolls over a surface, reducing friction when compared with dragging. A driven wheel is powered, and transmits energy to the surface as a means of achieving locomotion. Wheels are typically round, or nearly so, but this is not strictly necessary for a wheel to be an effective form of locomotion, as seen in the example of the square wheel.

Efficiency

While ship propellers typically have efficiencies around 60%, and aircraft propellers near 90%, much higher efficiencies, in the range of 96%–98%, can be achieved with an oscillating flexible foil, like a fish tail or bird wing.

Although stiff wheels are more energy efficient than other means of locomotion when traveling over hard, level terrain (such as paved roads), wheels have several distinct disadvantages when compared to limbed locomotion which make them unlikely to replace limbed locomotion of animals. These disadvantages stem largely from the fact that many natural environments are ill-suited to the use of wheels.

Rolling resistance

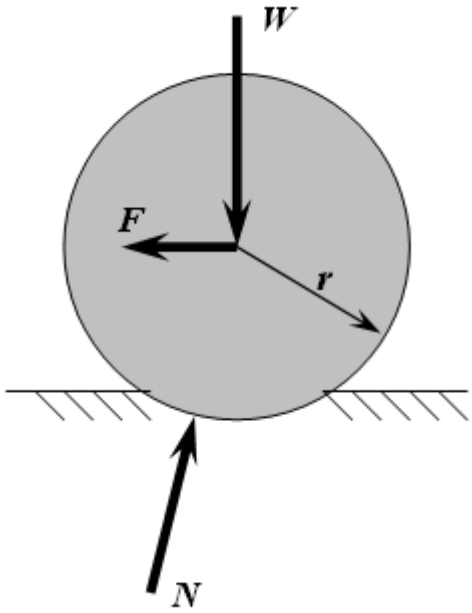


Diagram of a hard wheel rolling on and deforming a soft surface, resulting in the reaction force from the surface having a component that opposes the motion.

Wheels are not especially efficient on soft terrain such as soils, because they are vulnerable to rolling resistance. In rolling resistance, the wheel is robbed of energy by the deformation of the wheel and the surface on which it is rolling. Smaller wheels are especially susceptible to rolling resistance. Softer surfaces deform more and recover less than firm surfaces, resulting in greater resistance. Rolling resistance in sand, for example, is ten times higher than in concrete.

Rolling resistance is also the reason wheels are not seen in certain human civilizations. During the Roman Empire, wheeled chariots were common in the Middle East and North Africa, yet when the Roman Empire collapsed, wheels fell out of favor with the local populations, who turned to camels to transport goods in the sandy desert climate. Stephen

Jay Gould discusses this curiosity of history in his book *Hen's Teeth and Horse's Toes*, asserting that in the absence of maintained roads, camels required less manpower and water than a cart pulled by oxen.

Obstacle navigation

Wheels are poor at dealing with vertical obstacles, especially obstacles on the same scale as the wheel itself. The highest obstacle a passive-wheeled vehicle can surmount, assuming the vehicle cannot change its center of mass, is one quarter to one half the radius of the wheel. Even if the vehicle can move its center of mass, the limiting obstacle height for a passive wheel is one radius. Without articulation, a wheeled vehicle may become stuck on top of an obstacle, with the obstacle between the wheels, preventing them from contacting the ground. Limbs, in contrast, are useful for climbing, and equipped to deal with uneven terrain.

For unarticulated wheels, climbing obstacles will cause the body of the vehicle to rotate. If the rotation angle is too high, the vehicle will become statically unstable and tip over. At high speeds, a vehicle can become dynamically unstable, meaning that it can be tipped over by an obstacle smaller than its static stability limit. Without articulation, this can be an impossible position from which to recover.

Maneuverability

Most methods of steering wheeled vehicles involve some degree of skidding, or dragging of one or more wheels against the surface. This can impose limits on the achievable turning radius, thus limiting the ability of a vehicle to navigate around obstacles in areas with a high obstacle frequency. As Jared Diamond points out, most biological examples of rolling are found in wide open, hard-packed terrain, including the use of rolling by dung beetles and tumbleweeds.

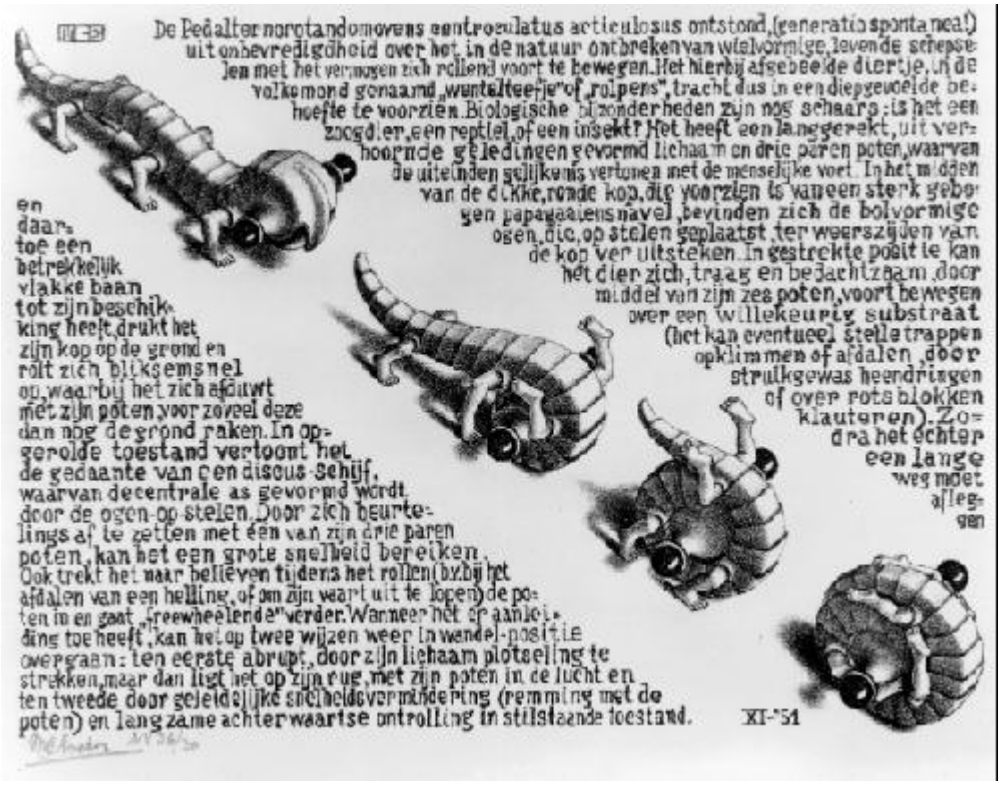
Traction

Wheels are prone to slipping, an inability to generate traction, on loose or slippery terrain. Slipping wastes energy, and can potentially lead to a loss of control or becoming stuck, as with an automobile on mud, ice, or snow. This disadvantage of wheels is apparent in the realm of human technology; legged vehicles find use in the logging industry, where they allow access to more challenging terrain than wheeled vehicles can navigate. Tracked vehicles suffer less from slipping than wheeled vehicles, due to their larger contact area with the ground, but they tend to have larger turning radii than wheeled vehicles, and are less efficient and more mechanically complex.

Versatility

Articulated limbs used by animals for locomotion are frequently also used for other purposes, such as grasping and kicking. With a lack of articulation, wheels would not be as versatile in this regard.

Rolling and wheeled creatures in fiction and legend



Escher's Curl-up

Rolling

The hoop snake is a legendary creature of the United States and Australia. The snake is said to grasp its tail in its mouth and roll like a wheel towards its prey. The Japanese Tsuchinoko is a similar mythical creature.

The Dutch graphic artist M. C. Escher invented a creature he called *Pedalternorotandomovens centroculatus articulosus*, which was capable of rolling itself forward like a wheel. He illustrated this creature in his 1951 lithograph *Wentelteefje*, or in English, *Curl-up*.

The 1944 science-fiction short story "Arena", by Fredric Brown, features a telepathic, alien creature called an "Outsider" which is roughly spherical and moves by rolling. The story was the basis for the 1967 Star Trek episode of the same name, and a 1964 The Outer Limits episode entitled "Fun and Games".

Tuf Voyaging, a science fiction novel by George R. R. Martin, first published in 1986, features an alien species called the "Rolleram", which kills its prey by rolling over it.

The 1995 short story "Microbe", by Kenyon College biologist and feminist science fiction writer Joan Slonczewski, describes an exploratory expedition to an alien world, whose plant and animal life consists entirely of doughnut-shaped organisms.

Sonic the Hedgehog and his friend Tails roll very fast.

Wheeled

L. Frank Baum's 1907 children's book *Ozma of Oz* features humanoid creatures with wheels instead of hands and feet, called "Wheelers".

The 1968 novel *The Goblin Reservation* by Clifford D. Simak features an intelligent alien race which uses biological wheels.

David Brin's *Uplift* Universe includes a wheeled species called the G'Kek, which are described in some detail in the novel *Brightness Reef*. The G'Kek are described as looking like "a squid in a wheelchair." They suffer from arthritic axles in their old age, particularly when living in a high gravity environment.

A 1997 novel in the Animorphs series, *The Andalite Chronicles*, involves a species called Mortrons, composed of two separate entities, a yellow and black bottom half with four wheels, and a red elongated head with razor-sharp teeth and concealed wings.

The 2000 novel *The Amber Spyglass*, by English author Philip Pullman, features an alien race known as the Mulefa, who have diamond-shaped bodies with one leg at the front and back and one on each side. The Mulefa use large, disc-shaped seed pods as wheels. They mount the pods on bone axles on their front and back legs, while propelling themselves with their side legs. The Mulefa have a symbiotic relationship with the seed pod trees, which depend on the rolling action to crack open the pods and allow the seeds to emerge.

In the 2000 novel *Wheelers*, by English mathematician Ian Stewart and reproductive biologist Jack Cohen, an alien species called "blimps" has developed the ability to biologically produce machines called *wheelers*, which use wheels for locomotion.

Piers Anthony's book *Cluster* and its sequels feature members of an alien species called Polarians who locomote by gripping and balancing atop a large ball. The ball is a living though temporarily separable portion of a Polarian's body.

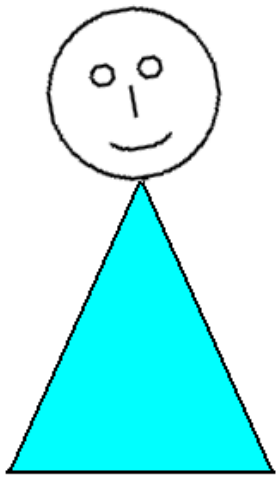
Chapter 10

Vestibulo Emotional Reflex and Work Loop

Vestibulo emotional reflex

Vestibulo-emotional reflex (**VER**) is a reflex 3d head movement that stabilized vertical equilibrium of head by producing head-neck muscle movement with the frequency depending on the emotional and psychophysiological state of a person. Vestibulo-emotional reflex is one of the vestibular reflexes, linking human physiology and emotions. Physiology and pathology of vestibular system and vestibular apparatus were researched by Robert Bárány receiving the 1914 Nobel Price in Physiology. The vestibular system, which contributes to human balance and our sense of spatial orientation, is the sensory system that provides the dominant input about movement and equilibrioception. Vertical human head position is controlled by vestibular system by the means of head-neck anatomy.

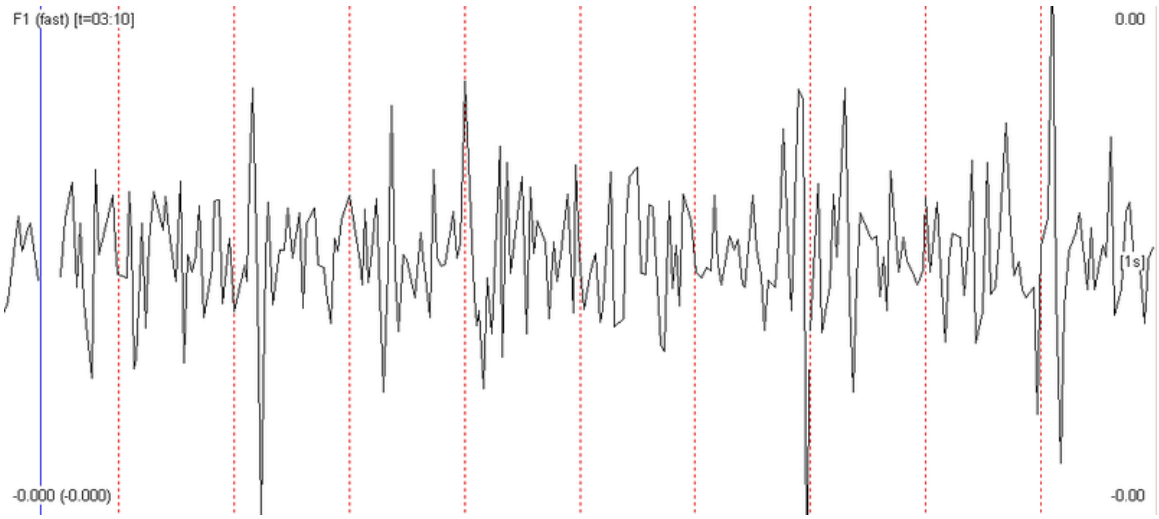
Biomechanics



VER model. Human head moves slowly when person is calm and still (white head image). Human head moves fast and frequently when person is active, aggressive, anxiety and nervous (red head image)

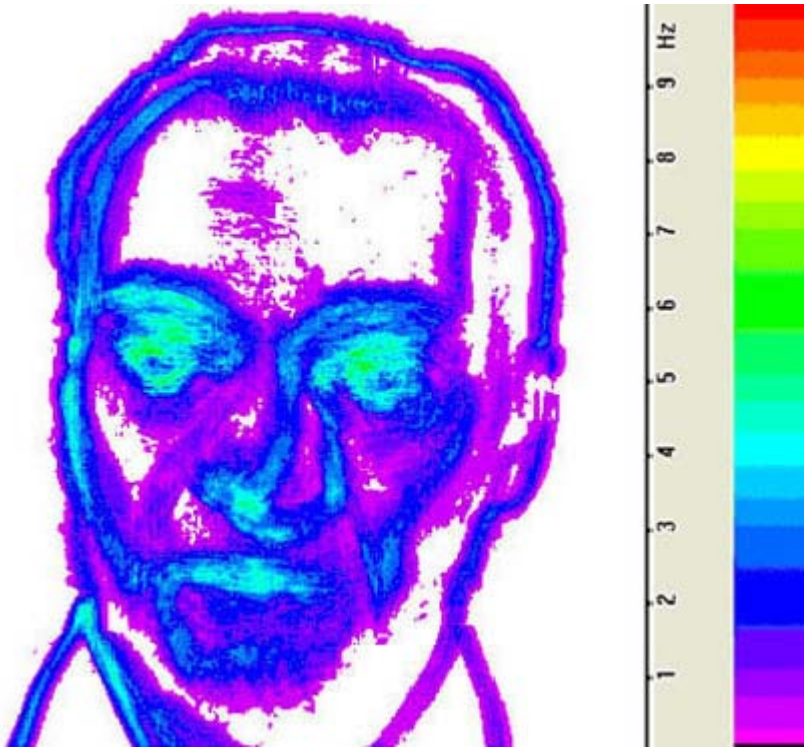
Two month old child begins to poise the head in vertical position on reflex level, firstly performs visible movements for it. An adult person also performs micromovements for poise vertical head position, because it is impossible to coordinate vertical mechanical balance of heavy object without movements. The trajectory of 3D head movement is enough complicated and used for different vestibular reflexes researches and human health diagnostics, because vestibular system links with sensory system, nervous system and every part of human body. Sensory systems code for four aspects of a stimulus; type, intensity, location, and duration. Certain receptors are sensitive to certain types of stimuli (for example, different mechanoreceptors respond best to different kinds of touch stimuli, like sharp or blunt objects). Receptors send impulses in certain patterns to send information about the intensity of a stimulus (for example, how loud a sound is). Russian neurophysiologist Nikolai Bernstein spent most part of his life to physiology of movement. He also coined the term biomechanics, the study of movement through the application of mechanical principles. The principles of biological feedback and discrete movement discovered by Bernstein, forms one of the VER bases and his calculation of human movement time discrete about 0.1 sec was confirmed by video image analysis.

Vestibular system as typical sensory system reacts to stimulus. But gravitation is constantly working stimulus, so vertical head coordination becomes constantly working and reflex process. This is the main physiology difference between vertical head coordination and any other sensory process that works sometimes. This difference transfers vertical head coordination into typical physiological process as heart rate (HR) measured by ECG and blood pressure, brain activity measured by electroencephalography (EEG), or thermoregulation measured by galvanic-skin respond (GSR). Biological evolution used head vertical coordination for energy regulation, because natural head movement is ideal vibration movement with high energy range. The other sample of nature vibration process for energy regulation is dog tail wagging, but humans have not tail and head movement is more optimal for it. It is understandable, that more high frequency head movement requests more energy, than low frequency movement. On sensor level it means, that signals send from vestibular receptors to autonomic nervous system, brain and muscles are going with different time delay, depends on biochemical human state. That means dependence between emotional state and vestibular head coordination or vestibulo-emotional reflex.



Head movement vestibulogram signal captured by low noise web camera with resolution 640x480 pixels and 30 f/s frequency

VER application



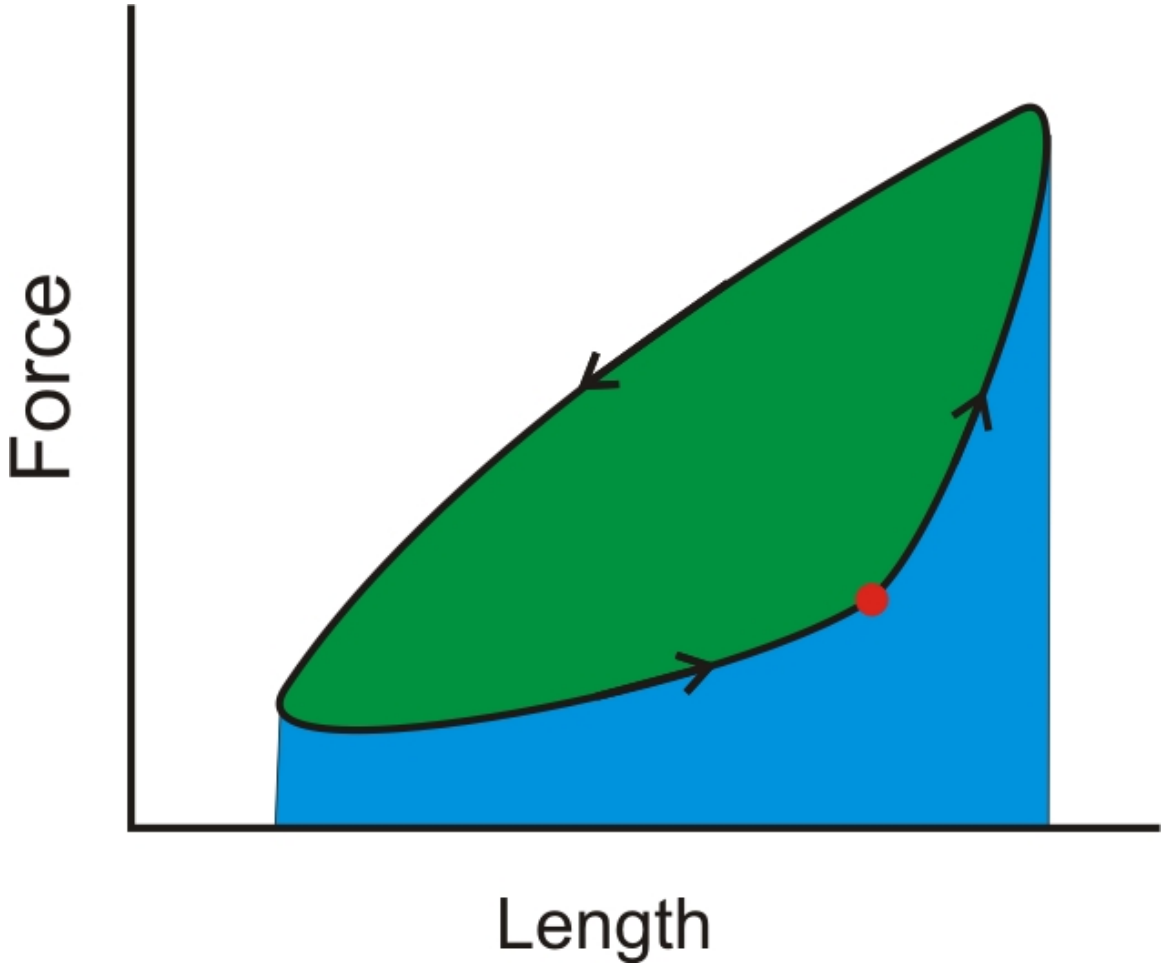
Facial vibraimage with frequency scale

VER gives functional information about person and could be apply for medical, eHealth, psychology and behavior testing, lie detection, emotion control, self-regulation, fitness, animals research are also providing by the different types of vibraimage system. Vibraimage system transforms biomechanics movement into emotional and physiological

data of person by video image processing. This process could be remote and hidden for user that is important for security applications, as aviation security.

Work Loop

A **work loop** is a technique used in muscle physiology to evaluate the mechanical work and power output of muscle during cyclical contractions via *in vitro* muscle testing.



A hypothetical work loop. Arrows indicate the timecourse of the work loop, with stimulation occurring at the red dot. Green shaded area is net work, blue shaded area is work lost to passive and active resistance, and green + blue is total work.

Work loops were first used by Josephson to evaluate properties of katydid flight muscles. Previously, attempts to understand muscle function during locomotion relied upon characterizing various aspects of muscle physiology in isolation, which made determining their interactions difficult - for instance, force-velocity relationships are

evaluated at constant velocities and loads, which is rarely the case in nature, and power measurements obtained from these tests could not take into account the activation and relaxation times of the muscle (which can comprise a significant time portion of the limb's overall movement cycle). By driving the muscle through cycles of a natural range of motion at a range of frequencies observed in natural behavior, all of these aspects would be integrated in a single resultant graph of force vs. displacement.

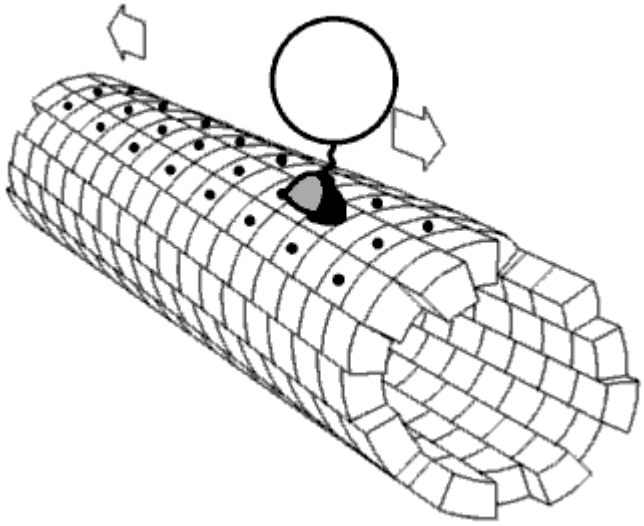
Since work is defined as force multiplied by distance, the area of the graph could determine mechanical output of the muscle. In a work-generating instance, the muscle would show a rapid curvilinear rise in force as it shortened, followed by a slower decline during or shortly before the muscle begins the lengthening phase of the cycle. The area under the shortening curve would give the total work done by the shortening muscle, while the area underneath the lengthening curve would represent the work absorbed by the muscle and turned into heat (done by either environmental forces or antagonistic muscles). Subtracting the latter from the former would give the net mechanical work output of the muscle cycle, and dividing that by the cycle duration would give mechanical power output. This technique allowed greater appreciation for the role of activation & relaxing kinetics in muscle power and work output - for instance, if a muscle turns on and off more slowly, the shortening and lengthening curves will be shallower and closer together, resulting in decreased work output. "Negative" work loops were also possible, showing a lengthening curve at higher force than the shortening curve and resulting in net energy absorption by the muscle, as in the case of deceleration or constant-speed downhill walking.

Originally, workloops imposed a sinusoidal length change on the muscle, with equal time lengthening and shortening. However, in vivo muscle length change often has greater than half the cycle shortening, and less than half lengthening. Imposing these "asymmetrical" stretch-shorten cycles can result in higher work and power outputs, as shown in treefrog calling muscles.

Chapter 11

Motor Protein

Motor proteins are a class of molecular motors that are able to move along the surface of a suitable substrate. They are powered by the hydrolysis of ATP and convert chemical energy into mechanical work.



Motor Protein

Cellular functions

The most prominent example of a motor protein is the muscle protein myosin which "motors" the contraction of muscle fibers in animals. Motor proteins are the driving force behind most active transport of proteins and vesicles in the cytoplasm. Kinesins and dyneins play essential roles in intracellular transport such as axonal transport and in the formation of the spindle apparatus and the separation of the chromosomes during mitosis and meiosis. Dynein is found in flagella and is crucial to cell motility, for example in spermatozoa.

Diseases associated with motor protein defects

The importance of motor proteins in cells becomes evident when they fail to fulfill their function. For example, kinesin deficiencies have been identified as cause for Charcot-Marie-Tooth disease and some kidney diseases. Dynein deficiencies can lead to chronic infections of the respiratory tract as cilia fail to function without dynein. Defects in muscular myosin predictably cause myopathies, whereas defects in unconventional myosin are the cause for Usher syndrome and deafness.

Cytoskeletal motor proteins

Motor proteins utilizing the cytoskeleton for movement fall into two categories based on their substrates: Actin motors such as myosin move along microfilaments through interaction with actin. Microtubule motors such as dynein and kinesin move along microtubules through interaction with tubulin. There are two basic types of microtubule motors: plus-end motors and minus-end motors, depending on the direction in which they "walk" along the microtubule cables within the cell.

Actin motors

Myosin

Myosins are actin motors and form myosin complexes consisting of two heavy chains with motor heads and two light chains. Derived from the Greek word for muscle, myosin is the protein responsible for generating muscle contraction. By non-processively walking along actin filaments, many molecules of myosin generate enough force to contract muscle tissue. Myosins are also vital in the process of cell division. They are also involved in cytoplasmic streaming, wherein movement along microfilament networks in the cell allows organelles and cytoplasm to stream in a particular direction. Eighteen different classes of myosins are known.

Genomic representation of myosin motors:

- Fungi (yeast): 5
- Plants (Arabidopsis): 17
- Insects (Drosophila): 13
- Mammals (human): 40

Microtubule motors

Kinesin

Kinesins are a group of related motor proteins that use a microtubule track along which to "walk." They are vital to movement of chromosomes during mitosis and are also responsible for shuttling mitochondria, Golgi bodies, and vesicles within eukaryotic cells. Kinesins typically contain two heavy chains with motor heads which move along

microtubules via a pseudo-processive asymmetric walking motion, that can be towards the plus-end or the minus-end, depending on the type of kinesin. Fourteen distinct kinesin families are known, with some additional kinesin-like proteins that cannot be classified into these families.

Genomic representation of kinesin motors:

- Fungi (yeast): 6
- Plants (Arabidopsis thaliana): 61
- Insects (Drosophila melanogaster): 25
- Mammals (human): 45

Dynein

Dyneins are microtubule motors capable of a sliding movement. Dynein complexes are much larger and more complex than kinesin and myosin motors. Dynein facilitates the movement of cilia and flagella. Compared to 15 types of dynein for this function, only two cytoplasmic forms are known.

Genomic representation of dynein motors:

- Fungi (yeast): 1
- Plants (Arabidopsis thaliana): 0
- Insects (Drosophila melanogaster): 13
- Mammals (human): 14-15

Plant-specific motors

In contrast to animals, fungi and non-vascular plants, the cells of flowering plants lack dynein motors. However, they contain a larger number of different kinesins. Many of these plant-specific kinesin groups are specialized for functions during plant cell mitosis. Plant cells differ from animal cells in that they have a cell wall. During mitosis, the new cell wall is built by the formation of a cell plate starting in the center of the cell. This process is facilitated by a phragmoplast, a microtubule array unique to plant cell mitosis. The building of cell plate and ultimately the new cell wall requires kinesin-like motor proteins.

Another motor protein essential for plant cell division is kinesin-like calmodulin-binding protein (KCBP), which is unique to plants and part kinesin and part myosin.

Other molecular motors

Besides the motor proteins above, there are many more types of proteins capable of generating forces and torque in the cell. Many of these molecular motors are ubiquitous in both prokaryotic and eukaryotic cells, although some, such as those involved with cytoskeletal elements or chromatin, are unique to eukaryotes. The motor protein prestin,

expressed in mammalian cochlear outer hair cells, produces mechanical amplification in the cochlea. It is a direct voltage-to-force converter, which operates at the microsecond rate and possesses piezoelectric properties.

Chapter 12

Arboreal Locomotion

Arboreal locomotion is the locomotion of animals in trees. In every habitat in which trees are present, animals have evolved to move in them. Some animals may only scale trees occasionally, while others are exclusively arboreal. These habitats pose numerous mechanical challenges to animals moving through them, leading to a variety of anatomical, behavioral and ecological consequences. Furthermore, many of these same principles may be applied to climbing without trees, such as on rock piles or mountains.

The earliest known tetrapod with specializations that adapted it for climbing trees, was *Suminia*, a synapsid of the late Permian, about 260 million years ago.

Biomechanics of arboreal locomotion

Arboreal habitats pose numerous mechanical challenges to animals moving in them, which have been solved diverse ways. These challenges include moving on narrow branches, moving up and down inclines, balancing, crossing gaps, and dealing with obstructions.

Diameter

Moving along a narrow surface poses special difficulties to animals. During locomotion on the ground, the location of the center of mass may swing from side to side, but during arboreal locomotion, this would result in the center of mass moving beyond the edge of the branch, resulting in a tendency to topple over. Additionally, foot placement is constrained by the need to make contact with the narrow branch. This narrowness severely restricts the range of movements and postures an animal can use to move.

Incline

Branches are frequently oriented at an angle to gravity in arboreal habitats, including being vertical, which poses special problems. As an animal moves up an inclined branch, they must fight the force of gravity to raise their body, making movement more difficult. Conversely, as the animal descends, it must also fight gravity to control its descent and prevent falling. Descent can be particularly problematic for many animals, and highly arboreal species often have specialized methods for controlling their descent.

Balance

Due to the height of many branches and the potentially disastrous consequences of a fall, balance is of primary importance to arboreal animals. On horizontal and gently sloped branches, the primary problem is tipping to the side due to the narrow base of support. The narrower the branch, the greater the difficulty in balancing a given animal faces. On steep and vertical branches, tipping becomes less of an issue, and pitching backwards or slipping downwards becomes the most likely failure. In this case, large-diameter branches pose a greater challenge, since the animal cannot place its forelimbs closer to the center of the branch than its hindlimbs.

Crossing gaps

Branches are not continuous, and any arboreal animal must be able to move between gaps in the branches, or even between trees. This can be accomplished by reaching across gaps, or by leaping across them.

Obstructions

Arboreal habitats often contain many obstructions, both in the form of branches emerging from the one being moved on and other branches impinging on the space the animal needs to move through. These obstructions may impede locomotion, or may be used as additional contact points to enhance it, but only one laboratory study has ever quantified the results of obstructions.

Anatomical specializations

Arboreal organisms display many specializations for dealing with the mechanical challenges of moving through their habitats.

Limb length

Arboreal animals frequently have elongated limbs that help them cross gaps, reach fruit or other resources, test the firmness of support ahead, and in some cases, to brachiate. However, some species of lizard have reduced limb size that helps them avoid limb movement being obstructed by impinging branches.

Prehensile tails

Many arboreal species, such as chameleons, spider monkeys, and possums, use prehensile tails to grasp branches. In the spider monkey and crested gecko, the tip of the tail has either a bare patch or adhesive pad, which provide increased friction.

Claws

Claws can be used to interact with rough substrates and re-orient the direction of forces the animal applies. This is what allows squirrels to climb tree trunks that are so large as to be essentially flat, from the perspective of such a small animal. However, claws can interfere with an animal's ability to grasp very small branches, as they may wrap too far around and prick the animal's own paw.

Adhesion

Adhesion is an alternative to claws, which works best on smooth surfaces. Wet adhesion is common in tree frogs and arboreal salamanders, and functions either by suction or by capilary adhesion. Dry adhesion is best typified by the specialized toes of geckos, which use van der Waals forces to adhere to many substrates, even glass.

Two *Cepaea nemoralis* on a tree trunk

Gripping

Frictional gripping is used by primates, relying upon hairless fingertips. Squeezing the branch between the fingertips generates frictional force that holds the animal's hand to the branch. However, this type of grip depends upon the angle of the frictional force, thus upon the diameter of the branch, with larger branches resulting in reduced gripping ability. Animals other than primates that use gripping in climbing include the chameleon, which has mitten-like grasping feet, and many birds that grip branches in perching or moving about.

Reversible feet

To control descent, especially down large diameter branches, some arboreal animals such as squirrels have evolved highly mobile ankle joints that permit rotating the foot into a 'reversed' posture. This allows the claws to hook into the rough surface of the bark, opposing the force of gravity.

Low center of mass

Many arboreal species lower their center of mass to reduce pitching and toppling movement when climbing. This may be accomplish by postural changes, altered body proportions, or smaller size.

Small size

Small size provides many advantages to arboreal species, such as increasing the relative size of branches to the animal, lower center of mass, increased stability, lower mass (allowing movement on smaller branches), and the ability to move through more cluttered habitat.

Hanging under perches

Some species of primate, bats, and all species of sloth achieve passive stability by hanging beneath the branch. Both pitching and tipping become irrelevant, as the only method of failure would be losing their grip.

Behavioral specializations

Arboreal species have behaviors specialized for moving in their habitats, most prominently in terms of posture and gait. Specifically, arboreal mammals take longer steps, extend their limbs further forwards and backwards during a step, adopt a more 'crouched' posture to lower their center of mass, and use a diagonal sequence gait.

Ecological consequences

Arboreal locomotion allows animals access to different resources, depending upon their abilities. Larger species may be restricted to larger-diameter branches that can support their weight, while smaller species may avoid competition by moving in the narrower branches.

Climbing without trees

Many animals climb in other habitats, such as in rock piles or mountains, and in those habitats, many of the same principles apply due to inclines, narrow ledges, and balance issues. However, less research has been conducted on the specific demands of locomotion in these habitats.

Perhaps the most exceptional of the animals that move on steep or even near vertical rock faces by careful balancing and leaping are the various types of mountain dwelling caprid such as the Barbary sheep, markhor, yak, ibex, tahr, rocky mountain goat, and chamois. Their adaptations may include a soft rubbery pad between their hooves for grip, hooves with sharp keratin rims for lodging in small footholds, and prominent dew claws. The snow leopard, being a predator of such mountain caprids, also has spectacular balance and leaping abilities; being able to leap up to ~17m (~50 ft). Other balancers and leapers include the mountain zebra, mountain tapir, and hyraxes.

Brachiation

Brachiation is a specialized form of arboreal locomotion, used by primates to moves very rapidly while hanging beneath branches. Arguably the epitome of arboreal locomotion, it involves swinging with the arms from one handhold to another. Only a few species are brachiators, and all of these are primates; it is a major means of locomotion among spider monkeys and gibbons, and is occasionally used by female orangutans. Gibbons are the experts of this mode of locomotion, swinging from branch to branch distances of up to 15 m (50 ft), and traveling at speeds of as much as 56 km/h (35 mph).

Gliding between trees

To bridge gaps between trees, many animals such as the flying squirrel have become adapted to glide.

Limbless climbing

Many species of snake are highly arboreal, and some have evolved specialized musculature for this habitat. While moving in arboreal habitats, snakes move slowly along bare branches using a specialized form of concertina locomotion, but when secondary branches emerge from the branch being moved on, snakes use lateral undulation, a much faster mode. As a result, snakes perform best on small perches in

cluttered environments, while limbed organisms seem to do best on large perches in uncluttered environments.

Arboreal animals

Many species of animals are arboreal, far too many to list individually. This list is of prominently or predominantly arboreal species and higher taxa.

- Primates
- Cats
- brushtail possums
- opossums
- Sloths
- Treeshrews
- Goats
- Colugos
- Kinkajous
- Viverrids
- Many rodents
- Parrots
- Geckos
- Chameleons
- Many other lizards
- Mambas
- Brown Tree Snakes
- Many other snakes
- Stick insects
- Many other arthropods

Chapter 13

Aquatic Locomotion and Fin & Flipper Locomotion

Aquatic locomotion

Swimming is biologically propelled motion through a liquid medium. Swimming has evolved a number of times in a range of organisms ranging from arthropods to fish to molluscs.

Basic swimming – jellyfish

All jellyfish are free-swimming, although many of these spend most of their time swimming passively. Passive swimming is akin to gliding; the organism floats, using currents where it can, and does not exert any energy into controlling its position or motion. Active swimming, in contrast, involves the expenditure of energy to travel to a desired location.

Swimming in fish

Some fish need to swim in order to maintain flotation; others float naturally by means of swim bladders or other organs. Swimming primarily achieves motion in a certain direction, and the method employed by fish is the most efficient for maintaining a high speed over a significant distance. The same method has been converged upon by cephalopods, who have progressively downplayed the role of jet propulsion in favour of more fish-like swimming.

Jet-propelled swimming

Octopuses swim headfirst, with arms trailing behind

All cephalopods can move by jet propulsion, but this is a very energy-consuming way to travel compared to the tail propulsion used by fish. The relative efficiency of jet propulsion decreases further as animal size increases. Since the Paleozoic, as competition with fish produced an environment where efficient motion was crucial to survival, jet propulsion has taken a back role, with fins and tentacles used to maintain a steady velocity. The stop-start motion provided by the jets, however, continues to be useful for providing bursts of high speed - not least when capturing prey or avoiding predators. Indeed, it makes cephalopods the fastest marine invertebrates, and they can outaccelerate most fish. Oxygenated water is taken into the mantle cavity to the gills and through muscular contraction of this cavity, the spent water is expelled through the hyponome, created by a fold in the mantle. Motion of the cephalopods is usually backward as water is forced out anteriorly through the hyponome, but direction can be controlled somewhat by pointing it in different directions.

Most cephalopods float (i.e. are neutrally buoyant), so do not need to swim to remain afloat.

Evolution of swimming

Swimming evolved a number of times in unrelated lineages, and the evolutionary pressures leading to its adoption are unknown. Supposed jellyfish fossils occur in the Ediacaran, but the first free-swimming animals appear in the Early to Middle Cambrian. These are mostly related to the arthropods, and include the Anomalocaridids, which swam by means of lateral lobes in a fashion reminiscent of today's cuttlefish. Cephalopods joined the ranks of the nekton in the late Cambrian, and chordates were probably swimming from the Early Cambrian.

Secondary evolution of swimming

While tetrapods lost many of their natural adaptations to swimming when they evolved onto the land, many have re-evolved the ability to swim or have indeed returned to a completely aquatic lifestyle.

Primarily or exclusively aquatic animals have re-evolved from terrestrial tetrapods multiple times: examples include amphibians such as newts, reptiles such as crocodiles, sea turtles, ichthyosaurs, plesiosaurs and mosasaurs, marine mammals such as whales, seals and otters, and birds such as penguins. Many species of snakes are also aquatic and live their entire lives in the water. Many insects swim on a regular basis and some insects, such as certain species of diving beetle, spend most of their time in the water. There are also aquatic spiders, although they tend to prefer other modes of locomotion under water than swimming proper.

A dog is swimming

Even though primarily terrestrial tetrapods have lost many of their adaptations to swimming, the ability to swim has been preserved or re-developed in many of them. It may never have been completely lost.

Examples are: Some breeds of dog swim recreationally. Umbra, a world record-holding dog, can swim 4 miles (6.4 km) in 73 minutes, placing her in the top 25% in human long-distance swimming competitions. Although most cats hate water, adult cats are good swimmers. The fishing cat is one wild species of cat that has evolved special adaptations for an aquatic or semi-aquatic lifestyle – webbed digits. Tigers and some individual jaguars are the only big cats known to go into water readily, though other big cats, including lions, have been observed swimming. A few domestic cat breeds also like swimming, such as the Turkish Van. In an unpublished research carried out 2002 at the University of Bern (Switzerland), Bender & Hirt showed that the Turkish Van has less inhibition to enter in shallow water compared to another breed, the Russian Blue. This behavior can be partially explained by the character of the Turkish Van, who seems to be more curious and enterprising than other cat breeds.

Horses, moose, and elk are very powerful swimmers, and can travel long distances in the water. Elephants are also capable of swimming, even in deep waters. Eyewitnesses have confirmed that camels, including Dromedary and Bactrian camels, can swim, despite the fact that there is little deep water in their natural habitats.

Both domestic and wild rabbits can swim. Domestic rabbits are sometimes trained to swim as a circus attraction. A wild rabbit famously swam in an apparent attack on U.S. President Jimmy Carter's boat when it was threatened in its natural habitat.

The Guinea pig (or cavy) is noted as having an excellent swimming ability. Mice can swim quite well. They do panic when placed in water, but many lab mice are used in the Morris water maze, a test to measure learning. When mice swim, they use their tails like flagella and kick with their legs.

Many snakes are excellent swimmers as well. Large adult anacondas spend the majority of their time in the water, and have difficulty moving on land.

Humans do not swim instinctively, but nonetheless often feel attracted to water, showing a broader range of swimming movements than other non-aquatic animals. In contrast, many monkeys can naturally swim and some, like the proboscis monkey, crab-eating macaque, and Rhesus macaque swim regularly.

Large primates other than humans generally do not like to swim. Wild chimpanzees and some gorillas will wade in very shallow water but will make no attempt to cross larger bodies of water. Orangutans don't swim instinctively but will attempt it under pressure or if learned.

Human swimming

Swimming has been known amongst humans since prehistoric times; the earliest record of swimming dates back to Stone Age paintings from around 7,000 years ago. Competitive swimming started in Europe around 1800 and was part of the first modern 1896 Summer Olympics in Athens, though not in a form comparable to the contemporary events. It was not until 1908 that regulations were implemented by the International Swimming Federation to produce competitive swimming.

Fin & flipper locomotion

Periophthalmus gracilis (from Malaysia to North Australia)

Fin and flipper locomotion occurs mostly in aquatic locomotion, and rarely in terrestrial locomotion. From the three common states of matter - gas, liquid and solid, these appendages are adapted for liquids, mostly Fresh or Saltwater and used in locomotion, steering and balancing of the body. Locomotion is important in order to escape predators, acquire food, find mates and bury for shelter, nest or food. Aquatic locomotion consists of swimming, whereas terrestrial locomotion encompasses walking, 'crutching', jumping, digging as well as covering. Some animals such as Sea turtles and Mudskippers utilize both environments for various purposes, for example to lay their nests, or to hunt for food.

Aquatic locomotion with fins and flippers

Aquatic locomotion of fish

Fish live in Fresh or Saltwater habitats and some exceptions are capable of coming on land (Mudskippers). Most fish have a line of muscle blocks, called myomeres, along each

side of the body. To swim, they alternately contract one side and relax the other side in a progression which goes from the head to the tail. In this way, an undulatory locomotion results, first bending the body one way in a wave which travels down the body, and then back the other way, with the contracting and relaxing muscles switching roles. They use their fins to propel themselves through the water in this swimming motion. Actinopterygians, the ray-finned fish show an evolutionary pattern of fine control ability to control the dorsal and ventral lobe of the caudal fin. Through developmental changes, intrinsic caudal muscles were added, which enable fish to exhibit such complex maneuvers such as control during acceleration, braking and backing. Studies have shown that the muscles in the caudal fin, have independent activity patterns from the myotomal musculature. These results show specific kinematic roles for different part of the fishs' musculature. A curious example of fish adaption is the Ocean sunfish, also known as the *Mola mola*. These fish have undergone significant developmental changes reducing their spinal cord, giving them a disk like appearance, and investing in two very large fins for propulsion. This adaptation usually gives them the appearance that they are as long as they are tall. They are also amazing fish in that they hold the world record in weight gain from fry to adult (600 million times its weight).

Aquatic locomotion of marine mammals

Swimming mammals, such as whales, dolphins,and sea lions to name a few, utilize their flippers to move forward through the water column. During swimming sea lions have a thrust phase, which lasts about 60% of the full cycle, and the recovery phase lasts the remaining 40%. A full cycle duration lasts about 0.5 to 1.0 seconds. Changing direction is a very rapid maneuver that is initiated by head movement towards the back of the animal that is followed by a spiral turn with the body. Due to their pectoral flippers being so closely located to their center of gravity, sea lions are capable of displaying astounding maneuverability in the pitch, roll, and yaw direction and are therefore not constrained, turning stochastically as they please. It is hypothesized that the increased level of maneuverability is caused by their complex habitat. Hunting occurs in difficult environments containing rocky inshore/kelp forest communities, with many niches for prey to hide, therefore requiring speed and maneuverability for capture. The complex skills of a sea lion are learned early on in ontogeny and most are perfected by the time the pups reach one year. Whales and dolphins are less maneuverable and more constrained in their movements. However, dolphins are capable of accelerating as fast as sea lions, but they are not capable of turning as quickly and as efficiently. For both whales and dolphins, their center of gravity does not line up with their pectoral flippers in a straight line, causing a much more rigid and stable swimming pattern.

Aquatic locomotion of marine reptiles

Aquatic reptiles such as sea turtles predominantly use their pectoral flippers to propulse through the water and their pelvic flippers for maneuvering. During swimming they move their pectoral flippers in a clapping motion underneath their body and pull them back up into an airplane position, causing forward motion. During the swimming motion it is really important that they rotate their front flipper in order to decrease drag through the

water column and increase their efficiency. Sea turtles exhibit a natural suite of behavior skills that help them direct themselves towards the ocean as well as identify the transition from sand to water after hatching. If rotated in the pitch, yaw or roll direction the hatchlings are capable of counteracting the forces acting upon them by correcting with either their pectoral or pelvic flippers and redirecting themselves towards the open ocean.

Terrestrial Locomotion

Terrestrial locomotion of fish

Mudskippers in The Gambia

Terrestrial locomotion poses new obstacles such as gravity and new media, including sand, mudd, twigs, logs, debris, grass and many more. Fins and flippers are aquatically adapted appendages and typically aren't very useful in such an environment.It could be hypothesized that fish would try to "swim" on land, but studies have shown that some fish evolved to cope with the terrestrial environment. Mudskippers, for example demonstrate a 'crutching' gait which enables them to 'walk' over muddy surfaces as well as dig burrows to hide in. Mudskippers are also able to jump up to 3 cm distances. This behavior is described as starting with a J-curvature of the body at about 2/3 of its body length (with its' tail wrapped towards the head), followed by a straightening of their body which propulses them like a projectile through the air. This behavior enables them to cope with the new environment and opens their habitat to new food sources as well as new predators.

Terrestrial locomotion of marine reptiles

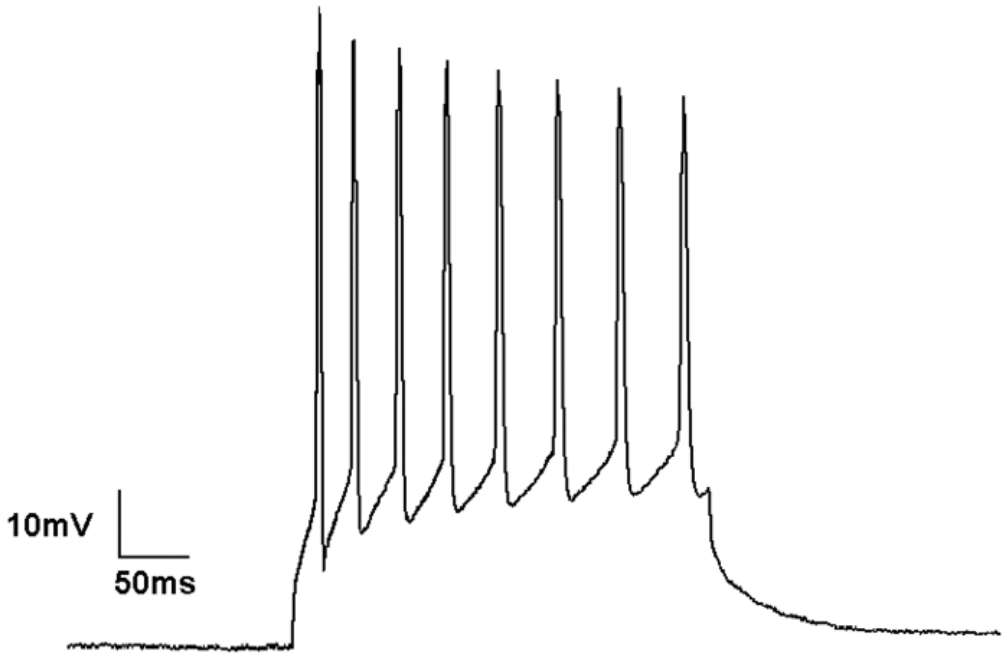
Caretta caretta Jekyll Island, GA

Reptiles, such as sea turtles spend most of their lives in the ocean. However, their life cycle requires the females to come on shore and lay their nests on the beach. Consequently, the hatchlings emerge out of the sand and have to run toward the water. Depending on their species, sea turtles are described to have either a symmetrical gait (diagonally opposite limbs are moving together) or an asymmetrical gait (Contra-lateral limbs move together). For example, loggerhead sea turtle hatchlings are commonly seen exhibiting symmetrical gait on sand, whereas, leatherback sea turtles employ the asymmetrical gait while on land. Notably, leatherbacks employ their front (pelvic) flippers more during forward terrestrial locomotion. Sea turtles can be seen nesting on subtropical and tropical beaches all around the world and exhibit such behavior such as arribada (Collective animal behavior). This is a phenomenon seen in Kemp's Ridley turtles which emerge all at once in one night only onto the beach to lay their nests.

Chapter 14

Electrophysiology

Electrophysiology (from Greek ἤλεκτρον, *ēlektron*, "amber"; φύσις, *physis*, "nature, origin"; and -λογία, *-logia*) is the study of the electrical properties of biological cells and tissues. It involves measurements of voltage change or electric current on a wide variety of scales from single ion channel proteins to whole organs like the heart. In neuroscience, it includes measurements of the electrical activity of neurons, and particularly action potential activity. Recordings of large-scale electric signals from the nervous system such as electroencephalography, may also be referred to as electrophysiological recordings.



"Current Clamp" is a common technique in electrophysiology. This is a whole-cell current clamp recording of a neuron firing due to it being depolarized by current injection

Definition and scope

Classical electrophysiological techniques

Electrophysiology is the science and branch of physiology that pertains to the flow of ions in biological tissues and, in particular, to the electrical recording techniques that enable the measurement of this flow. Classical electrophysiology techniques involve placing electrodes into various preparations of biological tissue. The principal types of electrodes are: 1) simple solid conductors, such as discs and needles (singles or arrays, often insulated except for the tip), 2) tracings on printed circuit boards, also insulated except for the tip, and 3) hollow tubes filled with an electrolyte, such as glass pipettes filled with potassium chloride solution or another electrolyte solution. The principal preparations include 1) living organisms, 2) excised tissue (acute or cultured), 3) dissociated cells from excised tissue (acute or cultured), 4) artificially grown cells or tissues, or 5) hybrids of the above.

If an electrode is small enough (micrometers) in diameter, then the electro-physiologist may choose to insert the tip into a single cell. Such a configuration allows direct observation and recording of the intracellular electrical activity of a single cell. However, at the same time such invasive setup reduces the life of the cell and causes a leak of substances across the cell membrane. Intracellular activity may also be observed using a specially formed (hollow) glass pipette containing an electrolyte. In this technique, the microscopic pipette tip is pressed against the cell membrane, to which it tightly adheres by an interaction between glass and lipids of the cell membrane. The electrolyte within the pipette may be brought into fluid continuity with the cytoplasm by delivering a pulse of pressure to the electrolyte in order to rupture the small patch of membrane encircled by the pipette rim (whole-cell recording). Alternatively, ionic continuity may be established by "perforating" the patch by allowing exogenous pore-forming agent within the electrolyte to insert themselves into the membrane patch (perforated patch recording). Finally, the patch may be left intact (patch recording).

The electrophysiologist may choose not to insert the tip into a single cell. Instead, the electrode tip may be left in continuity with the extracellular space. If the tip is small enough, such a configuration may allow indirect observation and recording of action potentials from a single cell, and is termed single-unit recording. Depending on the preparation and precise placement, an extracellular configuration may pick up the activity of several nearby cells simultaneously, and this is termed multi-unit recording.

As electrode size increases, the resolving power decreases. Larger electrodes are sensitive only to the net activity of many cells, termed local field potentials. Still larger electrodes, such as uninsulated needles and surface electrodes used by clinical and surgical neurophysiologists, are sensitive only to certain types of synchronous activity within populations of cells numbering in the millions.

Other classical electrophysiological techniques include single channel recording and amperometry.

Optical electrophysiological techniques

Optical electrophysiological techniques were created by scientists and engineers to overcome one of the main limitations of classical techniques. Classical techniques allow observation of electrical activity at approximately a single point within a volume of tissue. Essentially, classical techniques singularize a distributed phenomenon. Interest in the spatial distribution of bioelectric activity prompted development of molecules capable of emitting light in response to their electrical or chemical environment. Examples are voltage sensitive dyes and fluorescing proteins.

After introducing one or more such compounds into tissue via perfusion, injection or gene expression, the 1 or 2-dimensional distribution of electrical activity may be observed and recorded.

Many particular electrophysiological readings have specific names:

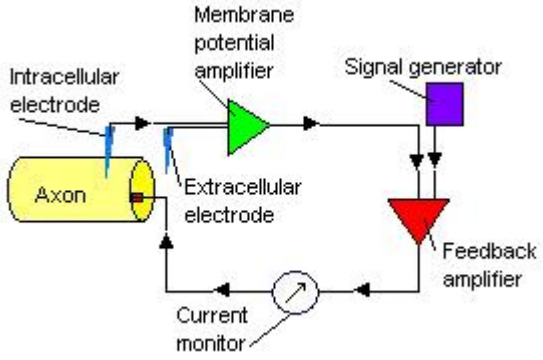
- Electrocardiography - for the heart
- Electroencephalography - for the brain
- Electrocorticography - from the cerebral cortex
- Electromyography - for the muscles
- Electrooculography - for the eyes
- Electroretinography - for the retina
- Electroantennography - for the olfactory receptors in arthropods
- Audiology - for the auditory system

Intracellular recording

Intracellular recording involves measuring voltage and/or current across the membrane of a cell. To make an intracellular recording, the tip of a fine (sharp) microelectrode must be inserted inside the cell, so that the membrane potential can be measured. Typically, the resting membrane potential of a healthy cell will be -60 to -80 mV, and during an action potential the membrane potential might reach +40 mV. In 1963, Alan Lloyd Hodgkin and Andrew Fielding Huxley won the Nobel Prize in Physiology or Medicine for their contribution to understanding the mechanisms underlying the generation of action potentials in neurons. Their experiments involved intracellular recordings from the giant axon of Atlantic squid (Loligo pealei), and were among the first applications of the "voltage clamp" technique. Today, most microelectrodes used for intracellular recording are glass micropipettes, with a tip diameter of < 1 micrometre, and a resistance of several megaohms. The micropipettes are filled with a solution that has a similar ionic composition to the intracellular fluid of the cell. A chlorided silver wire inserted in to the pipet connects the electrolyte electrically to the amplifier and signal processing circuit. The voltage measured by the electrode is compared to the voltage of a reference electrode, usually a silver chloride-coated silver wire in contact with the extracellular fluid around the cell. In general, the smaller the electrode tip, the higher its electrical resistance, so an electrode is a compromise between size (small enough to penetrate a

single cell with minimum damage to the cell) and resistance (low enough so that small neuronal signals can be discerned from thermal noise in the electrode tip).

Voltage clamp



The voltage clamp uses a negative feedback mechanism. The membrane potential amplifier measures membrane voltage and sends output to the feedback amplifier. The feedback amplifier subtracts the membrane voltage from the command voltage, which it receives from the signal generator. This signal is amplified and returned into the cell via the recording electrode.

The voltage clamp technique allows an experimenter to "clamp" the cell potential at a chosen value. This makes it possible to measure how much *ionic current* crosses a cell's membrane at any given voltage. This is important because many of the ion channels in the membrane of a neuron are voltage gated ion channels, which open only when the membrane voltage is within a certain range. Voltage clamp measurements of current are made possible by the near-simultaneous digital subtraction of transient capacitive currents that pass as the recording electrode and cell membrane are charged to alter the cell's potential.

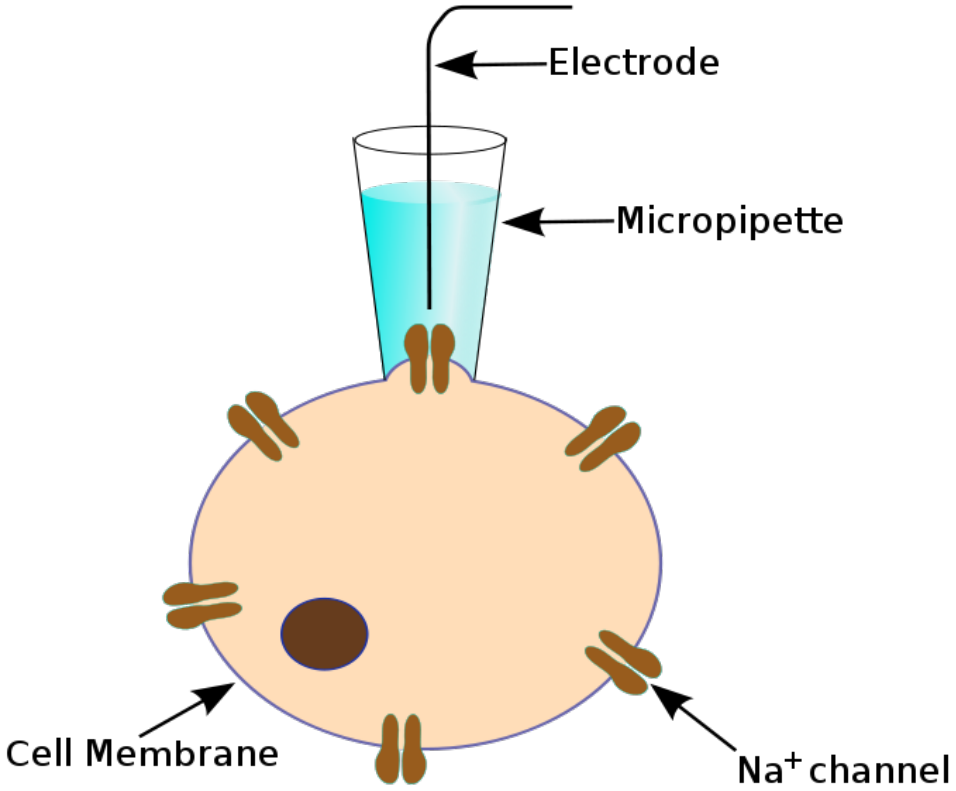
Current clamp

The current clamp technique records the membrane potential by injecting current into a cell through the recording electrode. Unlike in the voltage clamp mode, where the membrane potential is held at a level determined by the experimenter, in "current clamp" mode the membrane potential is free to vary, and the amplifier records whatever voltage the cell generates on its own or as a result of stimulation. This technique is used to study how a cell responds when electric current enters a cell; this is important for instance for understanding how neurons respond to neurotransmitters that act by opening membrane ion channels.

Most current-clamp amplifiers provide little or no amplification of the voltage changes recorded from the cell. The "amplifier" is actually an electrometer, sometimes referred to as a "unity gain amplifier"; its main job is to change the nature of small signals (in the mV range) produced by cells so that they can be accurately recorded by low-impedance electronics. The amplifier increases the current behind the signal while decreasing the

resistance over which that current passes. Consider this example based on Ohm's law: a voltage of 10 mV is generated by passing 10 nanoamperes of current across 1 MΩ of resistance. The electrometer changes this "high impedance signal" to a "low impedance signal" by using a voltage follower circuit. A voltage follower reads the voltage on the input (caused by a small current through a big resistor). It then instructs a parallel circuit that has a large current source behind it (the electrical mains) and adjusts the resistance of that parallel circuit to give the same output voltage, but across a lower resistance.

The patch-clamp technique



The cell-attached patch clamp uses a micropipette attached to the cell membrane to allow recording from a single ion channel.

This technique was developed by Erwin Neher and Bert Sakmann who received the Nobel Prize in 1991. Conventional intracellular recording involves impaling a cell with a fine electrode; patch-clamp recording takes a different approach. A patch-clamp microelectrode is a micropipette with a relatively large tip diameter. The microelectrode is placed next to a cell, and gentle suction is applied through the microelectrode to draw a piece of the cell membrane (the 'patch') into the microelectrode tip; the glass tip forms a high resistance 'seal' with the cell membrane. This configuration is the "cell-attached" mode, and it can be used for studying the activity of the ion channels that are present in the patch of membrane. If more suction is now applied, the small patch of membrane in the electrode tip can be displaced, leaving the electrode sealed to the rest of the cell. This "whole-cell" mode allows very stable intracellular recording. A disadvantage (compared

to conventional intracellular recording with sharp electrodes) is that the intracellular fluid of the cell mixes with the solution inside the recording electrode, and so some important components of the intracellular fluid can be diluted. A variant of this technique, the "perforated patch" technique, tries to minimise these problems. Instead of applying suction to displace the membrane patch from the electrode tip, it is also possible to make small holes on the patch with pore-forming agents so that large molecules such as proteins can stay inside the cell and ions can pass through the holes freely. Also the patch of membrane can be pulled away from the rest of the cell. This approach enables the membrane properties of the patch to be analysed pharmacologically.

Sharp electrode technique

In situations where one wants to record the potential inside the cell membrane with minimal effect on the ionic constitution of the intracellular fluid a sharp electrode can be used. These micropipettes (electrodes) are again like those for patch clamp pulled from glass capillaries, but the pore is much smaller so that there is very little ion exchange between the intracellular fluid and the electrolyte in the pipette. The resistance of the micropipette electrode is tens or hundreds of MΩ. Often the tip of the electrode is filled with various kinds of dyes like Lucifer yellow to fill the cells recorded from, for later confirmation of their morphology under a microscope. The dyes are injected by applying a positive or negative, DC or pulsed voltage to the electrodes depending on the polarity of the dye.

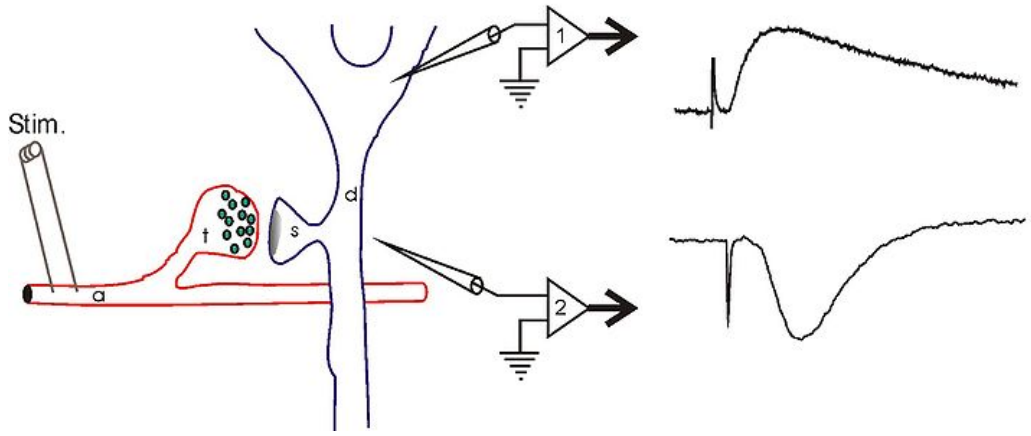
Extracellular recording

Single-unit recording

An electrode introduced into the brain of a living animal will detect electrical activity that is generated by the neurons adjacent to the electrode tip. If the electrode is a microelectrode, with a tip size of about 1 micrometre, the electrode will usually detect the activity of at most one neuron. Recording in this way is generally called "single-unit" recording. The action potentials recorded are very like the action potentials that are recorded intracellularly, but the signals are very much smaller (typically about 1 mV). Most recordings of the activity of single neurons in anesthetized animals are made in this way, and all recordings of single neurons in conscious animals. Recordings of single neurons in living animals have provided important insights into how the brain processes information. For example, David Hubel and Torsten Wiesel recorded the activity of single neurons in the primary visual cortex of the anesthetized cat, and showed how single neurons in this area respond to very specific features of a visual stimulus. Hubel and Wiesel were awarded the Nobel Prize in Physiology or Medicine in 1981. If the electrode tip is slightly larger, then the electrode might record the activity generated by several neurons. This type of recording is often called "multi-unit recording", and is often used in conscious animals to record changes in the activity in a discrete brain area during normal activity. Recordings from one or more such electrodes which are closely spaced can be used to identify the number of cells around it as well as which of the spikes come from which cell. This process is called spike sorting and is suitable in areas where there

are identified types of cells with well defined spike characteristics. If the electrode tip is bigger still, generally the activity of individual neurons cannot be distinguished but the electrode will still be able to record a field potential generated by the activity of many cells.

Field potentials



A schematic diagram showing a field potential recording from rat hippocampus. At the left is a schematic diagram of a presynaptic terminal and postsynaptic neuron. This is meant to represent a large population of synapses and neurons. When the synapse releases glutamate onto the postsynaptic cell, it opens ionotropic glutamate receptor channels. The net flow of current is inward, so a current sink is generated. A nearby electrode (#2) detects this as a negativity. An *intracellular* electrode placed inside the cell body (#1) records the change in membrane potential that the incoming current causes.

Extracellular field potentials are local current sinks or sources that are generated by the collective activity of many cells. Usually a field potential is generated by the simultaneous activation of many neurons by synaptic transmission. The diagram to the right shows hippocampal synaptic field potentials. At the right, the lower trace shows a negative wave that corresponds to a current sink caused by positive charges entering cells through postsynaptic glutamate receptors, while the upper trace shows a positive wave that is generated by the current that leaves the cell (at the cell body) to complete the circuit.

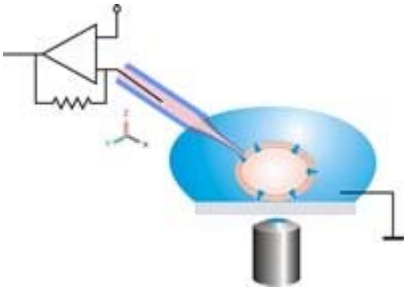
Amperometry

Amperometry uses a carbon electrode to record changes in the chemical composition of the oxidized components of a biological solution. Oxidation and reduction is accomplished by changing the voltage at the active surface of the recording electrode in a process known as "scanning". Because certain brain chemicals lose or gain electrons at

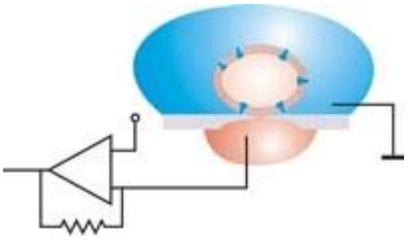
characteristic voltages, individual species can be identified. Amperometry has been used for studying exocytosis in the neural and endocrine systems. Many monoamine neurotransmitters, e.g., norepinephrine (noradrenalin), dopamine, serotonin (5-HT), are oxidizable. The method can also be used with cells that do not secrete oxidizable neurotransmitters by "loading" them with 5-HT or dopamine.

Planar patch clamp

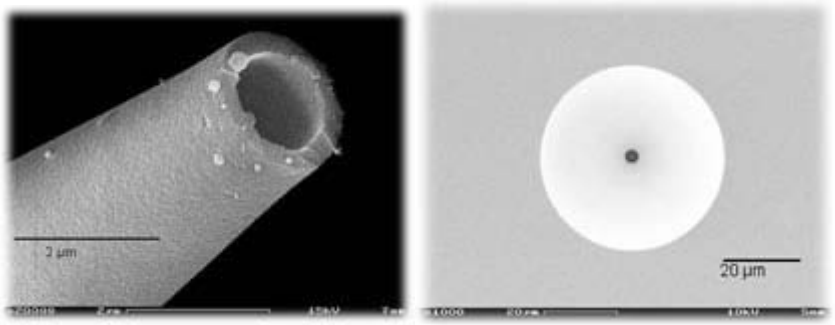
Planar patch clamp is a novel method developed for high throughput electrophysiology. Instead of positioning a pipette on an adherent cell, cell suspension is pipetted on a chip containing a microstructured aperture.



Schematic drawing of the classical patch clamp configuration. The patch pipette is moved to the cell using a micromanipulator under optical control. Relative movements between the pipette and the cell have to be avoided in order to keep the cell-pipette connection intact.



In planar patch configuration the cell is positioned by suction - relative movements between cell and aperture can then be excluded after sealing. An Antivibration table is not necessary.



A single cell is then positioned on the hole by suction and a tight connection (Gigaseal) is formed. The planar geometry offers a variety of advantages compared to the classical experiment: - it allows for integration of microfluidics, which enables automatic compound application for ion channel screening. - the system is accessible for optical or scanning probe techniques - perfusion of the intracellular side can be performed.

The Bioelectric Recognition Assay (BERA)

The **Bioelectric Recognition Assay (BERA)** is a novel method for determination of various chemical and biological molecules by measuring changes in the membrane potential of cells immobilized in a gel matrix. Apart from the increased stability of the electrode-cell interface, immobilization preserves the viability and physiological functions of the cells. BERA is primary used in biosensor applications in order to assay analytes which can interact with the immobilized cells by changing the cell membrane potential. In this way, when a positive sample is added to the sensor, a characteristic, 'signature-like' change in electrical potential occurs. BERA has been used for the detection for human viruses (Hepatitis B and C viruses, herpes viruses) and veterinary disease agents (foot and mouth disease virus, prions, blue tongue virus) and plants (tobacco and cucumber viruses) in a highly specific, rapid (1–2 minutes), reproducible and cost-efficient fashion. The method has also been used for the detection of environmental toxins, such as herbicides and the determination of very low concentrations of superoxide anion in clinical samples. A recent advance in the evolution of the BERA technology was the development of a technique called **Molecular Identification through Membrane Engineering (MIME)**. This technique allows for building cells with absolutely defined specificity for virtually any molecule of interest, by embedding thousand of artificial receptors into the cell membrane.

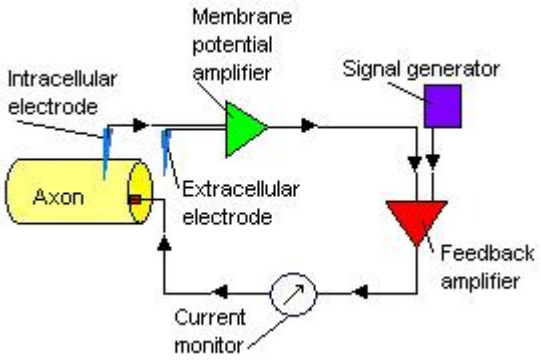
Reporting guidelines for electrophysiology experiments

Minimum Information (MI) standards or reporting guidelines specify the minimum amount of meta data (information) and data required to meet a specific aims or aims. Usually the aim is to provide enough meta data and data to enable the unambiguous reproduction and interpretation of an experiment. MI guidelines are normally informal human readable specifications that inform the development of formal data models (e.g. XML or UML), data exchange formats (e.g. FuGE, MAGE-ML, MAGE-TAB) or knowledge models such as an ontology (e.g. OBI, MGED-Ontology).

The Minimum Information about a Neuroscience investigation (MINI) family of reporting guideline documents, produced by community consultation and continually available for public comment aims to provide a consistent set of guidelines in order to report an electrophysiology experiment. A MINI module represents the minimum information that should be reported about a dataset to facilitate computational access and analysis to allow a reader to interpret and critically evaluate the processes performed and the conclusions reached, and to support their experimental corroboration. In practice a MINI module comprises a checklist of information that should be provided (for example about the protocols employed) whena data set is described for publication.

Chapter 15

Voltage Clamp



The voltage clamp operates by negative feedback. The membrane potential amplifier measures membrane voltage and sends output to the feedback amplifier; this subtracts the membrane voltage from the command voltage, which it receives from the signal generator. This signal is amplified and output is sent into the axon via the current electrode.

The **voltage clamp** is used by electrophysiologists to measure the ion currents across the membrane of excitable cells, such as neurons, while holding the membrane voltage at a set level. Cell membranes of excitable cells contain many different kinds of ion channels, some of which are voltage gated. The voltage clamp allows the membrane voltage to be manipulated independently of the ionic currents, allowing the current-voltage relationships of membrane channels to be studied.

The concept of the voltage clamp is due to Kenneth Cole and George Marmount in the 1940s. Cole discovered that it was possible to use two electrodes and a feedback circuit to keep the cell's membrane potential at a level set by the experimenter.

Alan Hodgkin realized that, to understand ion flux across the membrane, it was necessary to eliminate differences in membrane potential. After experiments with the voltage clamp, Hodgkin and Andrew Huxley outlined the ionic causes of the action potential in 1952, for which they shared the 1963 Nobel Prize in Physiology or Medicine.

Technique

The voltage clamp is a current generator with two electrodes. Transmembrane voltage is recorded through a "voltage electrode", relative to ground, and a "current electrode" passes current into the cell. The experimenter sets a "holding voltage", or "command potential", and the voltage clamp uses negative feedback to maintain the cell at this voltage. The electrodes are connected to an amplifier, which measures membrane potential and feeds the signal into a feedback amplifier. This amplifier also gets an input from the signal generator that determines the command potential, and it subtracts the membrane potential from the command potential ($V_{command} - V_m$), magnifies any difference, and sends an output to the current electrode. Whenever the cell deviates from the holding voltage, the operational amplifier generates an "error signal", that is the difference between the command potential and the actual voltage of the cell. The feedback circuit passes current into the cell to reduce the error signal to zero. Thus, the clamp circuit produces a current equal and opposite to the ionic current. This can be measured, giving an accurate reproduction of the currents flowing across the membrane.

Cole developed the voltage clamp technique before the era of microelectrodes, so his two electrodes consisted of fine wires twisted around an insulating rod. Because this type of electrode could be inserted into only the largest cells, early electrophysiological experiments were conducted almost exclusively on squid axons. Squid squirt jets of water when they need to move quickly, as when escaping a predator. To make this escape as fast as possible, they have an axon that can reach 1 mm in diameter (signals propagate more quickly down large axons). The squid giant axon was the first preparation that could be used to voltage clamp a transmembrane current, and it was the basis of Hodgkin and Huxley's pioneering experiments on the properties of the action potential.

Variations of the voltage clamp technique

A more detailed discussion of the below techniques can be found in the Axon Guide. The book is now out of print but can be downloaded in PDF form from Axon Instruments.

Two-electrode voltage clamp using microelectrodes

This works on the same principle, but the two electrodes are glass pipettes with very fine tips (smaller than 1 micrometer), allowing for clamping of cells smaller than the squid axon. However, microelectrodes are much poorer conductors than the wires used by Cole, and sometimes cannot pass current rapidly enough to compensate for cellular current. The faster the kinetics of the current (onset and offset), the more likely it is that the clamp will be unable to "follow" it faithfully. Another disadvantage involves "space clamp" issues. Cole's voltage clamp used a long wire that clamped the squid axon uniformly along its entire length. Microelectrodes can provide only a spatial point source of current that might not uniformly affect different parts of an irregularly shaped cell.

Single-electrode voltage clamp

In this, an electrode is placed inside a cell, and serves both the voltage-recording and current-passing duties.

Continuous single-electrode clamp (SEV-c)

The "patch-clamp" technique allows the study of individual ion channels. It uses an electrode with a relatively large tip (> 1 micrometer) which has a smooth surface (rather than a sharp tip). This is a "patch-clamp electrode" (as distinct from a "sharp electrode" used to impale cells). This electrode is pressed against a cell membrane and suction is applied to pull the cell's membrane inside the electrode tip. The suction causes the cell to form a tight seal with the electrode (a "gigaohm seal", as the resistance is more than a gigaohm).

SEV-c has the advantage is that you can record from small cells that would be impossible to impale with two electrodes. However:

1) Microelectrodes are imperfect conductors; they generally have a resistance of more than a million ohms. They rectify (i.e. change their resistance with voltage, often in an irregular manner), they sometimes have unstable resistance if clogged by cell contents. Thus they will not faithfully record the voltage of the cell, especially when it is changing quickly, nor will they faithfully pass current.

2) Voltage and current errors: SEV-c circuitry does not actually measure the voltage of the cell being clamped (as does a two-electrode clamp). The patch-clamp amplifier is like a two-electrode clamp, except the voltage measuring and current passing circuits are connected (in the two-electrode clamp, they are connected *through the cell*). The electrode is attached to a wire that contacts the current/voltage loop inside the amplifier. Thus the electrode has only an indirect influence on the feedback circuit. The amplifier reads only the voltage at the top of the electrode, and feeds back current to compensate. But, if the electrode is an imperfect conductor, the clamp circuity has only a distorted view of the membrane potential. Similarly, when the circuit passes back current to compensate for that (distorted) voltage, the current will be distorted by the electrode before it reaches the cell. To compensate for this, the electrophysiologist uses the lowest resistance electrode possible, makes sure that the electrode characteristics don't change during an experiment (so the errors will be constant), and avoids recording currents with kinetics likely to be too fast for the clamp to follow accurately. The accuracy of SEV-c goes up the slower and smaller are the voltage changes it is trying to clamp.

3) Series resistance errors: The currents passed to the cell must go to ground to complete the circuit. The voltages are recorded by the amplifier relative to ground. When a cell is clamped at its natural resting potential, there is no problem; the clamp is not passing current and the voltage is being generated only by the cell. But when clamping at a different potential, series resistance errors become a concern; the cell will pass current across its membrane in an attempt to return to its natural resting potential. The clamp

amplifier opposes this by passing current to maintain the holding potential. A problem arises because the electrode is between the amplifier and the cell, i.e. the electrode is *in series* with the resistor that is the cell's membrane. Thus, when passing current through the electrode and the cell, Ohm's Law tells us that this will cause a voltage to form across both the cell's and the electrode's resistance. As these resistors are in series, the voltage drops will add. If the electrode and the cell membrane have equal resistances (which they usually do not), and if the experimenter command a 40mV change from the resting potential, the amplifier will pass enough current until it reads that it has achieved that 40mV change. However, in this example, half of that voltage drop is across the electrode. The experimenter thinks he or she has moved the cell voltage by 40mV, but has moved it only by 20mV. The difference is the "series resistance error". Modern patch-clamp amplifiers have circuity to compensate for this error, but these compensate only 70-80% of it. The electrophysiologist can further reduce the error by recording at or near the cell's natural resting potential, and by using as low a resistance electrode as possible.

4) Capacitance errors. Microelectrodes are capacitors, and are particularly troublesome because they are non-linear. The capacitance arises because the electrolyte inside the electrode is separated by an insulator (glass) from the solution outside. This is, by definition and function, a capacitor. Worse, as the thickness of the glass changes the farther you get from the tip, the time constant of the capacitor will vary. This produces a distorted record of membrane voltage or current whenever they are changing. Amplifiers can compensate for this, but not entirely because the capacitance has many time-constants. The experimenter can reduce the problem by keeping the cell's bathing solution shallow (exposing less glass surface to liquid) and by coating the electrode with silicone, resin, paint, or another substance that will increase the distance between the inside and outside solutions.

5) Space clamp errors. A single electrode is a point source of current. In distant parts of the cell, the current passed through the electrode will be less influential than at nearby parts of the cell. This is particularly a problem when recording from neurons with elaborate dendritic structures. There is nothing one can do about space clamp errors except to temper the conclusions of the experiment.

Discontinuous single-electrode voltage-clamp

A single-electrode voltage clamp — discontinuous, or SEVC-d, has some advantages over SEVC-c for whole-cell recording. In this, a different approach is taken for passing current and recording voltage. A SEVC-d amplifier operates on a "time-sharing" basis, so the electrode regularly and frequently switches between passing current and measuring voltage. Effectively, there are two electrodes but each is only in operation for half of the time it is on. The oscillation between the two functions of the single electrode is termed a duty cycle. During each cycle, the amplifier measures the membrane potential and compares it with the holding potential. An operational amplifier measures the difference, and generates an error signal. This current is a mirror image of the current generated by the cell. The amplifier outputs feature sample and hold circuits, so each briefly sampled voltage is then held on the output until the next measurement in the next cycle.

Specifically, the amplifier measures voltage in the first few milliseconds of the cycle, generates the error signal, and spends the rest of the cycle passing current to reduce that error. At the start of the next cycle, voltage is measured again, a new error signal generated, current passed etc. The experimenter sets the cycle length, and it is possible to sample every 333 to 500 microseconds.

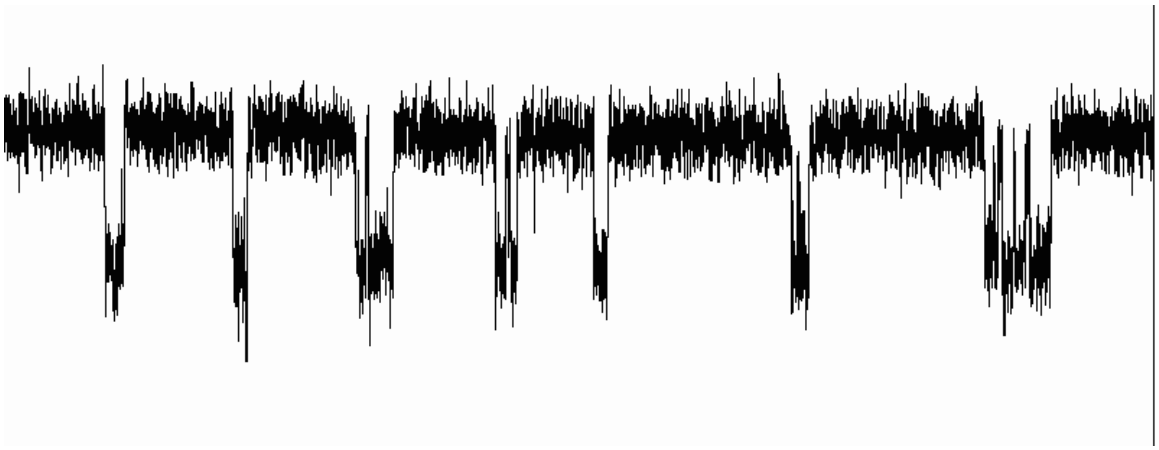
For this to work, the cell capacitance must be higher than the electrode capacitance by at least an order of magnitude. Capacitance slows the kinetics (the rise and fall times) of currents. If the electrode capacitance is much less than that of the cell, then when current is passed through the electrode, the electrode voltage will change faster than the cell voltage. Thus when you inject current and then turn it off (at the end of a duty cycle), the electrode voltage will decay faster than the cell voltage. As soon as the electrode voltage asymptotes to the cell voltage, the voltage can be sampled (again) and the next bolus of current applied. Thus the frequency of the duty cycle is limited to the speed at which the electrode voltage rises and decays while passing current. The lower the electrode capacitance, the faster one can cycle.

SEVC-d has a major advantage over SEVC-c in allowing the experimenter to measure membrane potential, and as it obviates passing current and measuring voltage at the same time, there is never a series resistance error. The main disadvantages are that the time resolution is limited, and the amplifier is unstable. If it passes too much current, so that the goal voltage is over-shot, it reverses the polarity of the current in the next duty cycle. This causes it to undershoot the target voltage, so the next cycle reverses the polarity of the injected current again. This error can grow with each cycle until the amplifier oscillates out of control (“ringing”); this usually results in the destruction of the cell being recorded. The investigator wants a short duty cycle to improve temporal resolution; the amplifier has adjustable compensators that will make the electrode voltage decay faster, but if these are set too high the amplifier will ring, so the investigator is always trying to “tune” the amplifier as close to the edge of uncontrolled oscillation as possible, in which case small changes in recording conditions can cause ringing. There are two solutions: to “back off” the amplifier settings into a safe range, or to be alert for signs that the amplifier is about to ring.

Chapter 16

Patch Clamp

Classical patch clamp setup, with microscope, antivibration table and micro manipulators



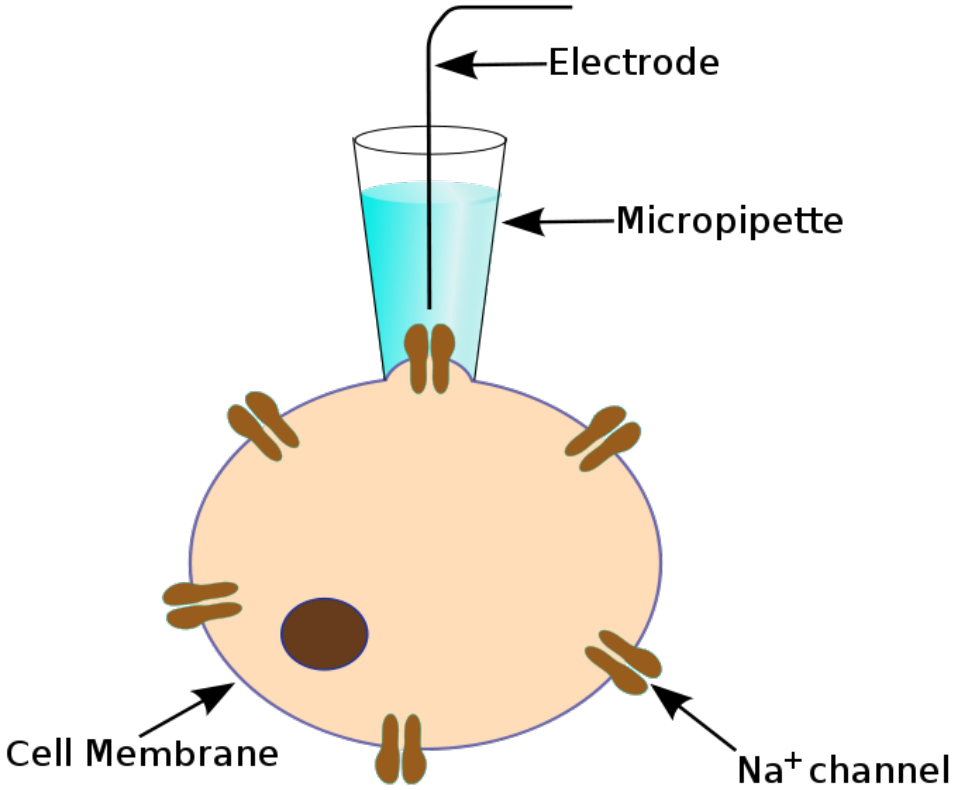
A patch clamp recording reveals transitions between two conductance states of a single ion channel: closed (at top) and open (at bottom).

The **patch clamp technique** is a laboratory technique in electrophysiology that allows the study of single or multiple ion channels in cells. The technique can be applied to a wide variety of cells, but is especially useful in the study of excitable cells such as neurons, cardiomyocytes, muscle fibers and pancreatic beta cells. It can also be applied to the study of bacterial ion channels in specially prepared giant spheroplasts.

The patch clamp technique is a refinement of the voltage clamp. Erwin Neher and Bert Sakmann developed the patch clamp in the late 1970s and early 1980s. This discovery

made it possible to record the currents of single ion channels for the first time, proving their involvement in fundamental cell processes such as action potential conduction. Neher and Sakmann received the Nobel Prize in Physiology or Medicine in 1991 for this work.

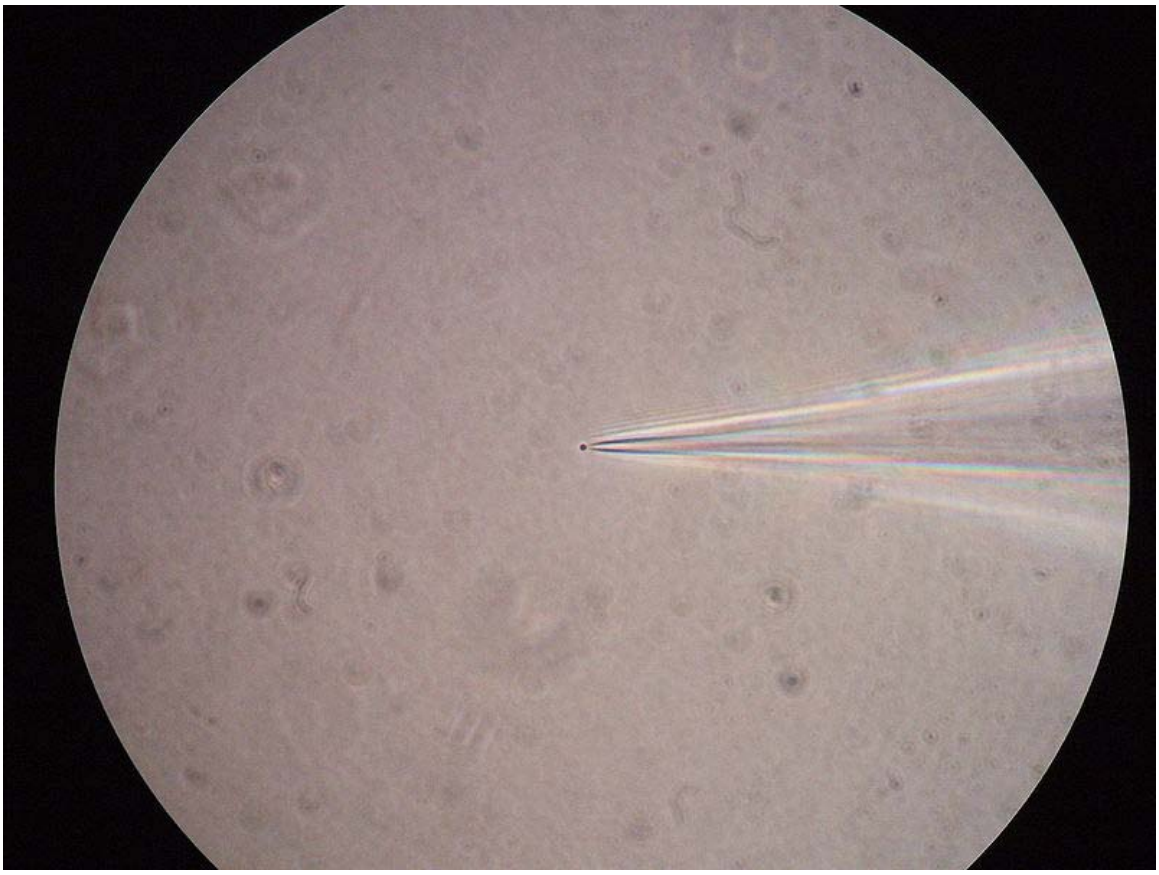
Basic technique



The cell-attached patch clamp uses a micropipette attached to the cell membrane to allow recording from a single ion channel.

Patch clamp recording uses, as an electrode, a glass micropipette that has an open tip diameter of about one micrometre, a size enclosing a membrane surface area or "patch" that often contains just one or a few ion channel molecules. This type of electrode is distinct from the "sharp microelectrode" used to impale cells in traditional intracellular recordings, in that it is sealed onto the surface of the cell membrane, rather than inserted through it. In some experiments, the micropipette tip is heated in a microforge to produce a smooth surface that assists in forming a high resistance seal with the cell membrane. The interior of the pipette is filled with a solution matching the ionic composition of the bath solution, as in the case of cell-attached recording, or the cytoplasm for whole-cell recording. A chlorided silver wire is placed in contact with this solution and conducts electric current to the amplifier. The investigator can change the composition of this solution or add drugs to study the ion channels under different conditions. The micropipette is pressed against a cell membrane and suction is applied to assist in the formation of a high resistance seal between the glass and the cell membrane (a "gigohm

seal" or "gigaseal," since the electrical resistance of that seal is in excess of a gigohm). The high resistance of this seal makes it possible to electronically isolate the currents measured across the membrane patch with little competing noise, as well as providing some mechanical stability to the recording.



A bacterial spheroplast patched with a glass pipette

Unlike traditional two-electrode voltage clamp recordings, patch clamp recording uses a single electrode to record currents. Many patch clamp amplifiers do not use true voltage clamp circuitry but instead are differential amplifiers that use the bath electrode to set the zero current level. This allows a researcher to keep the voltage constant while observing changes in current. Alternatively, the cell can be current clamped in whole-cell mode, keeping current constant while observing changes in membrane voltage.

Variations

Several variations of the basic technique can be applied, depending on what the researcher wants to study. The inside-out and outside-out techniques are called "excised patch" techniques, because the patch is excised (removed) from the main body of the cell. Cell-attached and both excised patch techniques are used to study the behavior of individual ion channels in the section of membrane attached to the electrode. Whole-cell

patch and perforated patch allow the researcher to study the electrical behavior of the entire cell.

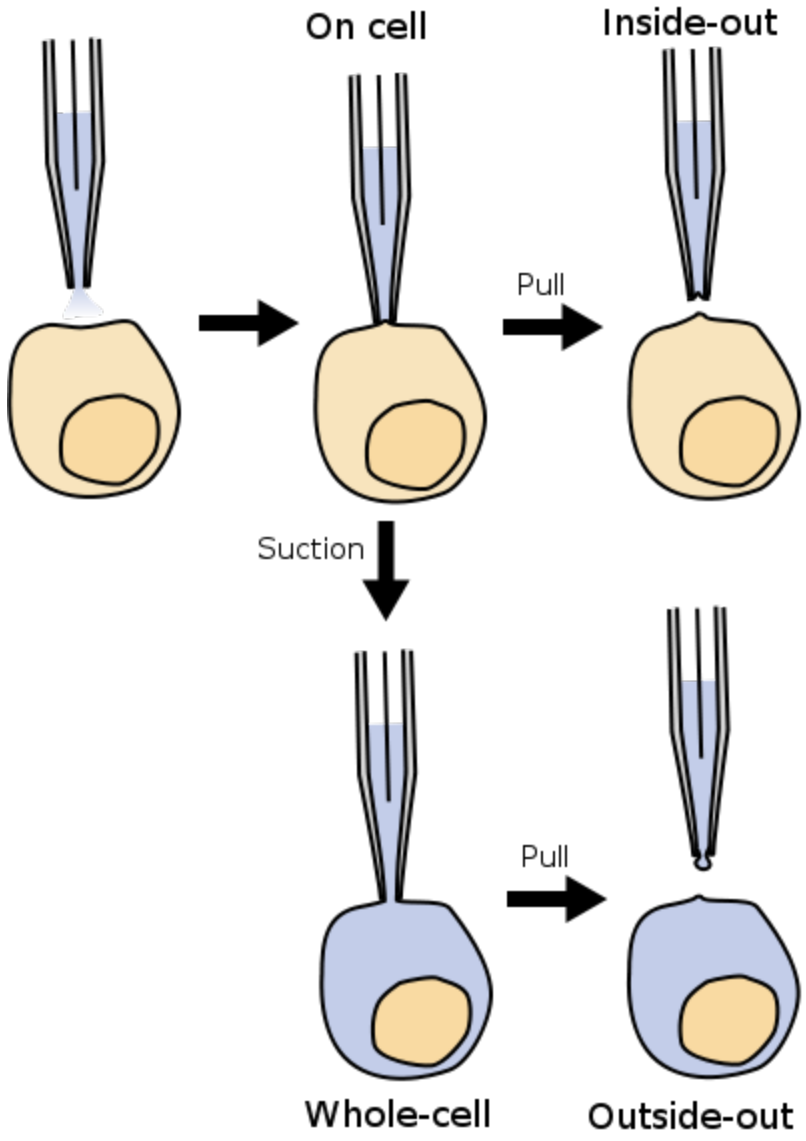


Diagram showing variations of the patch clamp technique

Cell-attached or on-cell patch

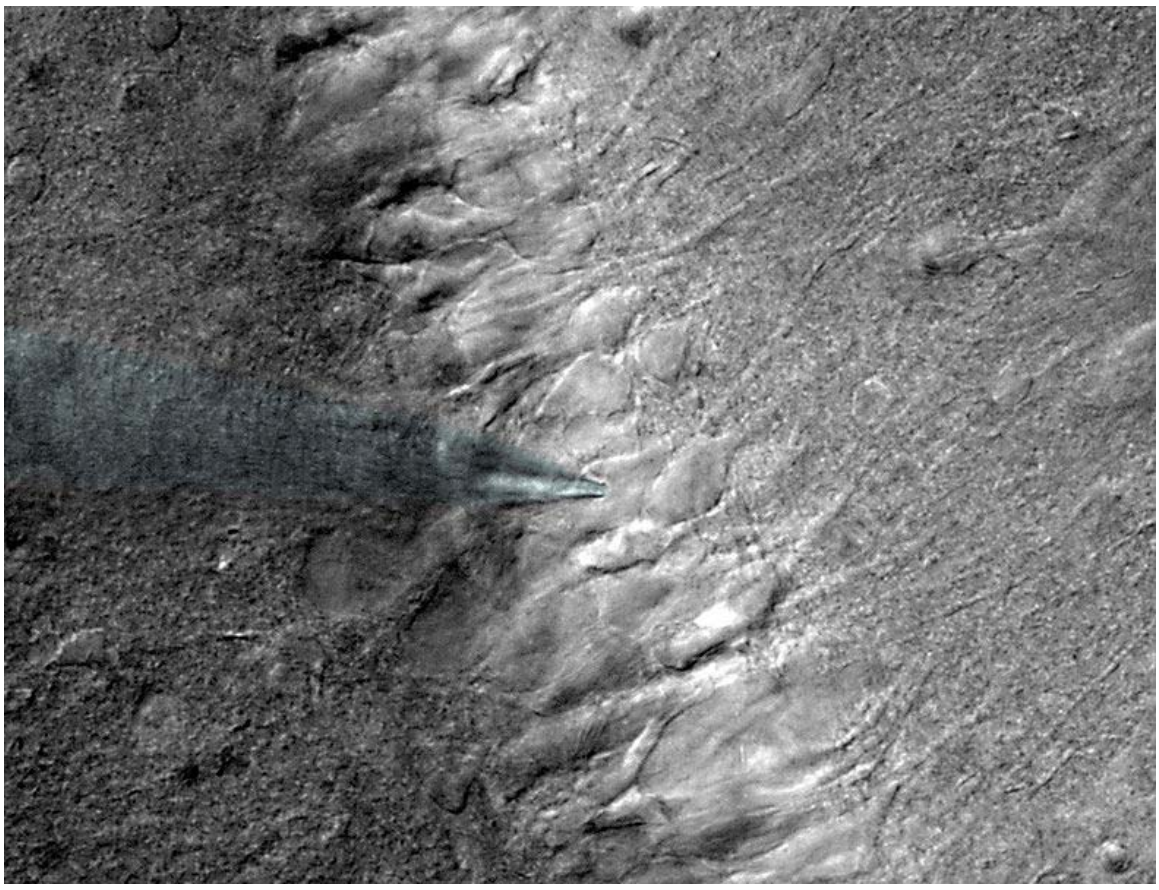
The electrode is sealed to the patch of membrane, and the cell remains intact. This allows for the recording of currents through single ion channels in that patch of membrane, without disrupting the interior of the cell. For ligand-gated ion channels or channels that are modulated by metabotropic receptors, the neurotransmitter or drug being studied is usually included in the pipette solution, where it can contact what had been the external surface of the membrane. While the resulting channel activity can be attributed to the drug being used, it is usually not possible to then change the drug concentration. The technique is thus limited to one point in a dose response curve per patch. Usually, the

dose response is accomplished using several cells and patches. However, voltage-gated ion channels can be clamped at different membrane potentials using the same patch. This results in graded channel activation, and a complete I-V (current-voltage) curve can be established with only one patch.

Inside-out patch

After the gigaseal is formed, the micropipette is quickly withdrawn from the cell, thus ripping the patch of membrane off the cell, leaving the patch of membrane attached to the micropipette, and exposing the intracellular surface of the membrane to the external media. This is useful when an experimenter wishes to manipulate the environment at the intracellular surface of ion channels. For example, channels that are activated by intracellular ligands can then be studied through a range of ligand concentrations.

Whole-cell recording or whole-cell patch



Whole cell recording of a nerve cell from the hippocampus. The pipette in the photograph has been marked with a slight blue colour.

Whole-cell recordings, in contrast, involve recording currents through multiple channels at once, over the membrane of the entire cell. The electrode is left in place on the cell, but more suction is applied to rupture the membrane patch, thus providing access to the

intracellular space of the cell. The advantage of whole-cell patch clamp recording over sharp microelectrode recording is that the larger opening at the tip of the patch clamp electrode provides lower resistance and thus better electrical access to the inside of the cell. A disadvantage of this technique is that the volume of the electrode is larger than the cell, so the soluble contents of the cell's interior will slowly be replaced by the contents of the electrode. This is referred to as the electrode "dialyzing" the cell's contents. Thus, any properties of the cell that depend on soluble intracellular contents will be altered. The pipette solution used usually approximates the high-potassium environment of the interior of the cell. Generally speaking, there is a period at the beginning of a whole-cell recording, lasting approximately 10 minutes, when one can take measurements before the cell has been dialyzed.

Outside-out patch

After the whole-cell patch is formed, the electrode can be slowly withdrawn from the cell, allowing a bulb of membrane to bleb out from the cell. When the electrode is pulled far enough away, this bleb will detach from the cell and reform as a convex membrane on the end of the electrode (like a ball open at the electrode tip), with the original outside of the membrane facing outward from the electrode. Single channel recordings are possible in this conformation if the bleb of membrane is small enough. Outside-out patching gives the experimenter the opportunity to examine the properties of an ion channel when it is isolated from the cell, and exposed to different solutions on the extracellular surface of the membrane. The experimenter can perfuse the same patch with different solutions, and if the channel is activated from the extracellular face, a dose-response curve can then be obtained. This is the distinct advantage of the outside-out patch relative to the cell-attached method. However, it is more difficult to accomplish, as more steps are involved in the patching process.

Perforated patch

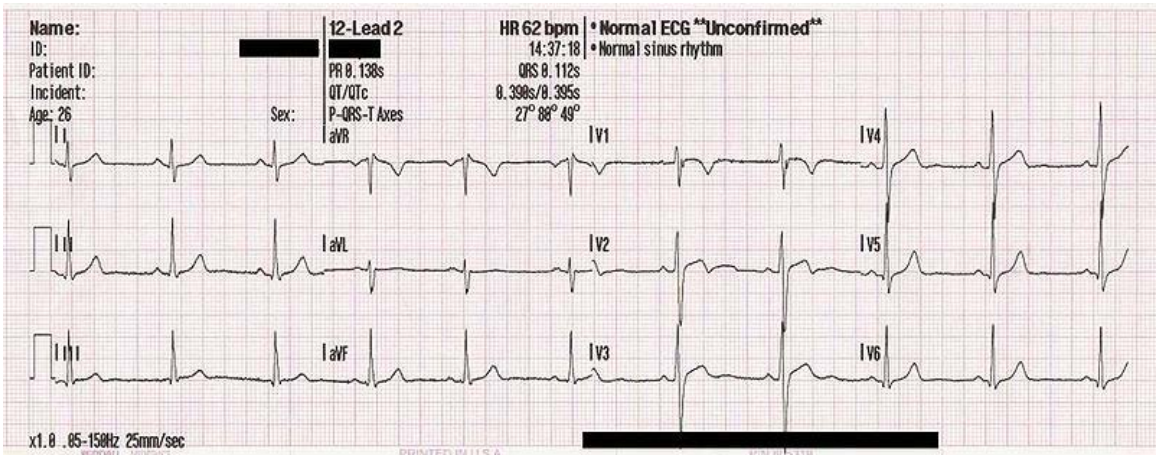
In this variation of whole-cell recording, the experimenter forms the gigohm seal, but does not use suction to rupture the patch membrane. Instead, the electrode solution contains small amounts of an antibiotic, such as amphothericin-B or gramicidin. As the antibiotic molecules diffuse into the membrane patch, they form small perforations in the membrane, providing electrical access to the cell interior. This has the advantage of reducing the dialysis of the cell that occurs in whole-cell recordings, but also has several disadvantages. First, the access resistance is higher, relative to whole-cell, due to the partial membrane occupying the tip of the electrode (access resistance being the sum of the electrode resistance and the resistance at the electrode-cell junction). This will decrease electrical access and thus decrease current resolution, increase recording noise, and magnify any series resistance error. Second, it can take a significant amount of time for the antibiotic to perforate the membrane (10–30 minutes, though this can be reduced with properly shaped electrodes). Third, the membrane under the electrode tip is weakened by the perforations formed by the antibiotic and can rupture. If the patch ruptures, the recording is then in whole-cell mode, with antibiotic contaminating the inside of the cell.

Loose patch

Loose patch clamp is different in that it employs a loose seal rather than the tight gigaseal used in the conventional technique. A significant advantage of the loose seal is that the pipette that is used can be repeatedly removed from the membrane after recording, and the membrane will remain intact. This allows for repeated measurements in a variety of locations on the same cell without destroying the integrity of the membrane. A major disadvantage is that the resistance between the pipette and the membrane is greatly reduced, allowing current to leak through the seal. This leakage can be corrected for, however, which offers the opportunity to compare and contrast recordings made from different areas on the cell of interest.

Chapter 17

Electrocardiography



12 Lead ECG of a 26-year-old male

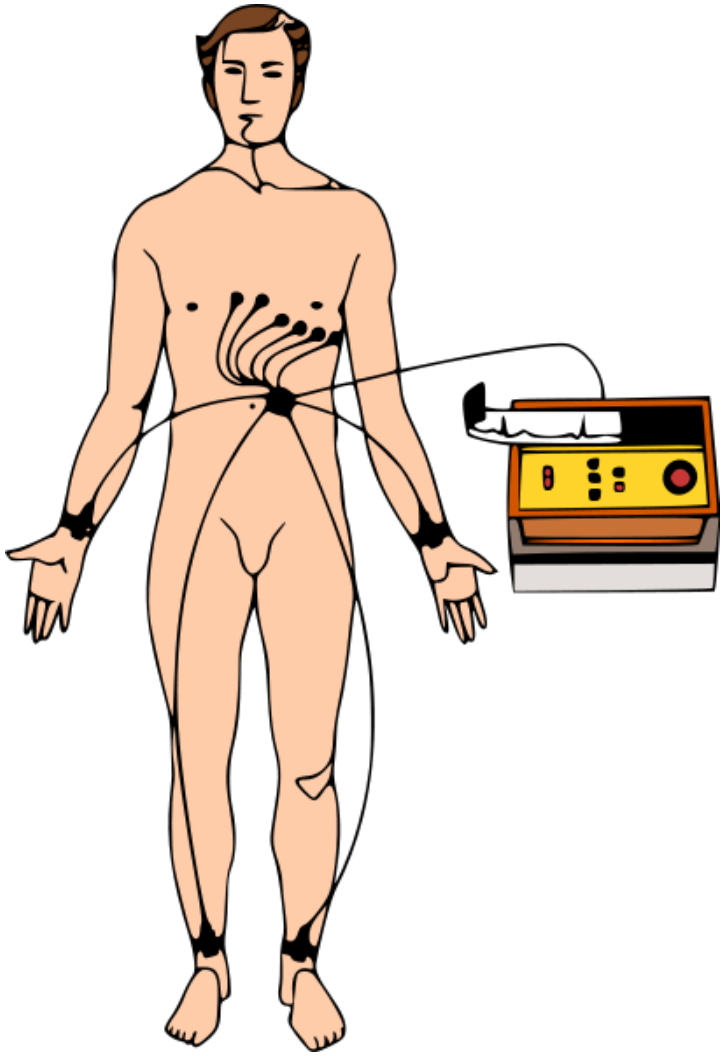


Image showing a patient connected to the 10 electrodes necessary for a 12-lead ECG

Electrocardiograph (*ECG,* or **EKG** [from the German *Elektrokardiogramm*]) is a transthoracic interpretation of the electrical activity of the heart over time captured and externally recorded by skin electrodes. It is a noninvasive recording produced by an electrocardiographic device. The etymology of the word is derived from the Greek *electro*, because it is related to electrical activity, *cardio*, Greek for heart, and *graph*, a Greek root meaning "to write". In English speaking countries, medical professionals often write EKG (the abbreviation for the German word elektrokardiogramm) in order to avoid confusion with EEG.

The ECG works mostly by detecting and amplifying the tiny electrical changes on the skin that are caused when the heart muscle "depolarizes" during each heart beat. At rest, each heart muscle cell has a charge across its outer wall, or cell membrane. Reducing this charge towards zero is called de-polarization, which activates the mechanisms in the cell that cause it to contract. During each heartbeat a healthy heart will have an orderly progression of a wave of depolarisation that is triggered by the cells in the sinoatrial

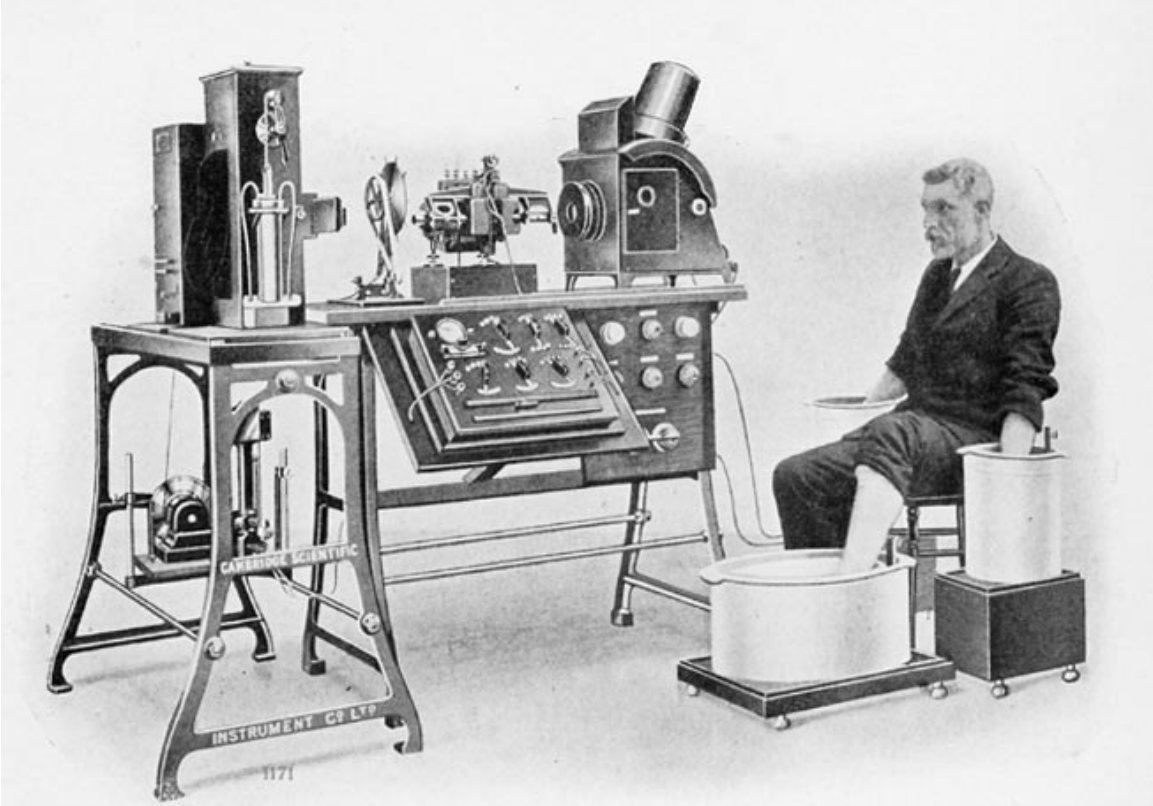
node, spreads out through the atrium, passes through "intrinsic conduction pathways" and then spreads all over the ventricles. This is detected as tiny rises and falls in the voltage between two electrodes placed either side of the heart which is displayed as a wavy line either on a screen or on paper. This display indicates the overall rhythm of the heart and weaknesses in different parts of the heart muscle.

Usually more than 2 electrodes are used and they can be combined into a number of pairs (For example: Left arm (LA), right arm (RA) and left leg (LL) electrodes form the pairs: LA+RA, LA+LL, RA+LL). The output from each pair is known as a **lead**. Each lead is said to look at the heart from a different angle. Different types of ECGs can be referred to by the number of leads that are recorded, for example 3-lead, 5-lead or 12-lead ECGs (sometimes simply "a 12-lead"). A 12-lead ECG is one in which 12 different electrical signals are recorded at approximately the same time and will often be used as a one-off recording of an ECG, typically printed out as a paper copy. 3- and 5-lead ECGs tend to be monitored continuously and viewed only on the screen of an appropriate monitoring device, for example during an operation or whilst being transported in an ambulance. There may, or may not be any permanent record of a 3- or 5-lead ECG depending on the equipment used.

It is the best way to measure and diagnose abnormal rhythms of the heart, particularly abnormal rhythms caused by damage to the conductive tissue that carries electrical signals, or abnormal rhythms caused by electrolyte imbalances. In a myocardial infarction (MI), the ECG can identify if the heart muscle has been damaged in specific areas, though not all areas of the heart are covered. The ECG cannot reliably measure the pumping ability of the heart, for which ultrasound-based (echocardiography) or nuclear medicine tests are used. It is possible to be in cardiac arrest with a normal ECG signal (a condition known as pulseless electrical activity).

History

Alexander Muirhead is reported to have attached wires to a feverish patient's wrist to obtain a record of the patient's heartbeat while studying for his Doctor of Science (in electricity) in 1872 at St Bartholomew's Hospital. This activity was directly recorded and visualized using a Lippmann capillary electrometer by the British physiologist John Burdon Sanderson. The first to systematically approach the heart from an electrical point-of-view was Augustus Waller, working in St Mary's Hospital in Paddington, London. His electrocardiograph machine consisted of a Lippmann capillary electrometer fixed to a projector. The trace from the heartbeat was projected onto a photographic plate which was itself fixed to a toy train. This allowed a heartbeat to be recorded in real time. In 1911 he still saw little clinical application for his work.



PHOTOGRAPH OF A COMPLETE ELECTROCARDIOGRAPH, SHOWING THE MANNER IN WHICH THE ELECTRODES ARE ATTACHED TO THE PATIENT, IN THIS CASE THE HANDS AND ONE FOOT BEING IMMERSED IN JARS OF SALT SOLUTION

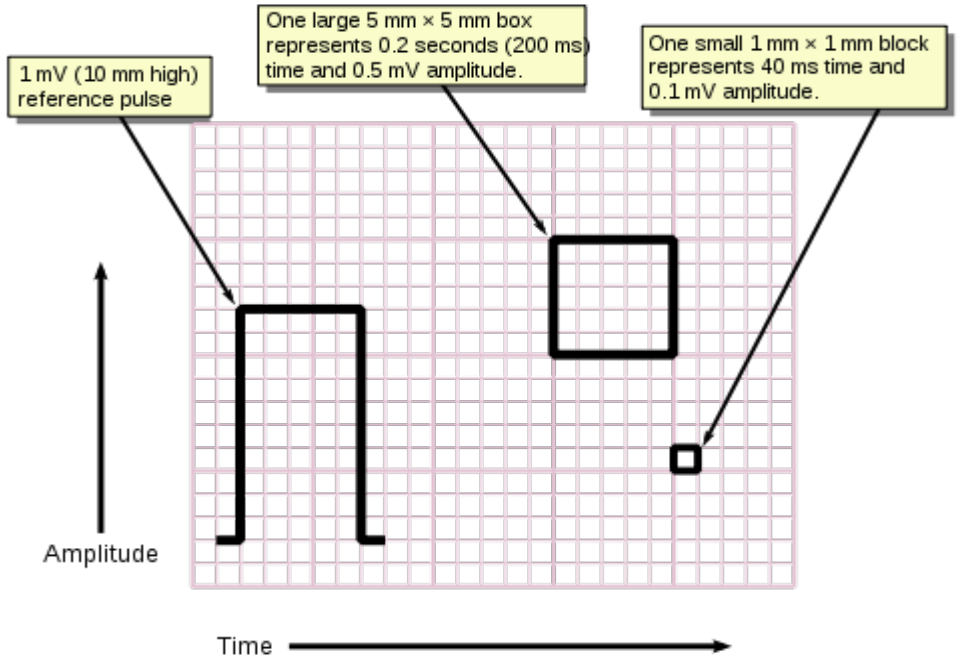
Einthoven's ECG device

An initial breakthrough came when Willem Einthoven, working in Leiden, Netherlands, used the string galvanometer that he invented in 1903. This device was much more sensitive than both the capillary electrometer that Waller used and the string galvanometer that had been invented separately in 1897 by the French engineer Clément Ader. Rather than using today's self-adhesive electrodes Einthoven's subjects would immerse each of their limbs into containers of salt solutions from which the ECG was recorded.

Einthoven assigned the letters P, Q, R, S and T to the various deflections, and described the electrocardiographic features of a number of cardiovascular disorders. In 1924, he was awarded the Nobel Prize in Medicine for his discovery.

Though the basic principles of that era are still in use today, there have been many advances in electrocardiography over the years. The instrumentation, for example, has evolved from a cumbersome laboratory apparatus to compact electronic systems that often include computerized interpretation of the electrocardiogram.

ECG graph paper



One second of ECG graph paper

The output of an ECG recorder is a graph (or sometimes several graphs, representing each of the leads) with time represented on the x-axis and voltage represented on the y-axis. A dedicated ECG machine would usually print onto graph paper which has a background pattern of 1mm squares (often in red or green), with bold divisions every 5mm in both vertical and horizontal directions. It is possible to change the output of most ECG devices but it is standard to represent each mV on the y axis as 1 cm and each second as 25mm on the x-axis (that is a paper speed of 25mm/s). Faster paper speeds can be used - for example to resolve finer detail in the ECG. At a paper speed of 25 mm/s, one small block of ECG paper translates into 40 ms. Five small blocks make up one large block, which translates into 200 ms. Hence, there are five large blocks per second. A calibration signal may be included with a record. A standard signal of 1 mV must move the stylus vertically 1 cm, that is two large squares on ECG paper.

Layout

By definition a 12-lead ECG will show a short segment of the recording of each of the 12-leads. This is often arranged in a grid of 4 columns by three rows, the first columns being the limb leads (I,II and III), the second column the augmented limb leads (aVR, aVL and aVF) and the last two columns being the chest leads (V1-V6). It is usually possible to change this layout so it is vital to check the labels to see which lead is represented. Each column will usually record the same moment in time for the three leads and then the recording will switch to the next column which will record the heart beats after that point. It is possible for the heart rhythm to change between the columns of leads.

Each of these segments is short, perhaps 1-3 heart beats only, depending on the heart rate and it can be difficult to analyse any heart rhythm that shows changes between heart beats. To help with the analysis it is common to print one or two "rhythm strips" as well. This will usually be lead II (which shows the electrical signal from the atrium, the P-wave, well) and shows the rhythm for the whole time the ECG was recorded (usually 5–6 seconds). Some ECG machines will print a second lead II along the very bottom of the paper in addition to the output described above. This printing of Lead II is continuous from start to finish of the process.

The term "rhythm strip" may also refer to the whole printout from a continuous monitoring system which may show only one lead and is either initiated by a clinician or in response to an alarm or event.

Leads

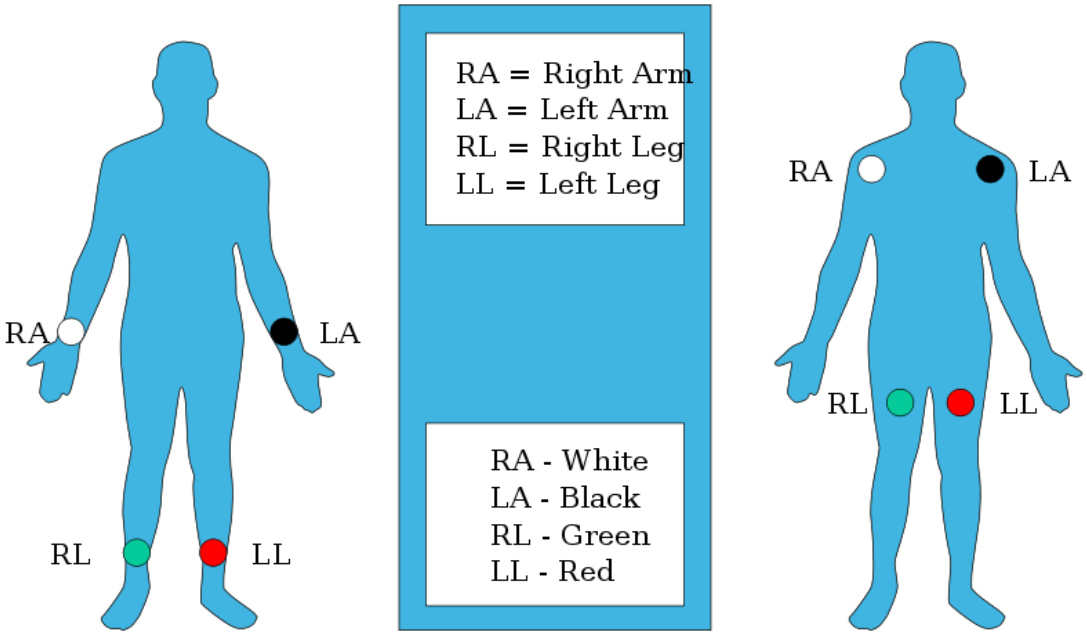
The term "lead" in electrocardiography causes much confusion because it is used to refer to two *different* things. In accordance with common parlance the word lead may be used to refer to the electrical cable attaching the electrodes to the ECG recorder. As such it may be acceptable to refer to the "left arm lead" as the electrode (and its cable) that should be attached at or near the left arm. There are usually ten of these electrodes in a standard "12-lead" ECG.

Alternatively (and some would say properly, in the context of electrocardiography) the word *lead* may refer to the tracing of the voltage difference between two of the electrodes and is what is actually produced by the ECG recorder. Each will have a specific name. For example "Lead I" (lead one) is the voltage between the right arm electrode and the left arm electrode, whereas "Lead II" (lead two) is the voltage between the right limb and the feet. (This rapidly becomes more complex as one of the "electrodes" may in fact be a composite of the electrical signal from a combination of the other electrodes. Twelve of this type of lead form a "12-lead" ECG

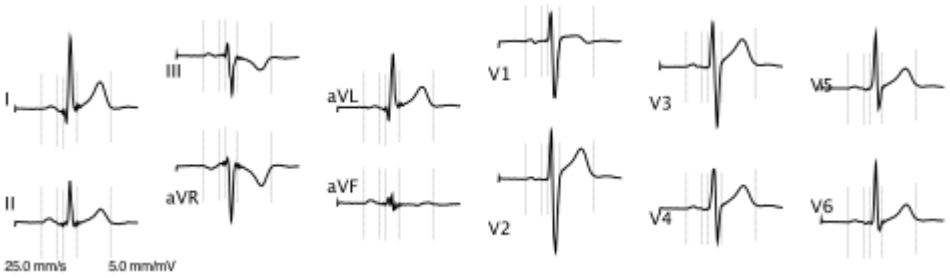
To cause additional confusion the term "limb leads" usually refers to the tracings from leads I, II and III rather than the electrodes attached to the limbs.

Placement of electrodes

Ten electrodes are used for a 12-lead ECG. The electrodes usually consist of a conducting gel, embedded in the middle of a self-adhesive pad onto which cables clip. Sometimes the gel also forms the adhesive. They are labeled and placed on the patient's body as follows:



Proper placement of the limb electrodes, color coded as recommended by the American Heart Association (a different colour scheme is used in Europe). Note that the limb electrodes can be far down on the limbs or close to the hips/shoulders, but they must be even (left vs right).



12 leads

Electrode label (in the USA)	Electrode placement
RA	On the right arm, avoiding thick muscle.
LA	In the same location that RA was placed, but on the left arm this time.
RL	On the right leg, lateral calf muscle
LL	In the same location that RL was placed, but on the left leg this time.

V_1	In the *fourth* intercostal space (between ribs 4 & 5) just to the *right* of the sternum (breastbone).
V_2	In the *fourth* intercostal space (between ribs 4 & 5) just to the *left* of the sternum.
V_3	Between leads V_2 and V_4.
V_4	In the fifth intercostal space (between ribs 5 & 6) in the mid-clavicular line (the imaginary line that extends down from the midpoint of the clavicle (collarbone)).
V_5	Horizontally even with V_4, but in the anterior axillary line. (The anterior axillary line is the imaginary line that runs down from the point midway between the middle of the clavicle and the lateral end of the clavicle; the lateral end of the collarbone is the end closer to the arm.)
V_6	Horizontally even with V_4 and V_5 in the midaxillary line. (The midaxillary line is the imaginary line that extends down from the middle of the patient's armpit.)

Additional electrodes

The classical 12-lead ECG can be extended in a number of ways in an attempt to improve its sensitivity in detecting myocardial infarction involving territories not normally "seen" well. This includes an rV_4 lead which uses the equivalent landmarks to the V_4 but on the *right* side of the chest wall and extending the chest leads onto the back with a V_7, V_8 and V_9.

Limb leads

In both the 5- and 12-lead configuration, leads I, II and III are called *limb leads*. The electrodes that form these signals are located on the limbs—one on each arm and one on the left leg. The limb leads form the points of what is known as *Einthoven's triangle*.

- Lead I is the voltage between the (positive) left arm (LA) electrode and right arm (RA) electrode:

  $I = LA - RA$.

- Lead II is the voltage between the (positive) left leg (LL) electrode and the right arm (RA) electrode:

  $II = LL - RA$.

- Lead III is the voltage between the (positive) left leg (LL) electrode and the left arm (LA) electrode:

  $III = LL - LA$.

Simplified electrocardiograph sensors designed for teaching purposes at e.g. high school level are generally limited to three arm electrodes serving similar purposes.

Unipolar vs. bipolar leads

There are two types of leads: *unipolar* and *bipolar*. Bipolar leads have one positive and one negative pole. In a 12-lead ECG, the limb leads (I, II and III) are bipolar leads. Unipolar leads also have two poles, as a voltage is measured; however, the negative pole is a composite pole (Wilson's central terminal, or WCT) made up of signals from lots of other electrodes. In a 12-lead ECG, all leads besides the limb leads are unipolar (aVR, aVL, aVF, V_1, V_2, V_3, V_4, V_5, and V_6).

Wilson's central terminal V_W is produced by connecting the electrodes, RA; LA; and LL, together, via a simple resistive network, to give an average potential across the body, which approximates the potential at infinity (i.e. zero):

$$V_W = \frac{1}{3}(RA + LA + LL).$$

Augmented limb leads

Leads aVR, aVL, and aVF are *augmented limb leads* (after their inventor Dr. Emanuel Goldberger known collectively as the *Goldberger's leads*). They are derived from the same three electrodes as leads I, II, and III. However, they view the heart from different angles (or vectors) because the negative electrode for these leads is a modification of Wilson's central terminal. This zeroes out the negative electrode and allows the positive electrode to become the "exploring electrode". This is possible because *Einthoven's Law* states that I + (−II) + III = 0. The equation can also be written I + III = II. It is written this way (instead of I − II + III = 0) because Einthoven reversed the polarity of lead II in Einthoven's triangle, possibly because he liked to view upright QRS complexes. Wilson's central terminal paved the way for the development of the augmented limb leads aVR, aVL, aVF and the precordial leads V_1, V_2, V_3, V_4, V_5 and V_6.

- Lead **augmented vector right (aVR)** has the positive electrode (*white*) on the right arm. The negative electrode is a combination of the left arm (black) electrode and the left leg (red) electrode, which "augments" the signal strength of the positive electrode on the right arm:

$$aVR = RA - \frac{1}{2}(LA + LL).$$

- Lead **augmented vector left (aVL)** has the positive (*black*) electrode on the left arm. The negative electrode is a combination of the right arm (white) electrode and the left leg (red) electrode, which "augments" the signal strength of the positive electrode on the left arm:

$$aVL = LA - \frac{1}{2}(RA + LL).$$

- Lead **augmented vector foot (aVF)** has the positive (*red*) electrode on the left leg. The negative electrode is a combination of the right arm (white) electrode and the left arm (black) electrode, which "augments" the signal of the positive electrode on the left leg:

$$aVF = LL - \frac{1}{2}(RA + LA).$$

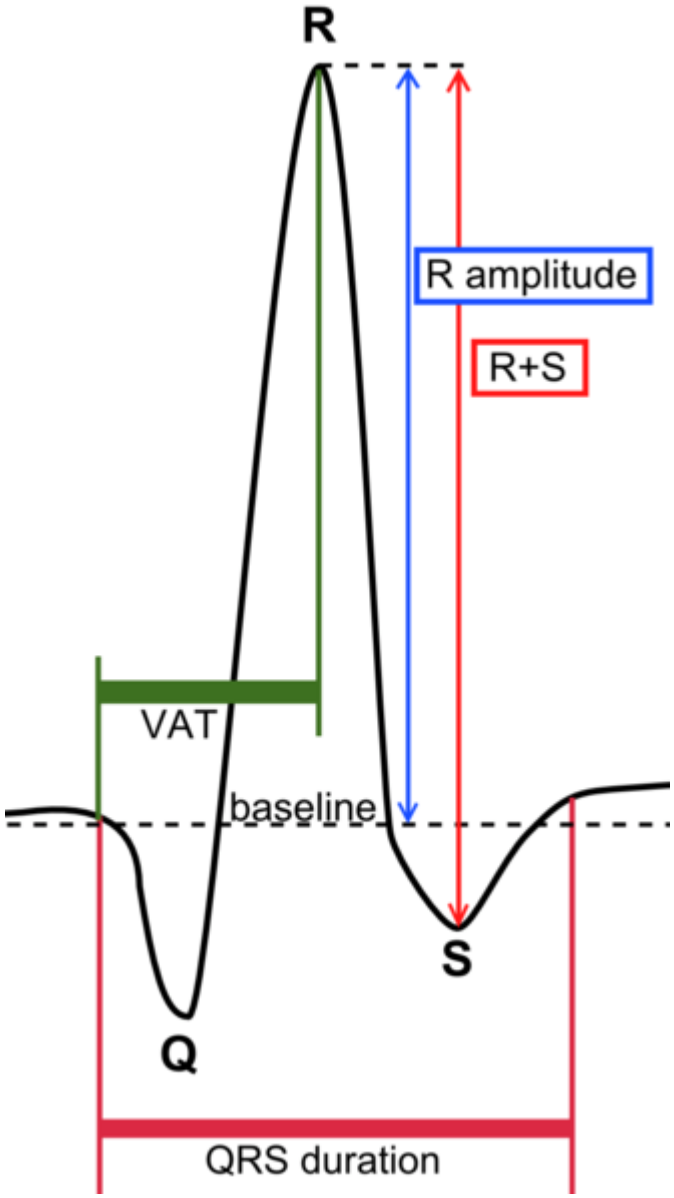
The augmented limb leads aVR, aVL, and aVF are amplified in this way because the signal is too small to be useful when the negative electrode is Wilson's central terminal. Together with leads I, II, and III, augmented limb leads aVR, aVL, and aVF form the basis of the *hexaxial reference system*, which is used to calculate the heart's electrical axis in the *frontal plane*. The aVR, aVL, and aVF leads can also be represented using the I and II limb leads:

$$aVR = -\frac{I + II}{2}$$
$$aVL = I - \frac{II}{2}$$
$$aVF = II - \frac{I}{2}$$

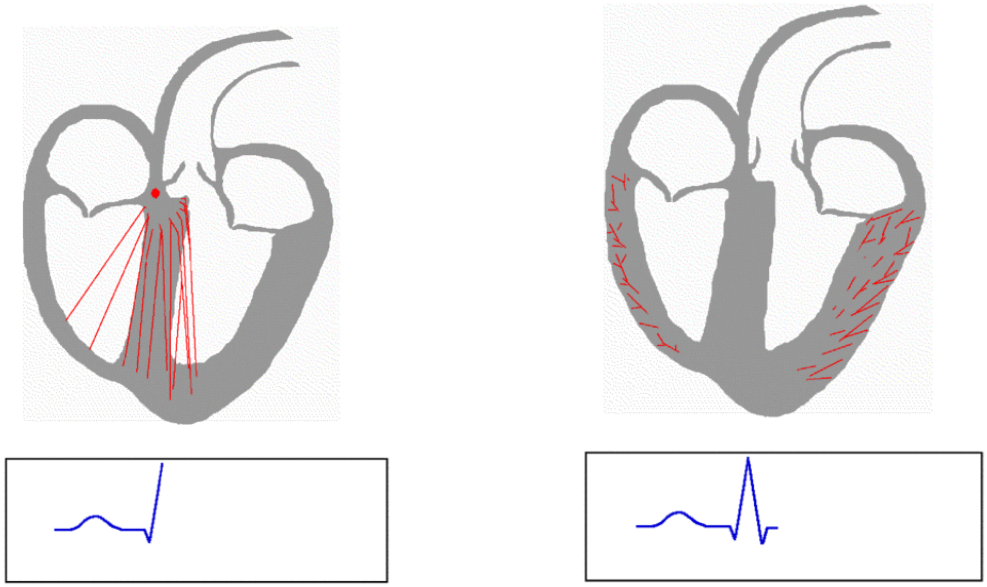
Precordial leads

The electrodes for the precordial leads (V_1, V_2, V_3, V_4, V_5 and V_6) are placed directly on the chest. Because of their close proximity to the heart, they do not require augmentation. Wilson's central terminal is used for the negative electrode, and these leads are considered to be *unipolar* (recall that Wilson's central terminal is the average of the three limb leads. This approximates common, or average, potential over the body). The precordial leads view the heart's electrical activity in the so-called *horizontal plane*. The heart's electrical axis in the horizontal plane is referred to as the *Z axis*.

Waves and intervals



Detail of the QRS complex, showing ventricular activation time (VAT) and amplitude



A normal ECG wave

A typical ECG tracing of the cardiac cycle (heartbeat) consists of a P wave, a QRS complex, a T wave, and a U wave which is normally visible in 50 to 75% of ECGs. The baseline voltage of the electrocardiogram is known as the *isoelectric line*. Typically the isoelectric line is measured as the portion of the tracing following the T wave and preceding the next P wave.

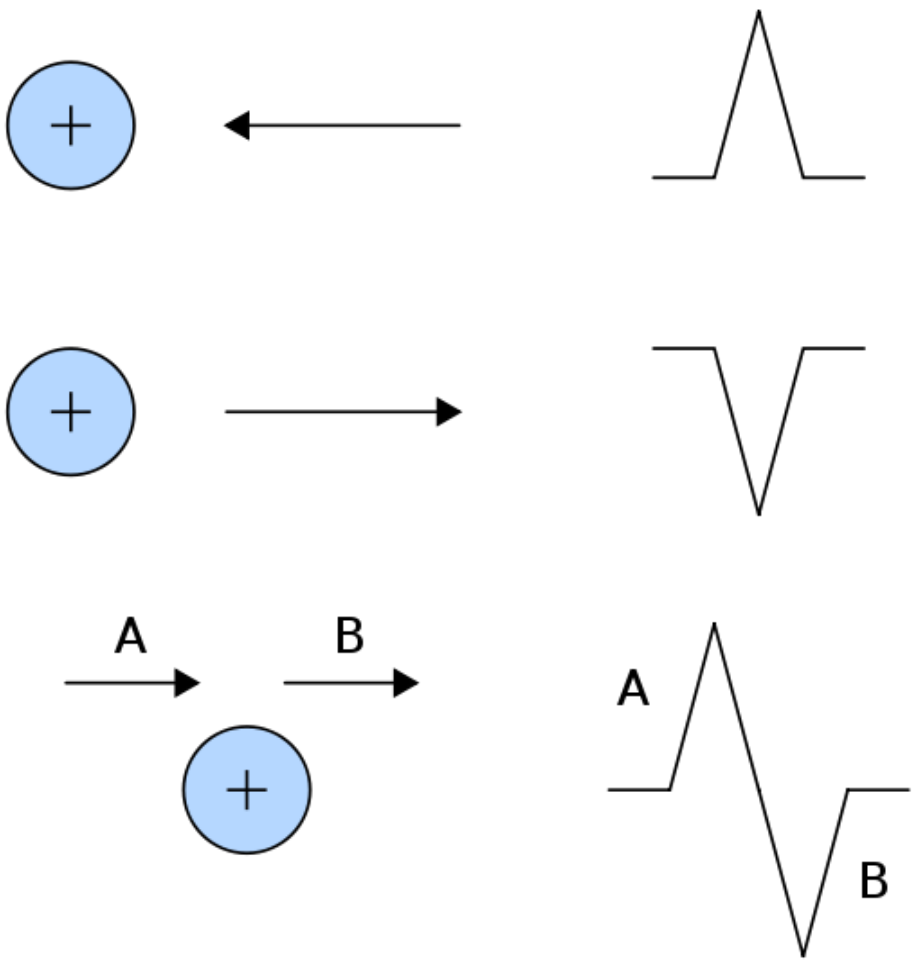
Feature	Description	Duration
RR interval	The interval between an R wave and the next R wave. Normal resting heart rate is between 60 and 100 bpm	0.6 to 1.2s
P wave	During normal atrial depolarization, the main electrical vector is directed from the SA node towards the AV node, and spreads from the right atrium to the left atrium. This turns into the P wave on the ECG.	80ms
PR interval	The PR interval is measured from the beginning of the P wave to the beginning of the QRS complex. The PR interval reflects the time the electrical impulse takes to travel from the sinus node through the AV node and entering the ventricles. The PR interval is therefore a good estimate of AV node function.	120 to 200ms
PR segment	The PR segment connects the P wave and the QRS complex. This coincides with the electrical conduction from the AV node to the bundle of His to the bundle branches and then to the Purkinje Fibers. This electrical activity does not produce a contraction directly and is merely traveling down towards the ventricles and this shows up flat on the ECG. The PR interval is more clinically relevant.	50 to 120ms
QRS	The QRS complex reflects the rapid depolarization of the right and	80 to

complex	left ventricles. They have a large muscle mass compared to the atria and so the QRS complex usually has a much larger amplitude than the P-wave.	120ms
J-point	The point at which the QRS complex finishes and the ST segment begins. Used to measure the degree of ST elevation or depression present.	N/A
ST segment	The ST segment connects the QRS complex and the T wave. The ST segment represents the period when the ventricles are depolarized. It is isoelectric.	80 to 120ms
T wave	The T wave represents the repolarization (or recovery) of the ventricles. The interval from the beginning of the QRS complex to the apex of the T wave is referred to as the *absolute refractory period*. The last half of the T wave is referred to as the *relative refractory period* (or vulnerable period).	160ms
ST interval	The ST interval is measured from the J point to the end of the T wave.	320ms
QT interval	The QT interval is measured from the beginning of the QRS complex to the end of the T wave. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death. It varies with heart rate and for clinical relevance requires a correction for this, giving the QTc.	300 to 430ms
U wave	The U wave is not always seen. It is typically low amplitude, and, by definition, follows the T wave.	
J wave	The J wave, elevated J-Point or Osborn Wave appears as a late delta wave following the QRS or as a small secondary R wave. It is considered pathognomonic of hypothermia or hypocalcemia.	

There were originally four deflections, but after the mathematical correction for artifacts introduced by early amplifiers, five deflections were discovered. Einthoven chose the letters P, Q, R, S, and T to identify the tracing which was superimposed over the uncorrected labeled A, B, C, and D.

In intracardiac electrocardiograms, such as can be acquired from pacemaker sensors, an additional wave that can be seen is the *H deflection*, which reflects the depolarization of the bundle of His. The *H-V interval*, in turn, is the duration from the beginning of the H deflection to the earliest onset of ventricular depolarization recorded in any lead.

Vectors and views



Graphic showing the relationship between positive electrodes, depolarization wavefronts (or mean electrical vectors), and complexes displayed on the ECG.

Interpretation of the ECG relies on the idea that different leads (by which we mean the ECG leads I,II,III, aVR, aVL, aVF and the chest leads) "view" the heart from different angles. This has two benefits. Firstly, leads which are showing problems (for example ST segment elevation) can be used to infer which region of the heart is affected. Secondly, the overall direction of travel of the wave of depolarisation can also be inferred which can reveal other problems. This is termed the cardiac **axis**. Determination of the cardiac axis relies on the concept of a vector which describes the motion of the depolarisation wave. This vector can then be described in terms of its components in relation to the direction of the lead considered. One component will be in the direction of the lead and this will be revealed in the behaviour of the QRS complex and one component will be at 90 degrees to this (which will not). Any net positive deflection of the QRS complex (i.e. height of the R-wave minus depth of the S-wave) suggests that the wave of depolarisation

is spreading through the heart in a direction that has some component (of the vector) in the same direction as the lead in question.

Axis

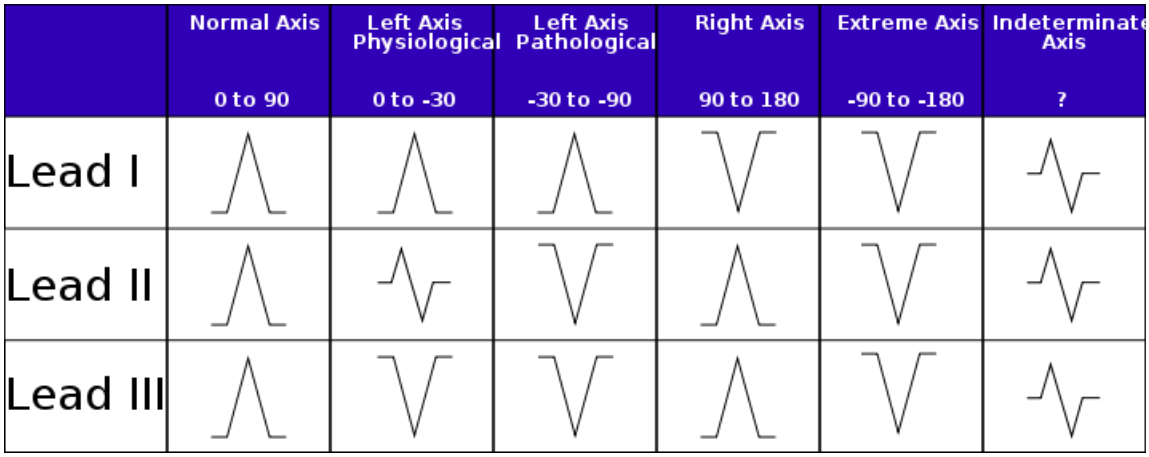
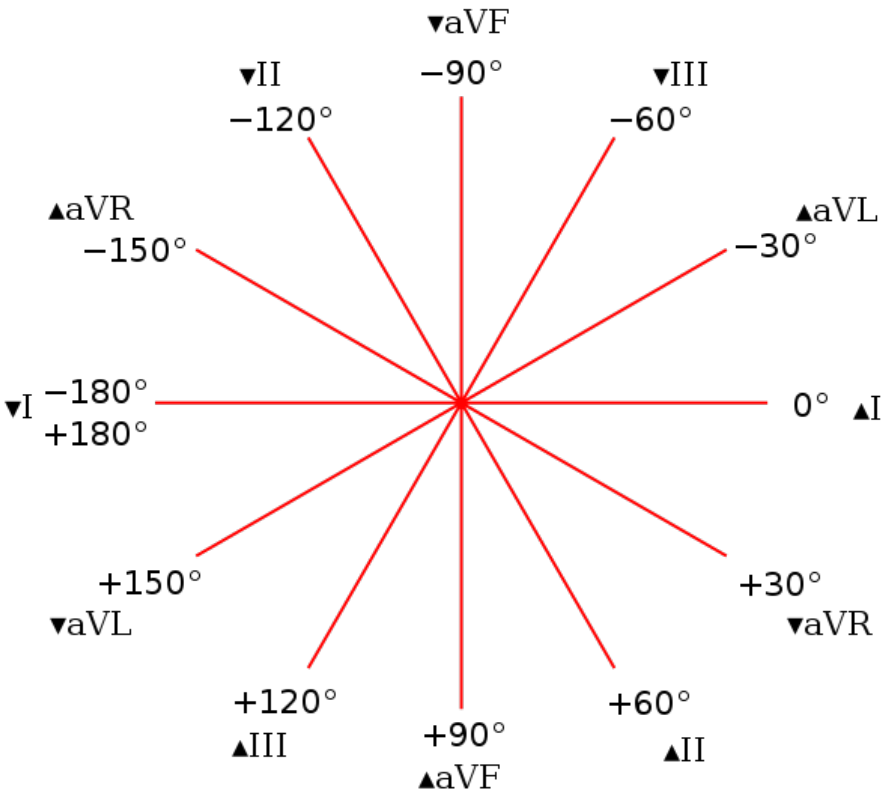


Diagram showing how the polarity of the QRS complex in leads I, II, and III can be used to estimate the heart's electrical axis in the frontal plane.

The heart's *electrical axis* refers to the general direction of the heart's depolarization wavefront (or *mean electrical vector*) in the frontal plane. With a healthy conducting system the cardiac axis is related to where the major muscle bulk of the heart lies. Normally this is the left ventricle with some contribution from the right ventricle. It is usually oriented in a right shoulder to left leg direction, which corresponds to the left inferior quadrant of the hexaxial reference system, although −30° to +90° is considered to be normal. If the left ventricle increases its activity or bulk then there is said to be "left axis deviation" as the axis swings round to the left beyond -30°, alternatively in conditions where the right ventricle is strained or hypertrophied then the axis swings round beyond +90° and "right axis deviation" is said to exist. Disorders of the conduction system of the heart can disturb the electrical axis without necessarily reflecting changes in muscle bulk.

Normal	−30° to 90°	Normal	Normal
Left axis deviation	−30° to −90°	May indicate left anterior fascicular block or Q waves from inferior MI.	Left axis deviation is considered normal in pregnant women and those with emphysema.
Right axis deviation	+90° to +180°	May indicate left posterior fascicular block, Q waves from high lateral MI, or a right ventricular strain pattern.	Right deviation is considered normal in children and is a standard effect of dextrocardia.
Extreme	+180°	Is rare, and considered an	

right axis deviation	to −90°	'electrical no-man's land'.	



The hexaxial reference system showing the orientation of each lead. For example, if the bulk of heart muscle is oriented at +60 degrees with respect to the SA node, lead II will show the greatest deflection and aVL the least.

In the setting of right bundle branch block, right or left axis deviation may indicate bifascicular block.

Clinical lead groups

There are twelve leads in total, each recording the electrical activity of the heart from a different perspective, which also correlate to different anatomical areas of the heart for the purpose of identifying acute coronary ischemia or injury. Two leads that look at neighbouring anatomical areas of the heart are said to be *contiguous* (see color coded chart). The relevance of this is in determining whether an abnormality on the ECG is likely to represent true disease or a spurious finding.

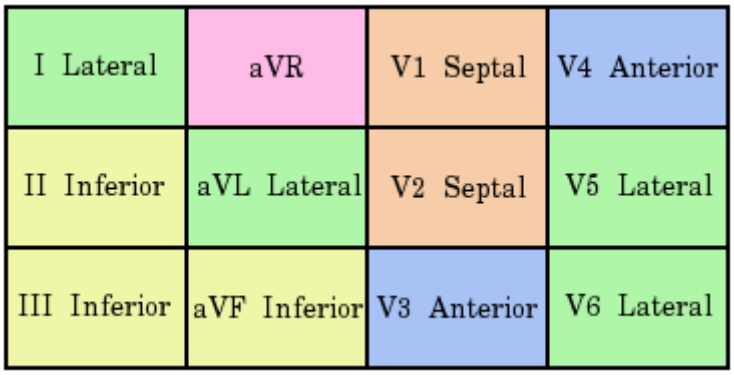
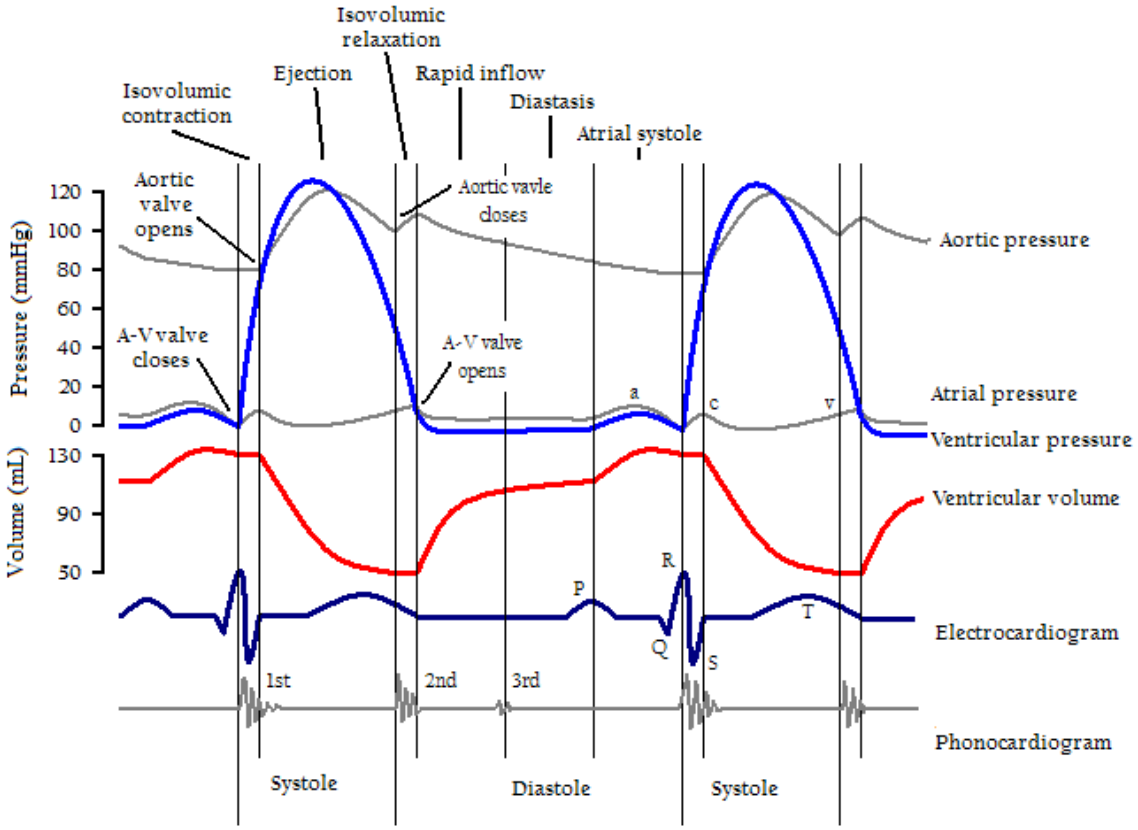


Diagram showing the contiguous leads in the same color

Category	Color on chart	Leads	Activity
Inferior leads	Yellow	Leads II, III and aVF	Look at electrical activity from the vantage point of the inferior surface (diaphragmatic surface of heart).
Lateral leads	Green	I, aVL, V_5 and V_6	Look at the electrical activity from the vantage point of the lateral wall of left ventricle. • The positive electrode for leads I and aVL should be located distally on the left arm and because of which, leads I and aVL are sometimes referred to as the *high lateral leads*. • Because the positive electrodes for leads V5 and V6 are on the patient's chest, they are sometimes referred to as the *low lateral leads*.
Septal leads	Orange	V_1 and V_2	Look at electrical activity from the vantage point of the septal wall of the ventricles (interventricular septum).
Anterior leads	Blue	V_3 and V_4	Look at electrical activity from the vantage point of the anterior surface of the heart (sternocostal surface of heart).

In addition, any two precordial leads that are next to one another are considered to be contiguous. For example, even though V4 is an anterior lead and V5 is a lateral lead, they are contiguous because they are next to one another.



Wiggers diagram, showing a normal ECG curve synchronized with other major events during the cardiac cycle.

Lead aVR offers no specific view of the left ventricle. Rather, it views the inside of the endocardial wall to the surface of the right atrium, from its perspective on the right shoulder.

Filter selection

Modern ECG monitors offer multiple filters for signal processing. The most common settings are monitor mode and diagnostic mode. In monitor mode, the low frequency filter (also called the high-pass filter because signals above the threshold are allowed to pass) is set at either 0.5 Hz or 1 Hz and the high frequency filter (also called the low-pass filter because signals below the threshold are allowed to pass) is set at 40 Hz. This limits artifact for routine cardiac rhythm monitoring. The high-pass filter helps reduce wandering baseline and the low-pass filter helps reduce 50 or 60 Hz power line noise (the power line network frequency differs between 50 and 60 Hz in different countries). In diagnostic mode, the high-pass filter is set at 0.05 Hz, which allows accurate ST segments to be recorded. The low-pass filter is set to 40, 100, or 150 Hz. Consequently, the monitor mode ECG display is more filtered than diagnostic mode, because its passband is narrower.

Indications

Symptoms generally indicating use of electrocardiography include:

- Cardiac murmurs
- Syncope or collapse
- Seizures
- Perceived cardiac dysrhythmias
- Symptoms of myocardial infarction.

It is also used to assess patients with systemic disease as well as monitoring during anesthesia and critically ill patients.

Some pathological entities which can be seen on the ECG

Shortened QT interval	Hypercalcemia, some drugs, certain genetic abnormalities.
Prolonged QT interval	Hypocalcemia, some drugs, certain genetic abnormalities.
Flattened or inverted T waves	Coronary ischemia, hypokalemia, left ventricular hypertrophy, digoxin effect, some drugs.
Hyperacute T waves	Possibly the first manifestation of acute myocardial infarction.
Prominent U waves	Hypokalemia.

Electrocardiogram heterogeneity

Electrocardiogram (ECG) heterogeneity is a measurement of the amount of variance between one ECG waveform and the next. This heterogeneity can be measured by placing multiple ECG electrodes on the chest and by then computing the variance in waveform morphology across the signals obtained from these electrodes. Recent research suggests that ECG heterogeneity often precedes dangerous cardiac arrhythmias.

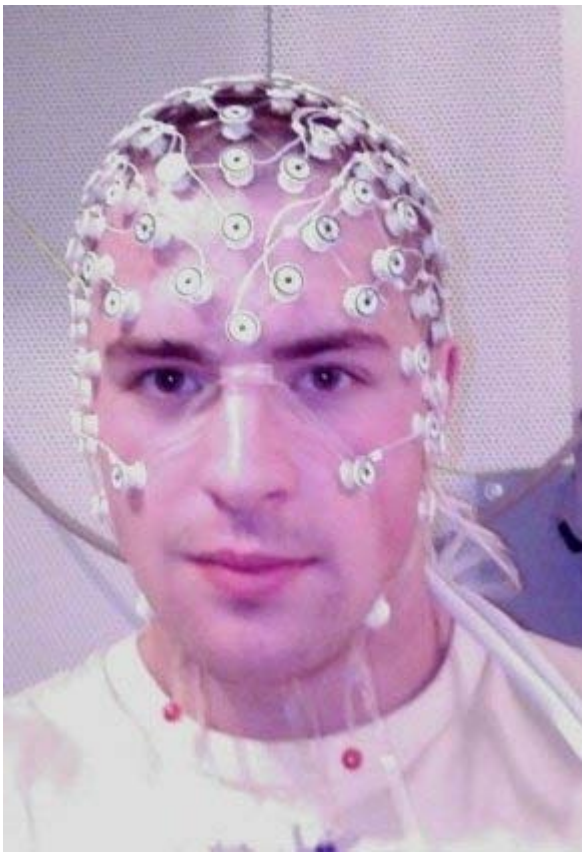
The time intervals between consecutive heart beats are customarily measured in the electrocardiogram from the beginning of a QRS complex to the beginning of the next QRS complex. Researchers obviously prefer to measure inter-beat intervals using the R-wave peak as the reference point since this measurement can be made with smaller errors. The obtained time series is usually named RR intervals. The measurements are being made for the purpose of studying RR or heart rate variability. The traditional method to study R-R intervals is performed in the frequency domain by using the FFT (Fast Fourier Method). Three bands are usually investigated for times series of R-R intervals relating five-six minutes of ECG recording. The band VLF usually covers the range 0.003-0.04 Hz, the band LF covers the range 0.04-0.15 Hz while the band HF covers the range 0.15-0.4 Hz. The LF and HF bands are of basic importance since they enable to investigate the modulations induced from the ANS (Autonomic Nervous System) on the cardiac rhythm and thus the balance between sympathetic and parasympathetic activity on heart. However, the FFT method has some basic limits linked to the basic features of the R-R signal that in fact is intrinsically non linear and non stationary. In order to overcome such basic limits, some researchers, Elio Conte, Antonio Federici and Joseph

P. Zbilut, have recently introduced a new method, called the CZF method. It makes it possible to analyze the R-R signal in normal subjects as well as in pathological conditions, and it may be conceived as giving a non invasive marker of ANS, inducing variability on R-R intervals.

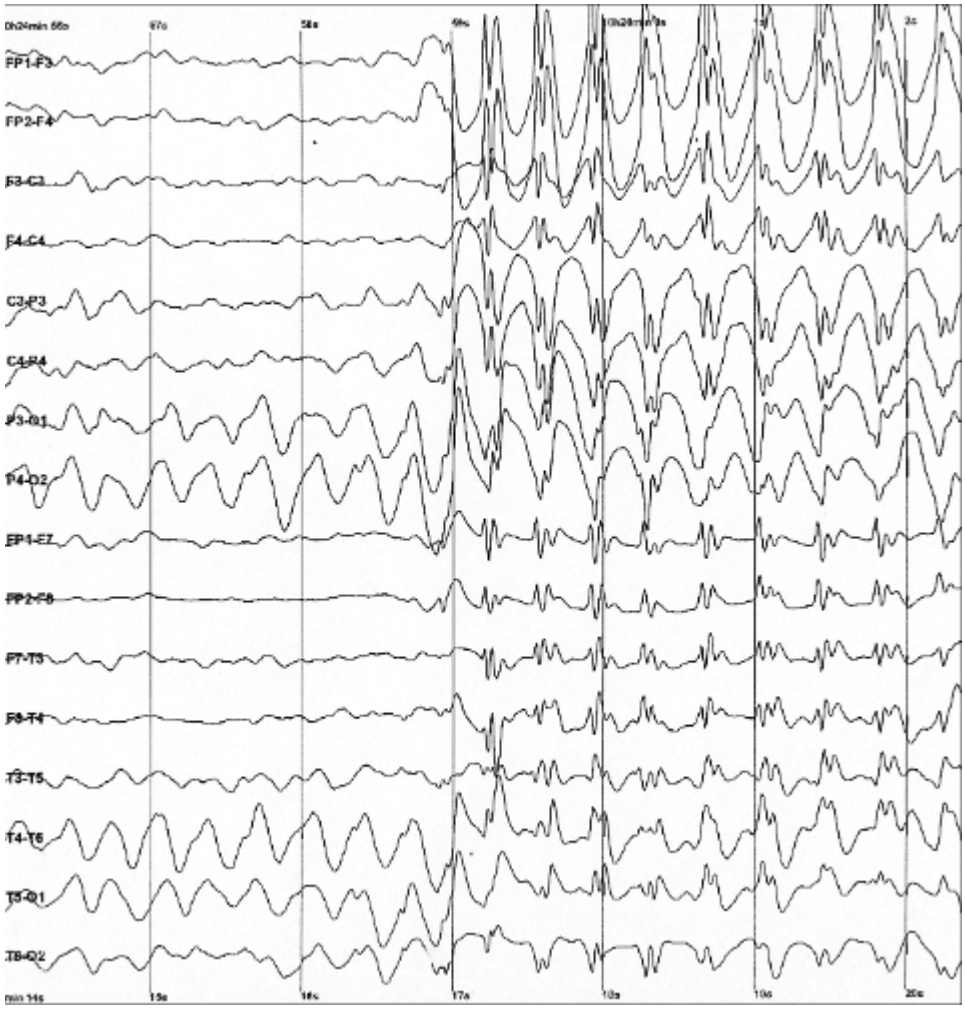
In the future, implantable devices may be programmed to measure and track heterogeneity. These devices could potentially help ward off arrhythmias by stimulating nerves such as the vagus nerve, by delivering drugs such as beta-blockers, and if necessary, by defibrillating the heart.

Chapter 18

Electroencephalography



An EEG recording net (Electrical Geodesics, Inc.) being used on a participant in a brain wave study



Epileptic spike and wave discharges monitored with EEG

Electroencephalography (**EEG**) is the recording of electrical activity along the scalp produced by the firing of neurons within the brain. In clinical contexts, EEG refers to the recording of the brain's spontaneous electrical activity over a short period of time, usually 20–40 minutes, as recorded from multiple electrodes placed on the scalp. In neurology, the main diagnostic application of EEG is in the case of epilepsy, as epileptic activity can create clear abnormalities on a standard EEG study. A secondary clinical use of EEG is in the diagnosis of coma, encephalopathies, and brain death. EEG used to be a first-line method for the diagnosis of tumors, stroke and other focal brain disorders, but this use has decreased with the advent of anatomical imaging techniques such as MRI and CT.

Derivatives of the EEG technique include evoked potentials (EP), which involves averaging the EEG activity time-locked to the presentation of a stimulus of some sort (visual, somatosensory, or auditory). Event-related potentials (ERPs) refer to averaged EEG responses that are time-locked to more complex processing of stimuli; this technique is used in cognitive science, cognitive psychology, and psychophysiological research.

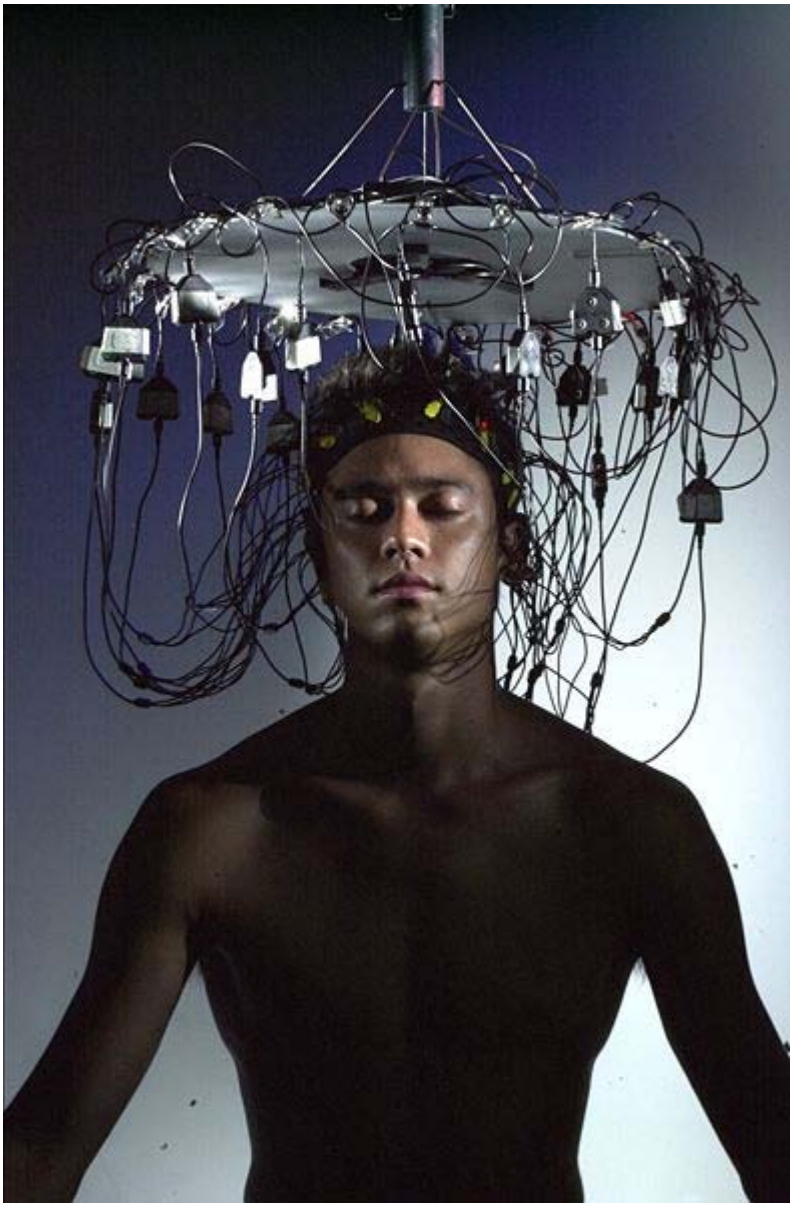
Source of EEG activity

The brain's electrical charge is maintained by billions of neurons. Neurons are electrically charged (or "polarized") by membrane transport proteins that pump ions across their membranes. When a neuron receives a signal from its neighbor via an action potential, it responds by releasing ions into the space outside the cell. Ions of like charge repel each other, and when many ions are pushed out of many neurons at the same time, they can push their neighbors, who push their neighbors, and so on, in a wave. This process is known as volume conduction. When the wave of ions reaches the electrodes on the scalp, they can push or pull electrons on the metal on the electrodes. Since metal conducts the push and pull of electrons easily, the difference in push, or voltage, between any two electrodes can be measured by a voltmeter. Recording these voltages over time gives us the EEG.

The electric potentials generated by single neurons are far too small to be picked by EEG or MEG. EEG activity therefore always reflects the summation of the synchronous activity of thousands or millions of neurons that have similar spatial orientation. If the cells do not have similar spatial orientation, their ions do not line up and create waves to be detected. Pyramidal neurons of the cortex are thought to produce most EEG signal because they are well-aligned and fire together. Because voltage fields fall off with the square of the distance, activity from deep sources is more difficult to detect than currents near the skull.

Scalp EEG activity shows oscillations at a variety of frequencies. Several of these oscillations have characteristic frequency ranges, spatial distributions and are associated with different states of brain functioning (e.g., waking and the various sleep stages). These oscillations represent synchronized activity over a network of neurons. The neuronal networks underlying some of these oscillations are understood (e.g., the thalamocortical resonance underlying sleep spindles), while many others are not (e.g., the system that generates the posterior basic rhythm). Research that measures both EEG and neuron spiking finds the relationship between the two is complex with the power of surface EEG only in two bands that of gamma and delta relating to neuron spike activity.

Clinical use



EEG electroencephalophone used during a music performance in which bathers from around the world were networked together as part of a collective musical performance, using their brainwaves to control sound, lighting, and the bath environment.

A routine clinical EEG recording typically lasts 20–30 minutes (plus preparation time) and usually involves recording from scalp electrodes. Routine EEG is typically used in the following clinical circumstances:

- to distinguish epileptic seizures from other types of spells, such as psychogenic non-epileptic seizures, syncope (fainting), sub-cortical movement disorders and migraine variants.

- to differentiate "organic" encephalopathy or delirium from primary psychiatric syndromes such as catatonia
- to serve as an adjunct test of brain death
- to prognosticate, in certain instances, in patients with coma
- to determine whether to wean anti-epileptic medications

At times, a routine EEG is not sufficient, particularly when it is necessary to record a patient while he/she is having a seizure. In this case, the patient may be admitted to the hospital for days or even weeks, while EEG is constantly being recorded (along with time-synchronized video and audio recording). A recording of an actual seizure (i.e., an ictal recording, rather than an inter-ictal recording of a possibly epileptic patient at some period between seizures) can give significantly better information about whether or not a spell is an epileptic seizure and the focus in the brain from which the seizure activity emanates.

Epilepsy monitoring is typically done:

- to distinguish epileptic seizures from other types of spells, such as psychogenic non-epileptic seizures, syncope (fainting), sub-cortical movement disorders and migraine variants.
- to characterize seizures for the purposes of treatment
- to localize the region of brain from which a seizure originates for work-up of possible seizure surgery

Additionally, EEG may be used to monitor certain procedures:

- to monitor the depth of anesthesia
- as an indirect indicator of cerebral perfusion in carotid endarterectomy
- to monitor amobarbital effect during the Wada test

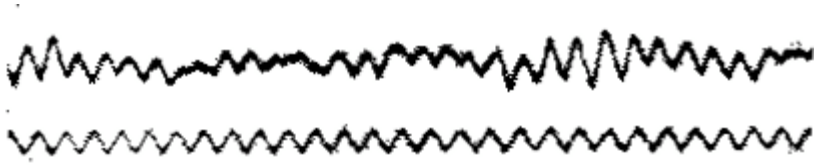
EEG can also be used in intensive care units for brain function monitoring:

- to monitor for non-convulsive seizures/non-convulsive status epilepticus
- to monitor the effect of sedative/anesthesia in patients in medically induced coma (for treatment of refractory seizures or increased intracranial pressure)
- to monitor for secondary brain damage in conditions such as subarachnoid hemorrhage (currently a research method)

If a patient with epilepsy is being considered for resective surgery, it is often necessary to localize the focus (source) of the epileptic brain activity with a resolution greater than what is provided by scalp EEG. This is because the cerebrospinal fluid, skull and scalp *smear* the electrical potentials recorded by scalp EEG. In these cases, neurosurgeons typically implant strips and grids of electrodes (or penetrating depth electrodes) under the dura mater, through either a craniotomy or a burr hole. The recording of these signals is referred to as electrocorticography (ECoG), subdural EEG (sdEEG) or intracranial EEG (icEEG)--all terms for the same thing. The signal recorded from ECoG is on a different

scale of activity than the brain activity recorded from scalp EEG. Low voltage, high frequency components that cannot be seen easily (or at all) in scalp EEG can be seen clearly in ECoG. Further, smaller electrodes (which cover a smaller parcel of brain surface) allow even lower voltage, faster components of brain activity to be seen. Some clinical sites record from penetrating microelectrodes.

Research use



The first human EEG recording obtained by Hans Berger in 1924. The upper tracing is EEG, and the lower is a 10 Hz timing signal.

EEG, and its derivative, ERPs, are used extensively in neuroscience, cognitive science, cognitive psychology, and psychophysiological research. Many techniques used in research contexts are not standardized sufficiently to be used in the clinical context.

A different method to study brain function is functional magnetic resonance imaging (fMRI). Some benefits of EEG compared to fMRI include:

- Hardware costs are significantly lower for EEG sensors versus an fMRI machine
- EEG sensors can be deployed into a wider variety of environments than can a bulky, immobile fMRI machine
- EEG enables higher temporal resolution, on the order of milliseconds, rather than seconds
- EEG is relatively tolerant of subject movement versus an fMRI (where the subject must remain completely still)
- EEG is silent, which allows for better study of the responses to auditory stimuli
- EEG does not aggravate claustrophobia

Limitations of EEG as compared with fMRI include:

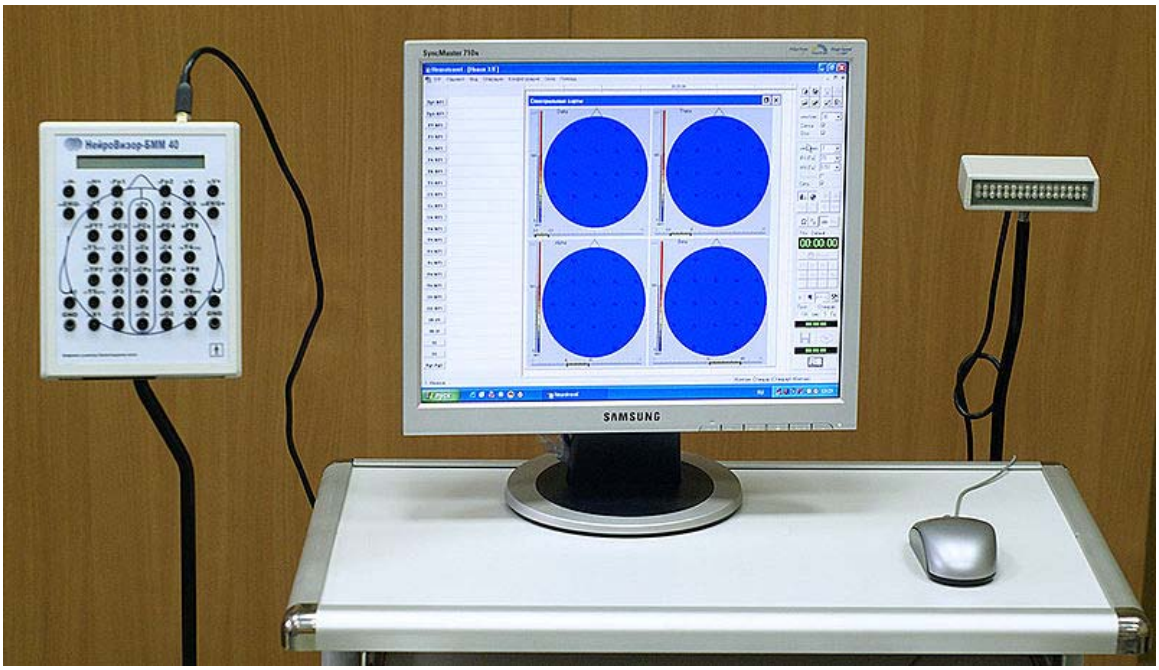
- Significantly lower spatial resolution
- ERP studies require relatively simple paradigms, compared with block-design fMRI studies

EEG recordings have been successfully obtained simultaneously with fMRI scans, though successful simultaneous recording requires that several technical issues be overcome, such as the presence of ballistocardiographic artifact, MRI pulse artifact and the induction of electrical currents in EEG wires that move within the strong magnetic fields of the MRI.

EEG also has some characteristics that compare favorably with behavioral testing:

- EEG can detect covert processing (i.e., processing that does not require a response)
- EEG can be used in subjects who are incapable of making a motor response
- Some ERP components can be detected even when the subject is not attending to the stimuli
- As compared with other reaction time paradigms, ERPs can elucidate stages of processing (rather than just the final end result)

Method



Computer Electroencephalograph *Neurovisor-BMM 40*

In conventional scalp EEG, the recording is obtained by placing electrodes on the scalp with a conductive gel or paste, usually after preparing the scalp area by light abrasion to reduce impedance due to dead skin cells. Many systems typically use electrodes, each of which is attached to an individual wire. Some systems use caps or nets into which electrodes are embedded; this is particularly common when high-density arrays of electrodes are needed.

Electrode locations and names are specified by the International 10–20 system for most clinical and research applications (except when high-density arrays are used). This system ensures that the naming of electrodes is consistent across laboratories. In most clinical applications, 19 recording electrodes (plus ground and system reference) are used. A smaller number of electrodes are typically used when recording EEG from neonates. Additional electrodes can be added to the standard set-up when a clinical or research application demands increased spatial resolution for a particular area of the brain. High-density arrays (typically via cap or net) can contain up to 256 electrodes more-or-less evenly spaced around the scalp.

Each electrode is connected to one input of a differential amplifier (one amplifier per pair of electrodes); a common system reference electrode is connected to the other input of each differential amplifier. These amplifiers amplify the voltage between the active electrode and the reference (typically 1,000–100,000 times, or 60–100 dB of voltage gain). In analog EEG, the signal is then filtered (next paragraph), and the EEG signal is output as the deflection of pens as paper passes underneath. Most EEG systems these days, however, are digital, and the amplified signal is digitized via an analog-to-digital converter, after being passed through an anti-aliasing filter. Analog-to-digital sampling typically occurs at 256–512 Hz in clinical scalp EEG; sampling rates of up to 20 kHz are used in some research applications.

During the recording, a series of activation procedures may be used. These procedures may induce normal or abnormal EEG activity that might not otherwise be seen. These procedures include hyperventilation, photic stimulation (with a strobe light), eye closure, mental activity, sleep and sleep deprivation. During (inpatient) epilepsy monitoring, a patient's typical seizure medications may be withdrawn.

The digital EEG signal is stored electronically and can be filtered for display. Typical settings for the high-pass filter and a low-pass filter are 0.5-1 Hz and 35–70 Hz, respectively. The high-pass filter typically filters out slow artifact, such as electrogalvanic signals and movement artifact, whereas the low-pass filter filters out high-frequency artifacts, such as electromyographic signals. An additional notch filter is typically used to remove artifact caused by electrical power lines (60 Hz in the United States and 50 Hz in many other countries). As part of an evaluation for epilepsy surgery, it may be necessary to insert electrodes near the surface of the brain, under the surface of the dura mater. This is accomplished via burr hole or craniotomy. This is referred to variously as "electrocorticography (ECoG)", "intracranial EEG (I-EEG)" or "subdural EEG (SD-EEG)". Depth electrodes may also be placed into brain structures, such as the amygdala or hippocampus, structures, which are common epileptic foci and may not be "seen" clearly by scalp EEG. The electrocorticographic signal is processed in the same manner as digital scalp EEG (above), with a couple of caveats. ECoG is typically recorded at higher sampling rates than scalp EEG because of the requirements of Nyquist theorem—the subdural signal is composed of a higher predominance of higher frequency components. Also, many of the artifacts that affect scalp EEG do not impact ECoG, and therefore display filtering is often not needed.

A typical adult human EEG signal is about 10μV to 100 μV in amplitude when measured from the scalp and is about 10–20 mV when measured from subdural electrodes.

Since an EEG voltage signal represents a difference between the voltages at two electrodes, the display of the EEG for the reading encephalographer may be set up in one of several ways. The representation of the EEG channels is referred to as a *montage.*

Bipolar montage

Each channel (i.e., waveform) represents the difference between two adjacent electrodes. The entire montage consists of a series of these channels. For example,

the channel "Fp1-F3" represents the difference in voltage between the Fp1 electrode and the F3 electrode. The next channel in the montage, "F3-C3," represents the voltage difference between F3 and C3, and so on through the entire array of electrodes.

Referential montage
: Each channel represents the difference between a certain electrode and a designated reference electrode. There is no standard position for this reference; it is, however, at a different position than the "recording" electrodes. Midline positions are often used because they do not amplify the signal in one hemisphere vs. the other. Another popular reference is "linked ears," which is a physical or mathematical average of electrodes attached to both earlobes or mastoids.

Average reference montage
: The outputs of all of the amplifiers are summed and averaged, and this averaged signal is used as the common reference for each channel.

Laplacian montage
: Each channel represents the difference between an electrode and a weighted average of the surrounding electrodes.

When analog (paper) EEGs are used, the technologist switches between montages during the recording in order to highlight or better characterize certain features of the EEG. With digital EEG, all signals are typically digitized and stored in a particular (usually referential) montage; since any montage can be constructed mathematically from any other, the EEG can be viewed by the electroencephalographer in any display montage that is desired.

The EEG is read by a neurologist, optimally one who has specific training in the interpretation of EEGs. This is done by visual inspection of the waveforms, called graphoelements. The use of computer signal processing of the EEG—so-called quantitative EEG—is somewhat controversial when used for clinical purposes (although there are many research uses).

Portable recording device for EEG

Limitations

EEG has several limitations. Most important is its poor spatial resolution. EEG is most sensitive to a particular set of post-synaptic potentials: those generated in superficial layers of the cortex, on the crests of gyri directly abutting the skull and radial to the skull. Dendrites, which are deeper in the cortex, inside sulci, in midline or deep structures (such as the cingulate gyrus or hippocampus), or producing currents that are tangential to the skull, have far less contribution to the EEG signal.

The meninges, cerebrospinal fluid and skull "smear" the EEG signal, obscuring its intracranial source.

It is mathematically impossible to reconstruct a unique intracranial current source for a given EEG signal, as some currents produce potentials that cancel each other out. This is referred to as the inverse problem. However, much work has been done to produce remarkably good estimates of, at least, a localized electric dipole that represents the recorded currents.

EEG vs fMRI and PET

EEG has several strong points as a tool for exploring brain activity. EEG's can detect changes within a millisecond timeframe, excellent considering an action potential takes approximately 0.5-130 milliseconds to propagate across a single neuron, depending on

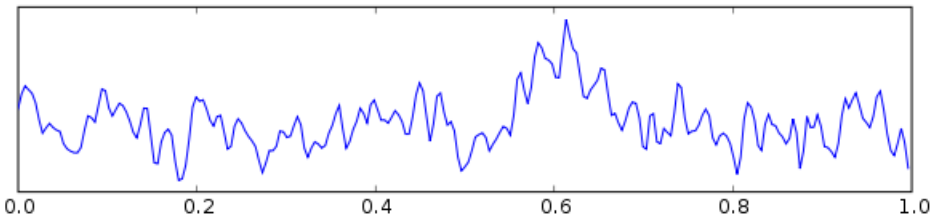
the type of neuron. Other methods of looking at brain activity, such as PET and fMRI have time resolution between seconds and minutes. EEG measures the brain's electrical activity directly, while other methods record changes in blood flow (e.g., SPECT, fMRI) or metabolic activity (e.g., PET), which are indirect markers of brain electrical activity. EEG can be used simultaneously with fMRI so that high-temporal-resolution data can be recorded at the same time as high-spatial-resolution data, however, since the data derived from each occurs over a different time course, the data sets do not necessarily represent exactly the same brain activity. There are technical difficulties associated with combining these two modalities, including the need to remove the *MRI gradient artifact* present during MRI acquisition and the ballistocardiographic artifact (resulting from the pulsatile motion of blood and tissue) from the EEG. Furthermore, currents can be induced in moving EEG electrode wires due to the magnetic field of the MRI.

EEG vs MEG

EEG reflects correlated synaptic activity caused by post-synaptic potentials of cortical neurons. The ionic currents involved in the generation of fast action potentials may not contribute greatly to the averaged field potentials representing the EEG. More specifically, the scalp electrical potentials that produce EEG are generally thought to be caused by the extracellular ionic currents caused by dendritic electrical activity, whereas the fields producing magnetoencephalographic signals are associated with intracellular ionic currents.

EEG can be recorded at the same time as MEG so that data from these complementary high-time-resolution techniques can be combined.

Normal activity



One second of EEG signal

The EEG is typically described in terms of (1) rhythmic activity and (2) transients. The rhythmic activity is divided into bands by frequency. To some degree, these frequency bands are a matter of nomenclature (i.e., any rhythmic activity between 8–12 Hz can be described as "alpha"), but these designations arose because rhythmic activity within a certain frequency range was noted to have a certain distribution over the scalp or a certain biological significance. Frequency bands are usually extracted using spectral methods (for instance Welch) as implemented for instance in freely available EEG software such as EEGLAB.

Most of the cerebral signal observed in the scalp EEG falls in the range of 1–20 Hz (activity below or above this range is likely to be artifactual, under standard clinical recording techniques).

Comparison table

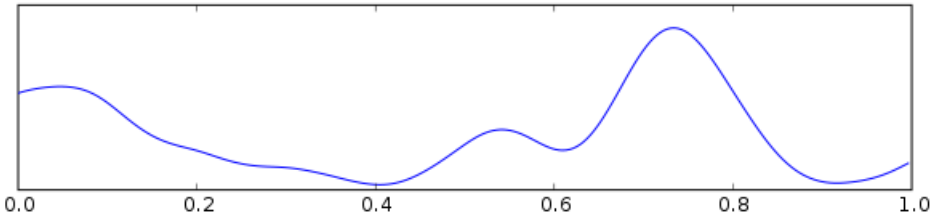
Comparison of EEG bands

Type	Frequency (Hz)	Location	Normally	Pathologically
Delta	up to 4	frontally in adults, posteriorly in children; high amplitude waves	• adults slow wave sleep • in babies • Has been found during some continuous attention tasks (Kirmizi-Alsan et al. 2006)	• subcortical lesions • diffuse lesions • metabolic encephalopathy hydrocephalus • deep midline lesions
Theta	4 – <8	Found in locations not related to task at hand	• young children • drowsiness or arousal in older children and adults • idling • Associated with inhibition of elicited responses (has been found to spike in situations where a person is actively trying to repress a response or action) (Kirmizi-Alsan et al. 2006).	• focal subcortical lesions • metabolic encephalopathy • deep midline disorders • some instances of hydrocephalus
Alpha	8 – 13	posterior regions of head, both sides, higher in amplitude on dominant side. Central sites (c3-c4) at rest.	• relaxed/reflecting • closing the eyes • Also associated with inhibition control, seemingly with the purpose of timing inhibitory activity in different locations across the brain (Klimesch, Sauseng, & Hanslmayr 2007;	• coma

			Coan & Allen 2008).	
Beta	>13 – 30	both sides, symmetrical distribution, most evident frontally; low amplitude waves	• alert/working • active, busy or anxious thinking, active concentration	• benzodiazepines
Gamma	30 – 100+	Somatosensory cortex	• Displays during cross-modal sensory processing (perception that combines two different senses, such as sound and sight) (Kisley & Cornwell 2006; Kanayama, Sato, & Ohira 2007; Nieuwenhuis, Yeung, & Cohen 2004) • Also is shown during short term memory matching of recognized objects, sounds, or tactile sensations (Herrmann, Frund, & Lenz 2009)	• A decrease in gamma band activity may be associated with cognitive decline, especially when related the theta band; however, this has not been proven for use as a clinical diagnostic measurement yet (Moretti et al. 2009).
Mu	8 – 13	Sensorimotor cortex	• Shows rest state motor neurons (Gastaut, 1952).	• Mu suppression could be indicative for motor mirror neurons working, and deficits in Mu suppression, and thus in mirror neurons, might play a role in autism. (Oberman et al., 2005)

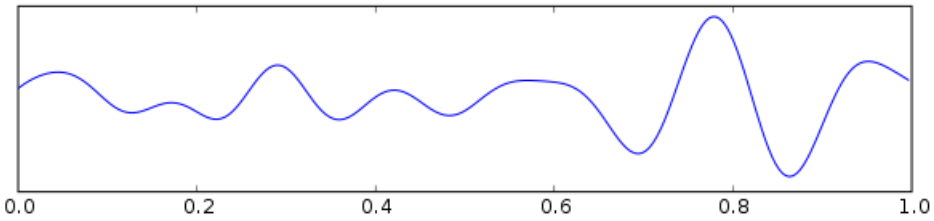
It should be noted that while these are the universally recognized ranges, they are not concrete definitions of the range of brain-waves. While researchers tend to follow these guidelines, many scholars use their own specific boundaries depending on the range they choose to focus on. Additionally, some researchers define the bands using decimal values rather than rounding to whole numbers (for example, one researcher may define the lower Beta band cut-off as 12.1, while another may use the value 13), while still others sometimes divide the bands into sub-bands. Generally, this is only done for the sake of analysis.

Wave patterns



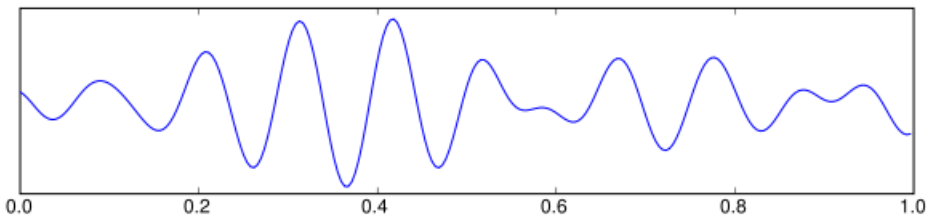
Delta waves

- Delta is the frequency range up to 4 Hz. It tends to be the highest in amplitude and the slowest waves. It is seen normally in adults in slow wave sleep. It is also seen normally in babies. It may occur focally with subcortical lesions and in general distribution with diffuse lesions, metabolic encephalopathy hydrocephalus or deep midline lesions. It is usually most prominent frontally in adults (e.g. FIRDA - Frontal Intermittent Rhythmic Delta) and posteriorly in children (e.g. OIRDA - Occipital Intermittent Rhythmic Delta).



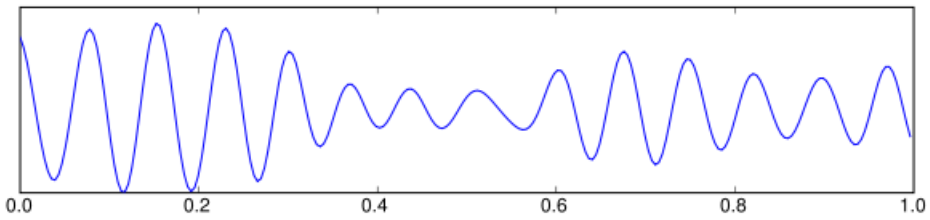
Theta waves

- Theta is the frequency range from 4 Hz to 7 Hz. Theta is seen normally in young children. It may be seen in drowsiness or arousal in older children and adults; it can also be seen in meditation. Excess theta for age represents abnormal activity. It can be seen as a focal disturbance in focal subcortical lesions; it can be seen in generalized distribution in diffuse disorder or metabolic encephalopathy or deep midline disorders or some instances of hydrocephalus. On the contrary this range has been associated with reports of relaxed, meditative, and creative states.



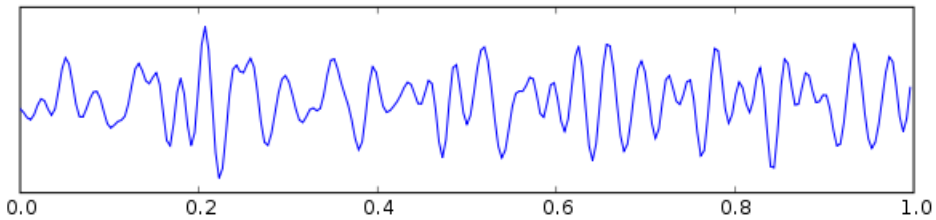
Alpha waves

- Alpha is the frequency range from 8 Hz to 12 Hz. Hans Berger named the first rhythmic EEG activity he saw as the "alpha wave". This was the "posterior basic rhythm" (also called the "posterior dominant rhythm" or the "posterior alpha rhythm"), seen in the posterior regions of the head on both sides, higher in amplitude on the dominant side. It emerges with closing of the eyes and with relaxation, and attenuates with eye opening or mental exertion. The posterior basic rhythm is actually slower than 8 Hz in young children (therefore technically in the theta range).



Sensorimotor rhythm aka mu rhythm

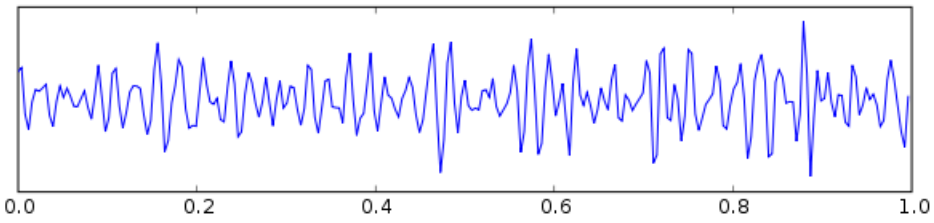
In addition to the posterior basic rhythm, there are other normal alpha rhythms such as the mu rhythm (alpha activity in the contralateral sensory and motor cortical areas that emerges when the hands and arms are idle; and the "third rhythm" (alpha activity in the temporal or frontal lobes). Alpha can be abnormal; for example, an EEG that has diffuse alpha occurring in coma and is not responsive to external stimuli is referred to as "alpha coma".



Beta waves

- Beta is the frequency range from 12 Hz to about 30 Hz. It is seen usually on both sides in symmetrical distribution and is most evident frontally. Beta activity is closely linked to motor behavior and is generally attenuated during active

movements. Low amplitude beta with multiple and varying frequencies is often associated with active, busy or anxious thinking and active concentration. Rhythmic beta with a dominant set of frequencies is associated with various pathologies and drug effects, especially benzodiazepines. It may be absent or reduced in areas of cortical damage. It is the dominant rhythm in patients who are alert or anxious or who have their eyes open.



Gamma waves

- Gamma is the frequency range approximately 30–100 Hz. Gamma rhythms are thought to represent binding of different populations of neurons together into a network for the purpose of carrying out a certain cognitive or motor function.

- Mu ranges 8–13 Hz., and partly overlaps with other frequencies. It reflects the synchronous firing of motor neurons in rest state. Mu suppression is thought to reflect motor mirror neuron systems, because when an action is observed, the pattern extinguishes, possibly because of the normal neuronal system and the mirror neuron system "go out of sync", and interfere with each other.

"Ultra-slow" or "near-DC" activity is recorded using DC amplifiers in some research contexts. It is not typically recorded in a clinical context because the signal at these frequencies is susceptible to a number of artifacts.

Some features of the EEG are transient rather than rhythmic. Spikes and sharp waves may represent seizure activity or interictal activity in individuals with epilepsy or a predisposition toward epilepsy. Other transient features are normal: vertex waves and sleep spindles are seen in normal sleep.

Note that there are types of activity that are statistically uncommon, but not associated with dysfunction or disease. These are often referred to as "normal variants." The mu rhythm is an example of a normal variant.

The normal Electroencephalography (EEG) varies by age. The neonatal EEG is quite different from the adult EEG. The EEG in childhood generally has slower frequency oscillations than the adult EEG.

The normal EEG also varies depending on state. The EEG is used along with other measurements (EOG, EMG) to define sleep stages in polysomnography. Stage I sleep (equivalent to drowsiness in some systems) appears on the EEG as drop-out of the

posterior basic rhythm. There can be an increase in theta frequencies. Santamaria and Chiappa cataloged a number of the variety of patterns associated with drowsiness. Stage II sleep is characterized by sleep spindles—transient runs of rhythmic activity in the 12–14 Hz range (sometimes referred to as the "sigma" band) that have a frontal-central maximum. Most of the activity in Stage II is in the 3–6 Hz range. Stage III and IV sleep are defined by the presence of delta frequencies and are often referred to collectively as "slow-wave sleep." Stages I-IV comprise non-REM (or "NREM") sleep. The EEG in REM (rapid eye movement) sleep appears somewhat similar to the awake EEG.

EEG under general anesthesia depends on the type of anesthetic employed. With halogenated anesthetics, such as halothane or intravenous agents, such as propofol, a rapid (alpha or low beta), nonreactive EEG pattern is seen over most of the scalp, especially anteriorly; in some older terminology this was known as a WAR (widespread anterior rapid) pattern, contrasted with a WAIS (widespread slow) pattern associated with high doses of opiates. Anesthetic effects on EEG signals are beginning to be understood at the level of drug actions on different kinds of synapses and the circuits that allow synchronized neuronal activity.

Artifacts

Biological artifacts

Electrical signals detected along the scalp by an EEG, but that originate from non-cerebral origin are called artifacts. EEG data is almost always contaminated by such artifacts. The amplitude of artifacts can be quite large relative to the size of amplitude of the cortical signals of interest. This is one of the reasons why it takes considerable experience to correctly interpret EEGs clinically. Some of the most common types of biological artifacts include:

- Eye-induced artifacts (includes eye blinks, eye movements and extra-ocular muscle activity)
- EKG (cardiac) artifacts
- EMG (muscle activation)-induced artifacts
- Glossokinetic artifacts

The most prominent eye-induced artifacts are caused by the potential difference between the cornea and retina, which is quite large compared to cerebral potentials. When the eyes and eyelids are completely still, this corneo-retinal dipole does not affect EEG. However, blinks occur several times per minute, the eyes movements occur several times per second. Eyelid movements, occurring mostly during blinking or vertical eye movements, elicit a large potential seen mostly in the difference between the Electrooculography (EOG) channels above and below the eyes. An established explanation of this potential regards the eyelids as sliding electrodes that short-circuit the positively charged cornea to the extra-ocular skin. Rotation of the eyeballs, and consequently of the corneo-retinal dipole, increases the potential in electrodes towards which the eyes are rotated, and decrease the potentials in the opposing electrodes. Eye movements called saccades also

generate transient electromyographic potentials, known as saccadic spike potentials (SPs). The spectrum of these SPs overlaps the gamma-band, and seriously confounds analysis of induced gamma-band responses, requiring tailored artifact correction approaches. Purposeful or reflexive eye blinking also generates electromyographic potentials, but more importantly there is reflexive movement of the eyeball during blinking that gives a characteristic artifactual appearance of the EEG.

Eyelid fluttering artifacts of a characteristic type were previously called Kappa rhythm (or Kappa waves). It is usually seen in the prefrontal leads, that is, just over the eyes. Sometimes they are seen with mental activity. They are usually in the Theta (4–7 Hz) or Alpha (8–13 Hz) range. They were named because they were believed to originate from the brain. Later study revealed they were generated by rapid fluttering of the eyelids, sometimes so minute that it was difficult to see. They are in fact noise in the EEG reading, and should not technically be called a rhythm or wave. Therefore, current usage in electroencephalography refers to the phenomenon as an eyelid fluttering artifact, rather than a Kappa rhythm (or wave).

Some of these artifacts can be useful in various applications. The EOG signals, for instance, can be used to detect and track eye-movements, which are very important in polysomnography, and is also in conventional EEG for assessing possible changes in alertness, drowsiness or sleep.

EKG artifacts are quite common and can be mistaken for spike activity. Because of this, modern EEG acquisition commonly includes a one-channel EKG from the extremities. This also allows the EEG to identify cardiac arrhythmias that are an important differential diagnosis to syncope or other episodic/attack disorders.

Glossokinetic artifacts are caused by the potential difference between the base and the tip of the tongue. Minor tongue movements can contaminate the EEG, especially in parkinsonian and tremor disorders.

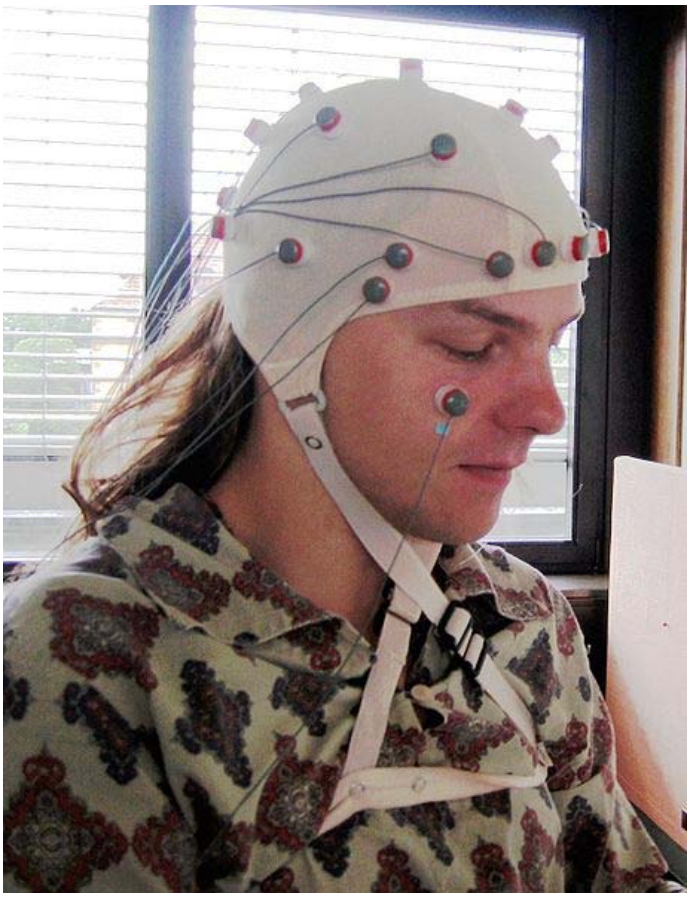
Environmental artifacts

In addition to artifacts generated by the body, many artifacts originate from outside the body. Movement by the patient, or even just settling of the electrodes, may cause *electrode pops*, spikes originating from a momentary change in the impedance of a given electrode. Poor grounding of the EEG electrodes can cause significant 50 or 60 Hz artifact, depending on the local power system's frequency. A third source of possible interference can be the presence of an IV drip; such devices can cause rhythmic, fast, low-voltage bursts, which may be confused for spikes.

Artifact correction

Recently, independent component analysis techniques have been used to correct or remove EEG contaminates. These techniques attempt to "unmix" the EEG signals into some number of underlying components. There are many source separation algorithms,

often assuming various behaviors or natures of EEG. Regardless, the principle behind any particular method usually allow "remixing" only those components that would result in "clean" EEG by nullifying (zeroing) the weight of unwanted components. Fully automated artifact rejection methods, which use ICA, have also been developed.



Person wearing electrodes for EEG

Abnormal activity

Abnormal activity can broadly be separated into epileptiform and non-epileptiform activity. It can also be separated into focal or diffuse.

Focal epileptiform discharges represent fast, synchronous potentials in a large number of neurons in a somewhat discrete area of the brain. These can occur as interictal activity, between seizures, and represent an area of cortical irritability that may be predisposed to producing epileptic seizures. Interictal discharges are not wholly reliable for determining whether a patient has epilepsy nor where his/her seizure might originate.

Generalized epileptiform discharges often have an anterior maximum, but these are seen synchronously throughout the entire brain. They are strongly suggestive of a generalized epilepsy.

Focal non-epileptiform abnormal activity may occur over areas of the brain where there is focal damage of the cortex or white matter. It often consists of an increase in slow frequency rhythms and/or a loss of normal higher frequency rhythms. It may also appear as focal or unilateral decrease in amplitude of the EEG signal.

Diffuse non-epileptiform abnormal activity may manifest as diffuse abnormally slow rhythms or bilateral slowing of normal rhythms, such as the PBR.

Intracortical Encephalogram electrodes and sub-dural electrodes can be used in tandem to discriminate and discretize artifact from epileptiform and other severe neurological events.

More advanced measures of abnormal EEG signals have also recently received attention as possible biomarkers for different disorders such as Alzheimer's disease.

History

A timeline of the history of EEG is given by Swartz. Richard Caton (1842–1926), a physician practicing in Liverpool, presented his findings about electrical phenomena of the exposed cerebral hemispheres of rabbits and monkeys in the British Medical Journal in 1875. In 1890, Polish physiologist Adolf Beck published an investigation of spontaneous electrical activity of the brain of rabbits and dogs that included rhythmic oscillations altered by light.

In 1912, Russian physiologist, Vladimir Vladimirovich Pravdich-Neminsky published the first animal EEG and the evoked potential of the mammalian (dog). In 1914, Napoleon Cybulski and Jelenska-Macieszyna photographed EEG-recordings of experimentally induced seizures.

German physiologist and psychiatrist Hans Berger (1873–1941) recorded the first human EEG in 1924. Expanding on work previously conducted on animals by Richard Caton and others, Berger also invented the electroencephalogram (giving the device its name), an invention described "as one of the most surprising, remarkable, and momentous developments in the history of clinical neurology". His discoveries were first confirmed by British scientists Edgar Douglas Adrian and B. H. C. Matthews in 1934 and developed by them.

In 1934, Fisher and Lowenback first demonstrated epileptiform spikes. In 1935 Gibbs, Davis and Lennox described interictal spike waves and the 3 cycles/s pattern of clinical absence seizures, which began the field of clinical electroencephalography. Subsequently, in 1936 Gibbs and Jasper reported the interictal spike as the focal signature of epilepsy. The same year, the first EEG laboratory opened at Massachusetts General Hospital.

Franklin Offner (1911–1999), professor of biophysics at Northwestern University developed a prototype of the EEG that incorporated a piezoelectric inkwriter called a Crystograph (the whole device was typically known as the Offner Dynograph).

In 1947, The American EEG Society was founded and the first International EEG congress was held. In 1953 Aserinsky and Kleitman describe REM sleep.

In the 1950s, William Grey Walter developed an adjunct to EEG called EEG topography, which allowed for the mapping of electrical activity across the surface of the brain. This enjoyed a brief period of popularity in the 1980s and seemed especially promising for psychiatry. It was never accepted by neurologists and remains primarily a research tool.

Various uses

The EEG has been used for many purposes besides the conventional uses of clinical diagnosis and conventional cognitive neuroscience. Long-term EEG recordings in epilepsy patients are used for seizure prediction. Neurofeedback remains an important extension, and in its most advanced form is also attempted as the basis of brain computer interfaces. The EEG is also used quite extensively in the field of neuromarketing. There are many commercial products substantially based on the EEG.

Honda is attempting to develop a system to move its Asimo robot using EEG, a technology it eventually hopes to incorporate into its automobiles.

EEGs have been used as evidence in trials in the Indian state of Maharastra.

EEG and Telepathy

DARPA budgeted $4 million in 2009 to investigate technology to enable soldiers on the battlefield to communicate via computer-mediated telepathy. The aim is to analyse neural signals that exist in the brain before words are spoken.

Chapter 19

Electrocorticography and Electroantennography

Electrocorticography

Electrocorticography (ECoG) is the practice of using electrodes placed directly on the exposed surface of the brain to record electrical activity from the cerebral cortex. ECoG may be performed either in the operating room during surgery (intraoperative ECoG) or outside of surgery (extraoperative ECoG). Because a craniotomy (a surgical incision into the skull) is required to implant the electrode grid, ECoG is an invasive procedure. ECoG is currently considered to be the "gold standard" for defining epileptogenic zones in clinical practice.

History

ECoG was pioneered in the early 1950's by Wilder Penfield and Herbert Jasper, neurosurgeons at the Montreal Neurological Institute. The two developed ECoG as part of their groundbreaking Montreal procedure, a surgical protocol used to treat patients with severe epilepsy. The cortical potentials recorded by ECoG were used to identify epileptogenic zones – regions of the cortex that generate epileptic seizures. These zones would then be surgically removed from the cortex during resectioning, thus destroying the brain tissue where epileptic seizures had originated. Penfield and Jasper also used electrical stimulation during ECoG recordings in patients undergoing epilepsy surgery under local anesthesia. This procedure was used to explore the functional anatomy of the brain, mapping speech areas and identifying the somatosensory and somatomotor cortex areas to be excluded from surgical removal.

Electrophysiological basis

ECoG signals are composed of synchronized postsynaptic potentials (local field potentials), recorded directly from the exposed surface of the cortex. The potentials occur primarily in cortical pyramidal cells, and thus must be conducted through several layers of the cerebral cortex, cerebrospinal fluid (CSF), pia mater, and arachnoid mater before reaching subdural recording electrodes placed just below the dura mater (outer cranial membrane). However, to reach the scalp electrodes of an electroencephalogram (EEG),

electrical signals must also be conducted through the skull, where potentials rapidly attenuate due to the low conductivity of bone. For this reason, the spatial resolution of ECoG is much higher than EEG, a critical imaging advantage for presurgical planning. ECoG offers a temporal resolution of approximately 5 ms and a spatial resolution of 1 cm.

Using depth electrodes, the local field potential gives a measure of a neural population in a sphere with a radius of 0.5-3 mm around the tip of the electrode. With a sufficiently high sampling rate (more than about 10 kHz), depth electrodes can also measure action potentials. In which case the spatial resolution is down to individual neurons, and the field of view of an individual electrode is approximately 0.05-0.35 mm.

Procedure

The ECoG recording is performed from electrodes placed on the exposed cortex. In order to access the cortex, a surgeon must first perform a craniotomy, removing a part of the skull to expose the brain surface. This procedure may be performed either under general anesthesia or under local anesthesia if patient interaction is required for functional cortical mapping. Electrodes are then surgically implanted on the surface of the cortex, with placement guided by the results of preoperative EEG and magnetic resonance imaging (MRI). Electrodes may either be placed outside the dura mater (epidural) or under the dura mater (subdural). ECoG electrode arrays typically consist of sixteen sterile, disposable stainless steel, carbon tip, platinum, or gold ball electrodes, each mounted on a ball and socket joint for ease in positioning. These electrodes are attached to an overlying frame in a “crown” or “halo” configuration. Subdural strip and grid electrodes are also widely used in various dimensions, having anywhere from 4 to 64 electrode contacts. The grids are transparent, flexible, and numbered at each electrode contact. Standard spacing between grid electrodes is 1 cm; individual electrodes are typically 5 mm in diameter. The electrodes sit lightly on the cortical surface, and are designed with enough flexibility to ensure that normal movements of the brain do not cause injury. A key advantage of strip and grid electrode arrays is that they may be slid underneath the dura mater into cortical regions not exposed by the craniotomy. Strip electrodes and crown arrays may be used in any combination desired. Depth electrodes may also be used to record activity from deeper structures such as the hippocampus.

DCES

Direct cortical electrical stimulation (DCES) is frequently performed in concurrence with ECoG recording for functional mapping of the cortex and identification of critical cortical structures. When using a crown configuration, a handheld wand bipolar stimulator may be used at any location along the electrode array. However, when using a subdural strip, stimulation must be applied between pairs of adjacent electrodes due to the nonconductive material connecting the electrodes on the grid. Electrical stimulating currents applied to the cortex are relatively low, between 2 to 4 mA for somatosensory stimulation, and near 15 mA for cognitive stimulation.

The functions most commonly mapped through DCES are primary motor, primary sensory, and language. The patient must be alert and interactive for mapping procedures, though patient involvement varies with each mapping procedure. Language mapping may involve naming, reading aloud, repetition, and oral comprehension; somatosensory mapping requires that the patient describe sensations experienced across the face and extremities as the surgeon stimulates different cortical regions.

Clinical applications

Since its development in the 1950's, ECoG has been used to localize epileptogenic zones during presurgical planning, map out cortical functions, and to predict the success of epileptic surgical resectioning. ECoG offers several advantages over alternative diagnostic modalities:

- Flexible placement of recording and stimulating electrodes

- Can be performed at any stage before, during, and after a surgery
- Allows for direct electrical stimulation of the brain, identifying critical regions of the cortex to be avoided during surgery
- Greater precision and sensitivity than an EEG scalp recording - spatial resolution is higher and signal-to-noise ratio is superior due to greater proximity to neural activity

Limitations of ECoG include:

- Limited sampling time – seizures (ictal events) may not be recorded during the ECoG recording period
- Limited field of view – electrode placement is limited by the area of exposed cortex and surgery time, sampling errors may occur
- Recording is subject to the influence of anesthetics, narcotic analgesics, and the surgery itself

Intractable epilepsy

Epilepsy is currently ranked as the third most commonly diagnosed neurological disorder, afflicting approximately 2.5 million people in the United States alone. Epileptic seizures are chronic and unrelated to any immediately treatable causes, such as toxins or infectious diseases, and may vary widely based on etiology, clinical symptoms, and site of origin within the brain. For patients with intractable epilepsy – epilepsy that is unresponsive to anticonvulsants – surgical treatment may be a viable treatment option.

Extraoperative ECoG

Before a patient can be identified as a candidate for resectioning surgery, MRI must be performed to demonstrate the presence of a structural lesion within the cortex, supported by EEG evidence of epileptogenic tissue. Once a lesion has been identified, ECoG may

be performed to determine the location and extent of the lesion and surrounding irritative region. The scalp EEG, while a valuable diagnostic tool, lacks the precision necessary to localize the epileptogenic region. ECoG is considered to be the gold standard for assessing neuronal activity in patients with epilepsy, and is widely used for presurgical planning to guide surgical resection of the lesion and epileptogenic zone. The success of the surgery depends on accurate localization and removal of the epileptogenic zone. ECoG data is assessed with regard to ictal spike activity – "diffuse fast wave activity" recorded during a seizure – and interictal epileptiform activity (IEA), brief bursts of neuronal activity recorded between epileptic events. ECoG is also performed following the resectioning surgery to detect any remaining epileptiform activity, and to determine the success of the surgery. Residual spikes on the ECoG, unaltered by the resection, indicate poor seizure control, and incomplete neutralization of the epileptogenic cortical zone. Additional surgery may be necessary to completely eradicate seizure activity.

Intraoperative ECoG

The objective of the resectioning surgery is to remove the epileptogenic tissue without causing unacceptable neurological consequences. In addition to identifying and localizing the extent of epileptogenic zones, ECoG used in conjunction with DCES is also a valuable tool for functional cortical mapping. It is vital to precisely localize critical brain structures, identifying which regions the surgeon must spare during resectioning (the "eloquent cortex") in order to preserve sensory processing, motor coordination, and speech. Functional mapping requires that the patient be able to interact with the surgeon, and thus is performed under local rather than general anesthesia. Electrical stimulation using cortical and acute depth electrodes is used to probe distinct regions of the cortex in order to identify centers of speech, somatosensory integration, and somatomotor processing. During the resectioning surgery, intraoperative ECoG may also be performed to monitor the epileptic activity of the tissue and ensure that the entire epileptogenic zone is resectioned.

Although the use of extraoperative and intraoperative ECoG in resectioning surgery has been an accepted clinical practice for several decades, recent studies have shown that the usefulness of this technique may very based on the type of epilepsy a patient exhibits. Kuruvilla and Flink reported that while intraoperative ECoG plays a critical role in tailored temporal lobectomies, in multiple subpial transections (MST), and in the removal of malformations of cortical development (MCDs), it has been found impractical in standard resection of medial temporal lobe epilepsy (TLE) with MRI evidence of mesial temporal sclerosis (MTS). A study performed by Wennberg, Quesney, and Rasmussen demonstrated the presurgical significance of ECoG in frontal lobe epilepsy (FLE) cases.

Research applications

ECoG has recently emerged as a promising recording technique for use in brain-computer interfaces (BCI). BCIs are direct neural interfaces that provide control of prosthetic, electronic, or communication devices via direct use of the individual's brain signals. Brain signals may be recorded either invasively, with recording devices implanted

directly into the cortex, or noninvasively, using EEG scalp electrodes. ECoG serves to provide a partially invasive compromise between the two modalities – while ECoG does not penetrate the blood-brain barrier like invasive recording devices, it features a higher spatial resolution and higher signal-to-noise ratio than EEG. A recent study by Shenoy et al. demonstrates the high movement classification accuracy potential of ECoG-based BCIs.

Recent advances in ECoG technology

The electrocorticogram is still considered to be the "gold" standard for defining epileptogenic zones; however, this procedure is risky and highly invasive. Recent studies have explored the development of a noninvasive cortical imaging technique for presurgical planning that may provide similar information and resolution of the invasive ECoG.

In one novel approach, Bin He et al. seek to integrate the information provided by a structural MRI and scalp EEG to provide a noninvasive alternative to ECoG. This study investigated a high-resolution subspace source localization approach, FINE (first principle vectors) to image the locations and estimate the extents of current sources from the scalp EEG. A thresholding technique was applied to the resulting tomography of subspace correlation values in order to identify epileptogenic sources. This method was tested in three pediatric patients with intractable epilepsy, with encouraging clinical results. Each patient was evaluated using structural MRI, long-term video EEG monitoring with scalp electrodes, and subsequently with subdural electrodes. The ECoG data was then recorded from implanted subdural electrode grids placed directly on the surface of the cortex. MRI and computed tomography images were also obtained for each subject.

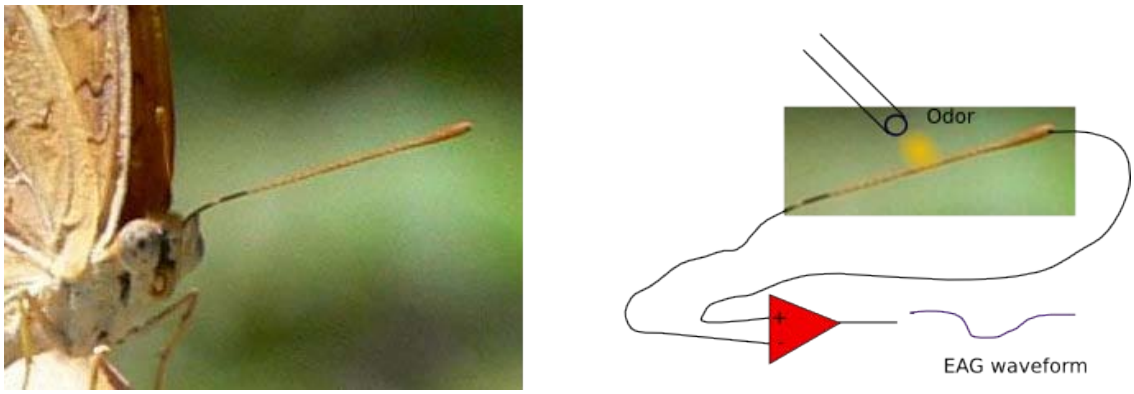
The epileptogenic zones identified from preoperative EEG data were validated by observations from postoperative ECoG data in all three patients. These preliminary results suggest that it is possible to direct surgical planning and locate epileptogenic zones noninvasively using the described imaging and integrating methods. EEG findings were further validated by the surgical outcomes of all three patients. After surgical resectioning, two patients are seizure-free and the third has experienced a significant reduction in seizures. Due to its clinical success, FINE offers a promising alternative to preoperative ECoG, providing information about both the location and extent of epileptogenic sources through a noninvasive imaging procedure.

Electroantennography

Electroantennogram or **EAG** is a technique by which we measure the average output of the antenna to the brain for a given odor. It is commonly used in the electrophysiology while studying the function of olfactory pathway in insects. The technique was invented in 1957 by German biologist Dietrich Schneider.

EAG is performed in two ways:

1. Remove the antenna from the animal and insert two chlorided silver wires for contact onto the two ends and amplify the voltage between them while applying an odor puff to see a deflection as in the figure.
2. Leave the animal intact and insert a ground wire (silver/silver chloride) or a glass electrode filled with a buffer solution to some part of the body, usually inserted into an eye, and another to the tip of the antenna. A large bore glass electrode can also be placed directly over the tip of the antenna, such as in *Drosophila melanogaster* (fruit fly) antenna recordings. The latter method is useful if one is doing an experiment on the animal as a whole while doing the antennogram.



Setup for antennogram

The technique is widely applied in screening of insect pheromones by examining the responses to fractions of a compound mixture separated using chromatography.

Usually the wire inserted into the antenna is a thin silver wire which is chlorided in bleach. This is an older practice. Commonly, tungsten wires which have been chemically sharpened are inserted into a single neuron in the antenna.

Further detailed examination of the odor response at the olfactory sensory level can be done by sensilla recording.

Chapter 20

Electromyography and Electroretinography

Electromyography

Electromyography (EMG) is a technique for evaluating and recording the electrical activity produced by skeletal muscles. EMG is performed using an instrument called an **electromyograph**, to produce a record called an **electromyogram**. An electromyograph detects the electrical potential generated by muscle cells when these cells are electrically or neurologically activated. The signals can be analyzed to detect medical abnormalities, activation level, recruitment order or to analyze the biomechanics of human or animal movement.

Electrical characteristics

The electrical source is the muscle membrane potential of about -90 mV. Measured EMG potentials range between less than 50 μV and up to 20 to 30 mV, depending on the muscle under observation.

Typical repetition rate of muscle motor unit firing is about 7–20 Hz, depending on the size of the muscle (eye muscles versus seat (gluteal) muscles), previous axonal damage and other factors. Damage to motor units can be expected at ranges between 450 and 780 mV.

History

The first documented experiments dealing with EMG started with Francesco Redi's works in 1666. Redi discovered a highly specialized muscle of the electric ray fish (Electric Eel) generated electricity. By 1773, Walsh had been able to demonstrate that the Eel fish's muscle tissue could generate a spark of electricity. In 1792, a publication entitled *De Viribus Electricitatis in Motu Musculari Commentarius* appeared, written by Luigi Galvani, in which the author demonstrated that electricity could initiate muscle contractions. Six decades later, in 1849, Dubois-Raymond discovered that it was also possible to record electrical activity during a voluntary muscle contraction. The first actual recording of this activity was made by Marey in 1890, who also introduced the

term electromyography. In 1922, Gasser and Erlanger used an oscilloscope to show the electrical signals from muscles. Because of the stochastic nature of the myoelectric signal, only rough information could be obtained from its observation. The capability of detecting electromyographic signals improved steadily from the 1930s through the 1950s, and researchers began to use improved electrodes more widely for the study of muscles. Clinical use of surface EMG (sEMG) for the treatment of more specific disorders began in the 1960s. Hardyck and his researchers were the first (1966) practitioners to use sEMG. In the early 1980s, Cram and Steger introduced a clinical method for scanning a variety of muscles using an EMG sensing device.

It is not until the middle of the 1980s that integration techniques in electrodes had sufficiently advanced to allow batch production of the required small and lightweight instrumentation and amplifiers. At present, a number of suitable amplifiers are commercially available. In the early 1980s, cables that produced signals in the desired microvolt range became available. Recent research has resulted in a better understanding of the properties of surface EMG recording. Surface electromyography is increasingly used for recording from superficial muscles in clinical or kinesiological protocols, where intramuscular electrodes are used for investigating deep muscles or localized muscle activity.

There are many applications for the use of EMG. EMG is used clinically for the diagnosis of neurological and neuromuscular problems. It is used diagnostically by gait laboratories and by clinicians trained in the use of biofeedback or ergonomic assessment. EMG is also used in many types of research laboratories, including those involved in biomechanics, motor control, neuromuscular physiology, movement disorders, postural control, and physical therapy.

Procedure

There are two kinds of EMG in widespread use: surface EMG and intramuscular (needle and fine-wire) EMG. To perform intramuscular EMG, a needle electrode or a needle containing two fine-wire electrodes is inserted through the skin into the muscle tissue. A trained professional (such as a neurologist, physiatrist, or physical therapist) observes the electrical activity while inserting the electrode. The insertional activity provides valuable information about the state of the muscle and its innervating nerve. Normal muscles at rest make certain, normal electrical signals when the needle is inserted into them. Then the electrical activity when the muscle is at rest is studied. Abnormal spontaneous activity might indicate some nerve and/or muscle damage. Then the patient is asked to contract the muscle smoothly. The shape, size, and frequency of the resulting motor unit potentials are judged. Then the electrode is retracted a few millimeters, and again the activity is analyzed until at least 10–20 units have been collected. Each electrode track gives only a very local picture of the activity of the whole muscle. Because skeletal muscles differ in the inner structure, the electrode has to be placed at various locations to obtain an accurate study.

Intramuscular EMG may be considered too invasive or unnecessary in some cases. Instead, a surface electrode may be used to monitor the general picture of muscle activation, as opposed to the activity of only a few fibres as observed using an intramuscular EMG. This technique is used in a number of settings; for example, in the physiotherapy clinic, muscle activation is monitored using surface EMG and patients have an auditory or visual stimulus to help them know when they are activating the muscle (biofeedback).

A motor unit is defined as one motor neuron and all of the muscle fibers it innervates. When a motor unit fires, the impulse (called an action potential) is carried down the motor neuron to the muscle. The area where the nerve contacts the muscle is called the neuromuscular junction, or the motor end plate. After the action potential is transmitted across the neuromuscular junction, an action potential is elicited in all of the innervated muscle fibers of that particular motor unit. The sum of all this electrical activity is known as a motor unit action potential (MUAP). This electrophysiologic activity from multiple motor units is the signal typically evaluated during an EMG. The composition of the motor unit, the number of muscle fibres per motor unit, the metabolic type of muscle fibres and many other factors affect the shape of the motor unit potentials in the myogram.

Nerve conduction testing is also often done at the same time as an EMG to diagnose neurological diseases.

Some patients can find the procedure somewhat painful, whereas others experience only a small amount of discomfort when the needle is inserted. The muscle or muscles being tested may be slightly sore for a day or two after the procedure.

Normal results

Muscle tissue at rest is normally electrically inactive. After the electrical activity caused by the irritation of needle insertion subsides, the electromyograph should detect no abnormal spontaneous activity (i.e., a muscle at rest should be electrically silent, with the exception of the area of the neuromuscular junction, which is, under normal circumstances, very spontaneously active). When the muscle is voluntarily contracted, action potentials begin to appear. As the strength of the muscle contraction is increased, more and more muscle fibers produce action potentials. When the muscle is fully contracted, there should appear a disorderly group of action potentials of varying rates and amplitudes (a complete recruitment and interference pattern).

Abnormal results

EMG is used to diagnose diseases that generally may be classified into one of the following categories: neuropathies, neuromuscular junction diseases and myopathies.

Neuropathic disease has the following defining EMG characteristics:

- An action potential amplitude that is twice normal due to the increased number of fibres per motor unit because of reinnervation of denervated fibres
- An increase in duration of the action potential
- A decrease in the number of motor units in the muscle (as found using motor unit number estimation techniques)

Myopathic disease has these defining EMG characteristics:

- A decrease in duration of the action potential
- A reduction in the area to amplitude ratio of the action potential
- A decrease in the number of motor units in the muscle (in extremely severe cases only)

Because of the individuality of each patient and disease, some of these characteristics may not appear in every case.

Abnormal results may be caused by the following medical conditions (please note this is nowhere near an exhaustive list of conditions that can result in abnormal EMG studies):

- Alcoholic neuropathy
- Amyotrophic lateral sclerosis
- Anterior compartment syndrome of the lower leg
- Axillary nerve dysfunction
- Becker's muscular dystrophy
- Brachial plexopathy
- Carpal tunnel syndrome
- Centronuclear myopathy
- Cervical spondylosis
- Charcot-Marie-Tooth disease
- Chronic Immune Demyelinating Poly[radiculo]neuropathy (CIDP)
- Common peroneal nerve dysfunction
- Denervation (reduced nervous stimulation)
- Duchenne muscular dystrophy
- Facioscapulohumeral muscular dystrophy (Landouzy-Dejerine)
- Familial periodic paralysis
- Femoral nerve dysfunction
- Fields condition
- Friedreich's ataxia
- Guillain-Barre
- Lambert-Eaton Syndrome
- Mononeuritis multiplex
- Mononeuropathy
- Motor neurone disease
- Multiple system atrophy
- Myasthenia gravis
- Myopathy (muscle degeneration, which may be caused by a number of disorders, including muscular dystrophy)
- Myotubular myopathy
- Neuromyotonia
- Peripheral neuropathy
- Poliomyelitis
- Polymyositis
- Radial nerve dysfunction
- Sciatic nerve dysfunction
- Sensorimotor polyneuropathy
- Sleep bruxism
- Spinal stenosis
- Thyrotoxic periodic paralysis
- Tibial nerve dysfunction
- Ulnar nerve dysfunction

- Dermatomyositis
- Distal median nerve dysfunction

EMG signal decomposition

EMG signals are essentially made up of superimposed motor unit action potentials (MUAPs) from several motor units. For a thorough analysis, the measured EMG signals can be decomposed into their constituent MUAPs. MUAPs from different motor units tend to have different characteristic shapes, while MUAPs recorded by the same electrode from the same motor unit are typically similar. Notably MUAP size and shape depend on where the electrode is located with respect to the fibers and so can appear to be different if the electrode moves position. EMG decomposition is non-trivial, although many methods have been proposed.

Applications of EMG

EMG signals are used in many clinical and biomedical applications. EMG is used as a diagnostics tool for identifying neuromuscular diseases, assessing low-back pain, kinesiology, and disorders of motor control. EMG signals are also used as a control signal for prosthetic devices such as prosthetic hands, arms, and lower limbs.

EMG can be used to sense isometric muscular activity where no movement is produced. This enables definition of a class of subtle motionless gestures to control interfaces without being noticed and without disrupting the surrounding environment. These signals can be used to control a prosthesis or as a control signal for an electronic device such as a mobile phone or PDA.

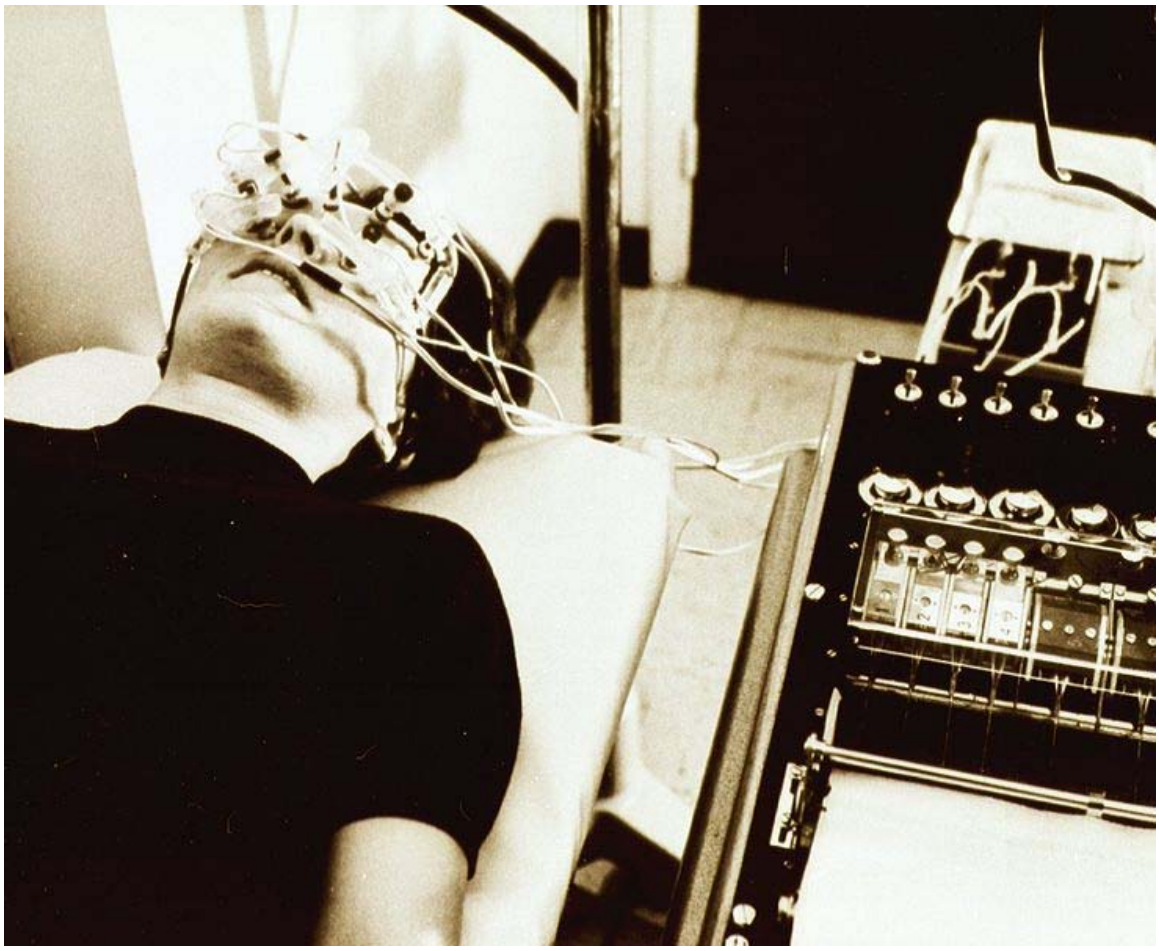
EMG signals have been targeted as control for flight systems. The Human Senses Group at the NASA Ames Research Center at Moffett Field, CA seeks to advance man-machine interfaces by directly connecting a person to a computer. In this project, an EMG signal is used to substitute for mechanical joysticks and keyboards. EMG has also been used in research towards a "wearable cockpit," which employs EMG-based gestures to manipulate switches and control sticks necessary for flight in conjunction with a goggle-based display.

Unvoiced speech recognition recognizes speech by observing the EMG activity of muscles associated with speech. It is targeted for use in noisy environments, and may be helpful for people without vocal cords and people with aphasia.

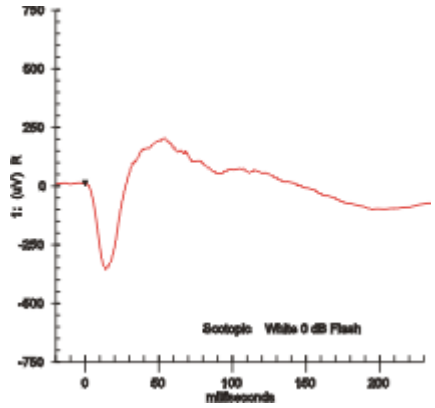
EMG has also been used as a control signal for computers and other devices. An interface device based on EMG could be used to control moving objects, such as mobile robots or an electric wheelchair. This may be helpful for individuals that cannot operate a joystick-controlled wheelchair. Surface EMG recordings may also be a suitable control signal for some interactive video games.

A joint project involving Microsoft, the University of Washington in Seattle, and the University of Toronto in Canada has explored using muscle signals from hand gestures as an interface device. A patent based on this research was submitted on June 26, 2008.

Electroretinography



A patient undergoing an electroretinogram



Maximal response ERG waveform from a dark adapted eye

Electroretinography measures the electrical responses of various cell types in the retina, including the photoreceptors (rods and cones), inner retinal cells (bipolar and amacrine cells), and the ganglion cells. Electrodes are usually placed on the cornea and the skin near the eye, although it is possible to record the ERG from skin electrodes. During a recording, the patient's eyes are exposed to standardized stimuli and the resulting signal is displayed showing the time course of the signal's amplitude (voltage). Signals are very small, and typically are measured in microvolts or nanovolts. The ERG is composed of electrical potentials contributed by different cell types within the retina, and the stimulus conditions (flash or pattern stimulus, whether a background light is present, and the colors of the stimulus and background) can elicit stronger response from certain components.

If a flash ERG is performed on a dark-adapted eye, the response is primarily from the rod system. Flash ERGs performed on a light adapted eye will reflect the activity of the cone system. Sufficiently bright flashes will elicit ERGs containing an a-wave (initial negative deflection) followed by a b-wave (positive deflection). The leading edge of the a-wave is produced by the photoreceptors, while the remainder of the wave is produced by a mixture of cells including photoreceptors, bipolar, amacrine, and Muller cells or Muller glia. The pattern ERG, evoked by an alternating checkerboard stimulus, primarily reflects activity of retinal ganglion cells.

Clinically used mainly by ophthalmologists and optometrists, the **electroretinogram (ERG)** is used for the diagnosis of various retinal diseases.

Inherited retinal degenerations in which the ERG can be useful include:

- Retinitis pigmentosa and related hereditary degenerations
- Retinitis punctata albescens
- Leber's congenital amaurosis
- Choroideremia
- Gyrate atrophy of the retina and choroid
- Goldman-Favre syndrome

- Congenital stationary night blindness - *normal a-wave indicates normal photoreceptors; absent b-wave indicates abnormality in the bipolar cell region.*
- X-linked juvenile retinoschisis
- Achromatopsia
- Cone dystrophy
- Disorders mimicking retinitis pigmentosa
- Usher Syndrome

Other ocular disorders in which the standard ERG provides useful information include:

- Diabetic retinopathy
- Other ischemic retinopathies including central retinal vein occlusion (CRVO), branch vein occlusion (BVO), and sickle cell retinopathy
- Toxic retinopathies, including those caused by Plaquenil and Vigabatrin. The ERG is also used to monitor retinal toxicity in many drug trials.
- Autoimmune retinopathies such as Cancer Associated Retinopathy (CAR), Melanoma Associated Retinopathy (MAR), and Acute Zonal Occult Outer Retinopathy (AZOOR)
- Retinal detachment
- Assessment of retinal function after trauma, especially in vitreous hemorrhage and other conditions where the fundus cannot be visualized.

The ERG is also used extensively in eye research, as it provides information about the function of the retina that is not otherwise available.

Other ERG tests, such as the Photopic Negative Response (PhNR) and pattern ERG (PERG) may be useful in assessing retinal ganglion cell function in diseases like glaucoma.

The **multifocal ERG** is used to record separate responses for different retinal locations.

The international body concerned with the clinical use and standardization of the ERG, EOG, and VEP is the International Society for the Clinical Electrophysiology of Vision.