Physiology

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CHARLES ASBURY; FRED RIEKE; BERTIL HILLE; MARK BOTHWELL; AND JOHN TUTHILL

Contents

Introduction to Physiology

CHARLES ASBURY; FRED RIEKE; BERTIL HILLE; MARK BOTHWELL; AND JOHN TUTHILL

Table of Contents

Learning Objective #5. Describe the dynamics of microtubules. Understand the importance of microtubule dynamics for mitosis, and why drugs that inhibit microtubule disassembly are used to treat cancer.

Extra Q&A from past students that might be helpful

Cell Membranes and Transport

FRED RIEKE AND BERTIL HILLE

Cell membranes

Learning Objective #1. Describe the structure and topology of cellular membranes.

Learning Objective #2. Contrast ion channels and ion transporters and the forces that drive them.

Learning Objective #3. Know the normal balance of Na^+, K^+, Cl^- and Ca^{2+} with respect to the plasma membrane.

Practice questions and review topics

G Protein Coupled Receptors

MARK BOTHWELL

Introduction: What is Cellular Signaling?

Learning Objective #1. Describe in detail the activation of GPCR signaling including receptors, ligands & heterotrimeric G-proteins.

Learning Objective #2. Know different G-proteins coupling to different second messengers.

Learning Objective #3. Describe two effector pathways: cAMP and PLC.
Learning Objective #4. Know how GPCR signaling is terminated.

Receptor tyrosine kinases and nuclear hormone receptors

MARK BOTHWELL

Introduction

Learning objective #1. Describe the structure and function of RTKs.

Learning objective #2. Describe signaling mechanisms used by RTK signaling (MAPK cascades).

Learning objective #3. Describe crosstalk and interactions between pathways.

Learning objective #4. Describe steroid hormone receptors.

Learning objective #5. Describe steroid hormone receptor mechanisms of activity.

Conclusion

Membrane Potentials

FRED RIEKE AND BERTIL HILLE

Learning Objective #1. Describe the elements of ionic electricity: ions, charge, potential gradients, forces, current, and conductance.
Learning Objective #2. Know how ion diffusion in ion channels establishes membrane potentials.

Learning Objective #3. Recognize that fluxes needed to make typical membrane potentials are extremely small: membrane capacitance.
Learning Objective #4. Explain with Na+, K+, Cl−, and Ca2+ what contribution each could make individually to electrical potential changes in the plasma membrane of excitable cells.
Review questions
Practice questions

Action Potential, Threshold, Refractory Period

FRED RIEKE
Overview
Key properties of action potentials
Quick review of membranes, ions and channels (see also Membrane Transport
and Membrane Potentials chapters)
Resting potential
Learning Objective #1. Explain the ionic basis of the action potential
Learning Objective #2. Explain the concept of threshold in terms of the underlying ion channel activity
Learning Objective #3. Describe the refractory period and how it is produced mechanistically
Practice questions

Action Potential Propagation

FRED RIEKE

Synapses and Neurotransmitter Receptors

Sensory Receptors

JOHN TUTHILL

Learning Objective #1: To know the general properties of sensory receptors

Learning Objective #2. To understand the labeled-line principle of signal detection

Learning Objective #3: To compare the mechanisms of sensory transduction in different types of sensory receptors

Light Receptors (Vision)

Chemoreceptors (Smell and Taste)

Mechanoreceptors (Touch and Proprioception)

Hair Cell Receptors (Hearing and Balance)

Learning Objective #4: To appreciate how the intensity and duration of a stimulus are reflected in the receptor potential and action potential discharge rate of a sensory afferent neuron.

Learning objective #5. To understand how sensory receptors adapt to a constant stimulus.

Autonomic Nervous System Physiology

JOHN TUTHILL

Learning objective #1: Compare and contrast the neurotransmitters and receptor types in the somatic motor, parasympathetic autonomic motor, and sympathetic autonomic motor divisions of the nervous system. Include the neurotransmitter- receptor pairs in the ANS two-neuron pathways.

Learning objective #2: Identify epinephrine / norepinephrine receptor types and their effects on various target organs

Learning Objective #3: Compare nicotinic and muscarinic

acetylcholine receptor activation and identify acetylcholine receptor types and their effects on various target organs.
Learning objective #4: Identify the role of ATP and nitric oxide in smooth-muscle relaxation and blood-vessel dilation.
Learning objective #5: Describe the Baroreceptor reflex in response to high or low blood pressure.
Learning objective #6: Define orthostatic hypotension and discuss how the baroreflex counters it.

Nociception and Spinal Reflexes

JOHN TUTHILL
Summary: Nociception and Spinal Reflexes
Learning Objective #1: To know how nociceptors are activated and sensitized.
Learning Objective #2: To understand difference between reflexes and other types of movements
Learning Objective #3: To differentiate the neural circuits underlying the flexion and stretch reflexes
Learning Objective #4: To understand how reflex testing can be used clinically to diagnose neuropathologies that affect motor and sensory function.

Muscle Physiology

CHARLES ASBURY
Introduction: Why is muscle physiology important?
Learning Objective #1: Explain the mechanism by which muscle

contracts, outlining how the sliding of actin filaments in sarcomeres is driven by ATP-dependent chemo-mechanical cycling of myosin motor proteins.

Learning Objective #2: Explain excitation-contraction coupling and relaxation in skeletal muscle by identifying the roles of the t-tubules, calcium channels (Cav1.1 and the ryanodine receptor), thin filament regulators (troponin and tropomyosin), and ATP-dependent calcium pumps.

Learning Objective #3: Compare twitch contractions for slow/type 1 and fast/type 2 skeletal muscle fibers and explain the molecular bases for the differences in twitch behavior. Define isometric and isotonic contractions.

Learning Objective #4: Explain how smooth, graded contractions of a skeletal muscle are produced by changes in stimulus intensity and by the size principle of motor unit recruitment.

Learning Objective #5: Understand the differences in excitation-contraction coupling between skeletal, cardiac, and smooth muscle. Describe the two-stage phospho-regulatory cascade that initiates smooth muscle contraction.

Learning Objective #6: Compare and contrast how skeletal, cardiac and smooth muscle are controlled by the nervous system. Define single-unit vs. multi-unit smooth muscle types.

Extra Q&A from past students that might be helpful

Cytoskeleton, Molecular Motors, and Cell Motility

CHARLES ASBURY

What is Cell Motility?

Cell motility refers to the movement of cells and the transport of organelles and other particles within cells. The most familiar example of cell motility is the action of muscle cells. There are also medically important forms of non-muscle motility. Examples of non-muscle motility are the beating of cilia lining the lungs, the movement of vesicles inside nerve axons, and mitosis, the separation of duplicated chromosomes in dividing cells. Many forms of life exist without muscle cells, but every living thing depends on motility of some form. The sarcomeres inside skeletal muscle cells are essentially permanent structures with nearly crystalline regularity. In contrast, the molecular machinery of non-muscle motility is often transiently assembled and then dismantled after use.

Session Learning Objectives

1. Compare molecular architectures of the three types of

cytoskeletal filaments: microtubules, actin filaments, and intermediate filaments. Understand the structural polarity of microtubules and actin filaments, and why intermediate filaments are not polar.

2. Describe how cytoskeletal filaments provide frameworks that underlie specialized structures within cells, including the role of cell-cell and cell-matrix junctions for transmitting forces between cells. Describe how junctional defects can cause human disease.

3. Describe how a molecular motor produces movement and force via its chemo-mechanical cycle. Understand how filament polarity determines the directionality of motor movement.

4. Understand how the molecular motor dynein drives the beating motions of cilia and flagella. Describe how dysfunctional cilia can cause human disease.

5. Describe the dynamics of microtubules. Understand the importance of microtubule dynamics for mitosis, and why drugs that inhibit microtubule disassembly are used to treat cancer.

Learning Objective #1. *Compare molecular architectures of the three types of cytoskeletal filaments, microtubules, actin filaments, and intermediate filaments. Understand the structural polarity of microtubules and actin filaments, and why intermediate filaments are not polar.*

Most cells in our bodies are packed with filament networks, which are collectively known as the **cytoskeleton**. These filaments provide cells with rigidity and toughness to withstand mechanical perturbations. There are three types of filaments, called **F-actin**, **microtubules**, and **intermediate filaments**. All three are protein polymers. They are composed of thousands of identical protein subunits that assemble into much larger filamentous structures. The assembly of both F-actin and microtubules is coupled to the hydrolysis of nucleotide triphosphates (either adenosine triphosphate (ATP) or guanine triphosphate (GTP)), which provide a source of energy that allows the filaments to grow and shorten in a very dynamic fashion.

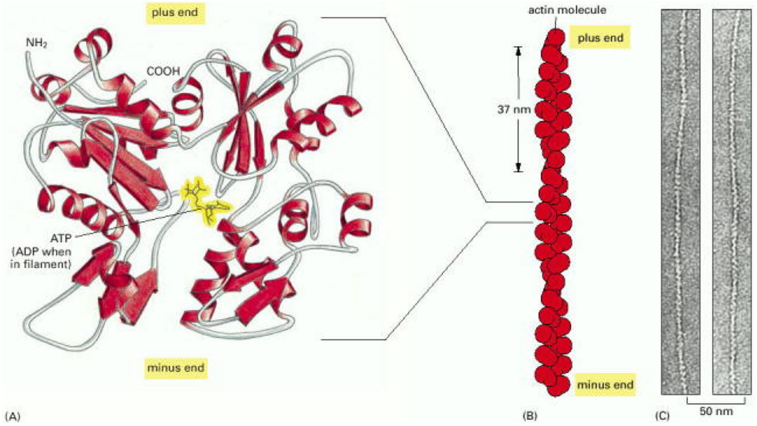

Figure 1. Globular actin molecules assemble into helical polymers. (*From Alberts, Molecular Biology of the Cell*)

First, consider the structure of F-actin filaments (see Figure 1). These are assembled from a compact, globular protein called **G-actin**. (The G- stands for globular, and the F- stands for filamentous.) An individual G-actin molecule is like a very tiny LEGO block, only a few nanometers across. It has a **polarity**, and it has several facets where it binds to other G-actin molecules. As a consequence of the arrangement of these facets, the molecules

assemble into two rows that gently spiral around one another. A typical F-actin filament may contain several hundred molecules, spanning a micrometer (10^{-6} m) in length. Because the individual subunits have polarity, the two ends of the filament are different. Often, the two ends are labeled 'plus' and 'minus' ends. The plus ends grow more quickly than the minus ends. (Caution: 'plus' does ***not*** refer to electrical charge.) The head-to-tail arrangement of subunits also gives structural polarity to filaments, allowing them to act as substrates (tracks) for directional motor transport (as discussed below in Learning Objective #3).

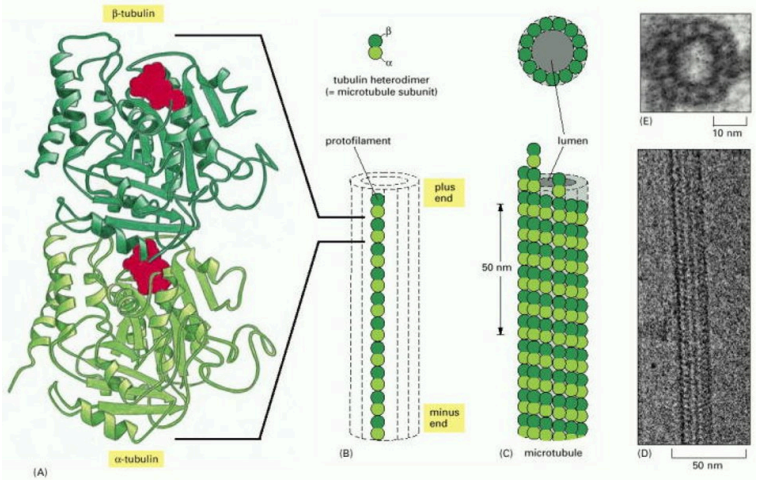

Figure 2. Tubulin subunits assemble into microtubules. (*From Alberts, Molecular Biology of the Cell*)

The structure of a microtubule filament (Figure 2) is conceptually similar to that of F-actin, but slightly more complicated. The elementary building block of a microtubule is a pair of globular proteins called **α- and β tubulin**, which associate very tightly with one another to form a heterodimer, a few nanometers in length. Each tubulin heterodimer has facets that bind other tubulin heterodimers (again like LEGO blocks), allowing them to associate

in a head-to-tail fashion, and also side-to-side, to form a tiny cylindrical tube. Within the walls of the tube, heterodimers are arranged head-to-tail in longitudinal rows, called protofilaments, which run parallel to the long axis of the tube. The side-to-side bonds between heterodimers hold these protofilaments together laterally. A typical microtubule might contain several thousand heterodimers, spanning several micrometers in length. Each microtubule, like each F-actin filament, has two ends which are different, called the plus and minus ends. (Again, the structural polarity allows microtubules to act as substrates for directional motor transport, as discussed below, under Learning Objective #3. And the plus ends grow more quickly than the minus ends, a concept that will be covered in more detail under Learning Objective #5.)

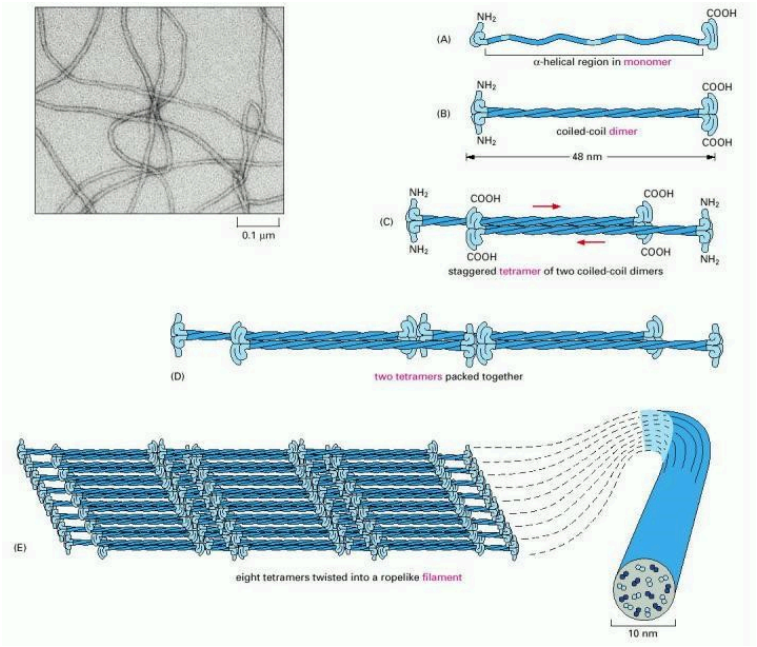

Figure 3. Architecture of an intermediate filament. (*From Alberts, Molecular Biology of the Cell*)

Intermediate filaments are assembled in an altogether different manner than microtubules or actin filaments. The elementary subunits do not bind nucleotides, and they are slender fibrils (rather than compact globular proteins), which pack together in a staggered arrangement (Figure 3). The subunits bind one another in an anti-parallel fashion and form filaments that lack structural polarity. As a consequence of their lack of polarity, intermediate filaments cannot provide useful tracks for motor protein-based transport. However, intermediate filaments are very important for the toughness of cells and tissues, so they can resist forces. A variety of different intermediate filaments exist, including **keratins** (found in skin and hair), **desmins** (found in muscle), and **neurofilaments** (brain and other neurons). The diversity of intermediate filaments makes them useful for identification of different types of cells.

Simple exercises to check what you recall

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Learning Objective #2. *Describe how cytoskeletal filaments provide frameworks that underlie specialized structures within cells, including the role of cell-cell and cell-matrix junctions for transmitting forces between cells. Describe how junctional defects can cause human disease.*

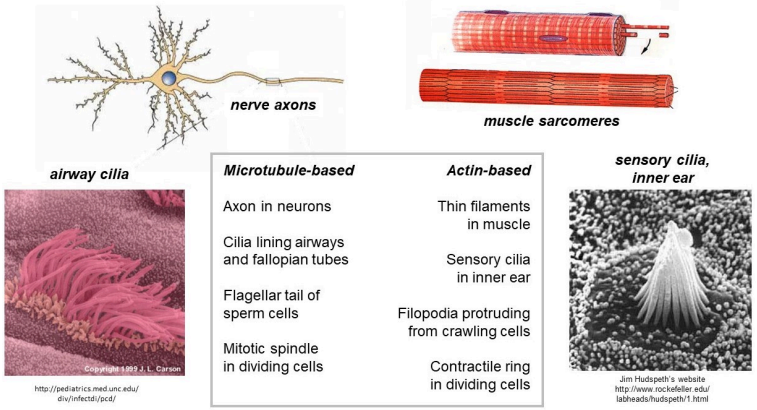

Figure 4: Polarized arrays of cytoskeletal filaments provide the framework around which many specialized cellular structures are built.

Cytoskeletal filaments provide the framework for various specialized structures in cells (Figure 4). The filaments in these structures often have identical **polarity**, with plus ends pointing in the same direction. One such structure is the muscle **sarcomere**. The thin filaments within sarcomeres are composed primarily of **F-actin** filaments with their plus ends all embedded in the z-bands (z-disks) on the left and right sides, and their minus ends pointing toward the middle (the m-line). Other examples of specialized structures that contain polarized F-actin are (i) the sensory cilia in

the inner ear, responsible for hearing, (ii) the filopodia that protrude from crawling cells (e.g., of our immune systems), and (iii) the contractile ring which drives fission of dividing cells. Polarized arrays of **microtubules** are found in (a) the axons of nerve cells, (b) the cilia lining our airways, (c) the flagellar tail of sperm cells, and (d) the mitotic spindle that drives chromosome separation in dividing cells.

Cytoskeletal junctions allow force transmission between neighboring cells

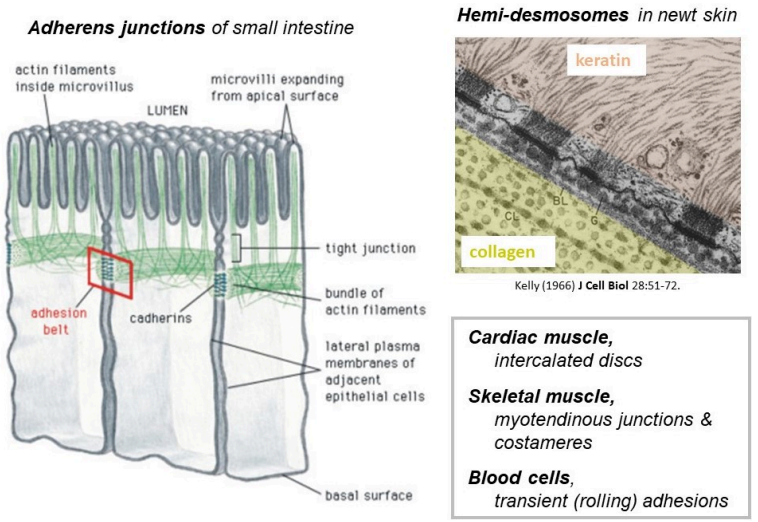

Figure 5: Junctions connect the cytoskeletal filaments of neighboring cells, allowing force transmission. (From Alberts, *Molecular Biology of the Cell*)

The cytoskeletal filaments described above are found exclusively inside cells and, as already mentioned, they provide toughness that allows the cells to withstand mechanical forces. The forces carried by cytoskeletal filaments are transmitted from one cell to a

neighboring cell, or to the extracellular matrix, by specialized junctions (Figure 5). The junctions are collections of membrane-spanning proteins that connect to cytoskeletal filaments inside the cell, to identical junctional proteins in the plasma membrane of a neighboring cell, or to extracellular matrix proteins. There are many different types of junctions found in different cellular contexts. Some examples are the **adherens junctions** that connect F-actin filaments in neighboring epithelial cells lining the lumen of your small intestine, and the **hemi-desmosomes** that connect keratin intermediate filaments inside your skin cells to extracellular matrix fibers such as laminin on the outside. The **intercalated discs** that connect cardiac muscle cells to one another, and the **myotendinous junctions** that connect skeletal muscles to tendons are also examples of cytoskeletal junctions.

Pathology note: The importance of cytoskeletal junctions for human health is illustrated by pemphigus, an autoimmune disease where antibodies target desmoglein. Desmoglein is a transmembrane protein (a 'cadherin') that links intermediate filaments in neighboring skin epithelial cells. The anti-desmoglein antibodies found in pemphigus patients disrupt these junctions, allowing epithelial cells to be torn apart much more easily than they should be, which causes severe blistering.

Simple exercises to check what you recall

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Learning Objective #3. *Describe how a molecular motor produces movement and force via its chemo-mechanical cycle. Understand how filament polarity determines the directionality of motor movement.*

One way that F-actin and microtubule filaments participate in cell motility is by serving as tracks along which molecular motors move. The most familiar example occurs in muscle contraction, where F-actin (in the thin filaments) serves as a track along which myosin motor proteins drive movement (of the thick filaments). Two examples of non-muscle movement driven in essentially this same way are the translocation of vesicles in nerve axons, and the beating of cilia, which are discussed below.

Axonal transport: single motors hauling vesicles

Because of their highly elongated shape, neurons rely heavily on intracellular transport. Smaller cells can rely on diffusion to deliver proteins, metabolites, second messengers, and so forth, from their sites of synthesis to their sites of action. But diffusion is far too slow in long, slender neurons. Consider, for example, the extraordinary length of individual motor neurons innervating your toe. These cells have axons that project all the way from the base of your spine to your toe, about 1 meter away. Even the smallest, fastest diffusing metabolites would take years to diffuse from where they are made, in the cell body (the soma, in your spine) to where they are needed, at the end of the axon (neuromuscular junction, in your toe). These extremely long cells must rely on active transport to carry cargo down their axons.

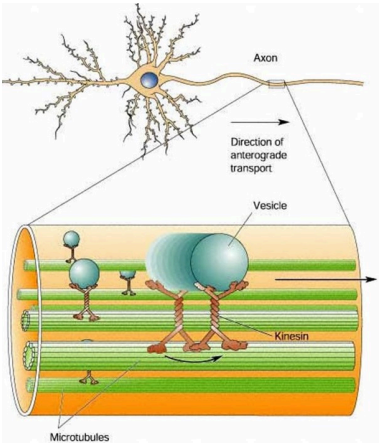

Figure 6: Cytoskeletal filaments provide polarized tracks for molecular motor-driven transport. Microtubules inside nerve axons, for example, have their minus ends pointing toward the cell body, and their plus ends pointing away from the cell body.

Fast axonal transport can be observed in living neurons. Some particles (typically, membrane-bound vesicles) move outward from the cell body toward the axon terminus, in the **anterograde** direction. These contain molecules important for synaptic transmission. Other particles move back toward the soma, in the **retrograde** direction. These might contain recycled synapse components, or signaling molecules for communicating the state of the nerve terminus back to the soma. Anterograde and retrograde transport both occur along microtubules, which are densely packed within axons (Figure 6), always with their plus ends

pointing away from the soma. Anterograde movement is driven by plus-end-directed motors called **kinesin**. Retrograde movement is driven by minus-end-directed motors called **dynein**. Many of the particles are probably driven by single motor molecules.

What defines a molecular motor?

Molecular motors are amazing enzymes that convert chemical energy, usually in the form of adenosine triphosphate (ATP), into mechanical work. The myosins that power muscle contraction are the most famous molecular motors. But hundreds of other motors are found in human cells. Luckily, most of them operate by the same general principles (Figure 7).

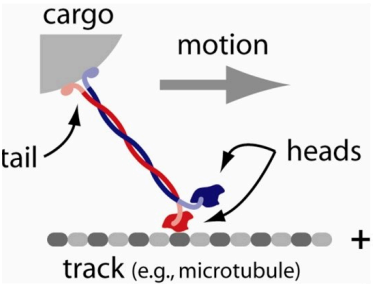

Figure 7: The basic elements of a molecular motor (in this case, kinesin).

Generally, a molecular motor…

(i) has a specific track and directionality,

(ii) has one or more domains, sometimes called heads, which attach directly to the track,

(iii) has a tail domain that attaches cargo,

(iv) requires a chemical fuel, usually ATP, to produce movement and force, and

(v) moves by a cyclical process where the heads repeatedly attach to the track, undergo a power stroke (i.e., take a step), detach from the track, and then reset for the next cycle.

The action of a motor protein is strikingly sophisticated, and similar in many ways to human-made machines: The mechanical cycle of a motor protein's heads (attach, stroke, detach, reset) is tightly coupled to a biochemical cycle that includes binding of an ATP (fuel) molecule, hydrolysis (breakage) of the high-energy gamma-phosphate bond of the ATP to produce ADP and phosphate, and then release of these reaction products.

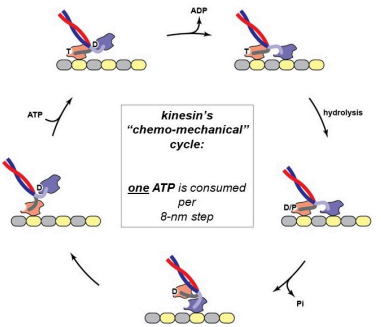

Figure 8: The chemo-mechanical cycle of the motor protein, kinesin. An animation of this kinesin cycle can be found here: https://www.youtube.com/watch?v=YAva4g3Pk6k

Because chemical and mechanical activities are coupled together, this process is called the **chemo-mechanical cycle** of the motor. The chemo-mechanical cycle of kinesin is diagrammed at right (Figure 8) and animated in the 2-minute video below (*includes narration*).

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The movement produced by molecular motors can be observed directly in a microscope using an **in vitro motility assay.** For example, kinesin motors attached to a glass surface will drive ATP-dependent gliding of microtubule filaments, as seen in this 14-second video (*this one has no sound*):

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Most motors that participate in cell motility fall into three families, the **kinesin**, **dynein** and **myosin** families (see Table 1). These families are defined by the detailed molecular architecture of the motors, which is beyond the scope of this class. However, a few general points are worth remembering. First, all motors from the myosin family move along F-actin filaments, and all motors from the kinesin and dynein families move along microtubules. Second, motors with opposite directionality can be found within the families. That is, there are some kinesin motors that move toward the microtubule plus end, and some that move toward the minus end. The same goes for myosins, and probably also for dyneins.

Motor	Track & direction	Examples of cellular function
Kinesin 1	microtubules, to ***plus*** end	vesicle transport in neurons
Kinesin 2	microtubules, to ***plus*** end	axoneme assembly
Kinesin 5 ("Eg5")	microtubules, to ***plus*** end	mitotic spindle assembly
Kinesin 14 ("Ncd")	microtubules, to ***minus*** end	meiotic spindle assembly
Dynein, Axonemal	microtubules, to ***minus*** end	beating of cilia, flagella
Dynein, Cytoplasmic	microtubules, to ***minus*** end	vesicle transport
Myosin II	F-actin, to ***plus*** end	muscle contraction
Myosin V	F-actin, to ***plus*** end	vesicle transport
Myosin VI	F-actin, to ***minus*** end	stereocilia (ear) development

Table 1: Examples of the three major families of molecular motors, kinesins (red), dyneins (green), and myosins (blue).

Some motors work in groups, others work alone

Some cell movements are driven by large numbers of motors working together, while others are driven by individual motor molecules working alone. Large numbers of myosin motors work together to drive muscle contraction. Similarly, large numbers of

dynein motors work together to produce the whip-like movement of a cilium (as discussed below, under Learning Objective #4). In contrast, single kinesin and dynein motors working alone can transport vesicles in neurons.

Motors that work alone have a special property, called **processivity**, which allows them to efficiently drive transport at the single molecule level. Processivity is defined as the ability of a motor molecule to generate many stepwise movements along its track (implying many rounds of ATP hydrolysis) without detaching from the filament. Processive motors literally walk along their filamentous tracks using a mechanism that is sometimes called 'hand-over-hand', because of its similarity to how one climbs a rope, or how a child at the playground travels across 'monkey bars'. (The mechanism is illustrated in the 'Kinesin walking' video above.)

Simple exercises to check what you recall

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Learning Objective #4. *Understand how the molecular motor dynein drives the beating motions of cilia and flagella. Describe how dysfunctional cilia can cause human disease.*

The whip-like movements of cilia and flagella are medically relevant examples of cell motility driven by molecular motors. Motile cilia are important for clearing mucus (and embedded pathogens) from our airways, for moving eggs through the fallopian tube to the uterus, and for circulating cerebrospinal fluid through the brain and spinal cord. Flagella, which are morphologically and functionally identical to cilia, drive sperm movement. Both cilia and flagella are driven by large collections of dynein motors. We will focus on cilia; but the same principles apply to flagella and cilia.

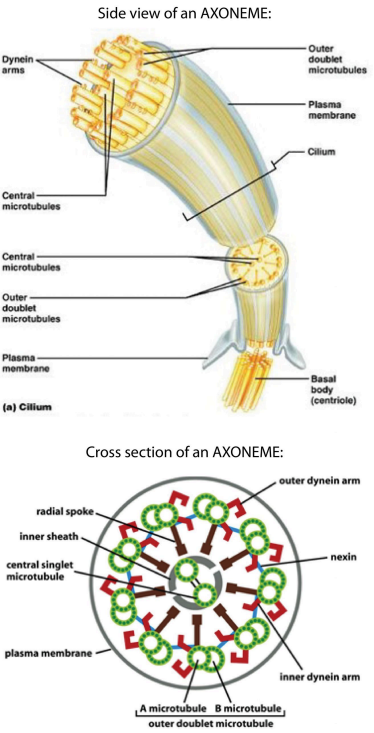

Figure 9. Arrangement of microtubules and dynein motors within the axoneme of a motile cilium or flagellum. (*Side view from website of Dr. Michael Blaber, cross section from studyblue.com*)

How ciliary movement is generated

To understand how cilia beat, one must understand the

arrangement of their cytoskeletons. All cilia contain a specialized, microtubule-based structure called an **axoneme** (Figure 9). Eleven parallel microtubules usually span the length of the structure, with their minus ends anchored at the base and their plus ends at the tip. Dynein motors, attached at regular intervals along the doublet microtubules, serve as the engines that drive whip-like movement of motile cilia.

Each ciliary dynein motor is permanently fastened by its tail to one microtubule. Its head can reach across to a neighboring microtubule, making transient attachments that move toward the minus end at the base of the axoneme. These movements tend to drive the filaments to slide past one another (similar to the relative sliding of thick and thin filaments in muscle). However, in the axoneme the filaments are anchored together at their base. This anchoring resists the sliding movement and, as a consequence, the sliding is converted into bending (Figure 10).

Non-motile cilia often serve as signaling 'antennas'

Not all cilia exhibit the motility described above. Non-motile cilia also play key roles in sensory perception and intercellular signaling. The outer segments of rod and cone photoreceptors, which sense light in our retinas, are specialized cilia. We sense odors through cilia that protrude from olfactory receptor neurons in our noses. The sensory receptor molecules of phototransduction and olfaction (which are G-protein coupled receptors, or GPCRs) are therefore found primarily in these specialized cilia. Moreover, almost every human cell carries a single, non-motile cilium called a **primary cilium**. The non-motile cilia are built around microtubule-based axonemes, just like those inside motile cilia but lacking certain elements, such as dyneins, that would be required for generating movement. The ubiquitous primary cilia were assumed for decades to be functionless, vestigial

remnants of evolution. But recent work shows that they are vital antennas for intercellular signaling, particularly during embryo development. A core developmental signaling pathway called the Hedgehog pathway is completely dependent on primary cilia. Hedgehog receptors and their downstream molecular targets are found in primary cilia, where the initial steps of Hedgehog signal transduction occur. Cilia contain no ribosomes. Thus the receptor molecules and their downstream effectors cannot be produced there. Instead, these molecules are delivered via an **intra-flagellar transport** system involving kinesin and dynein motor proteins.

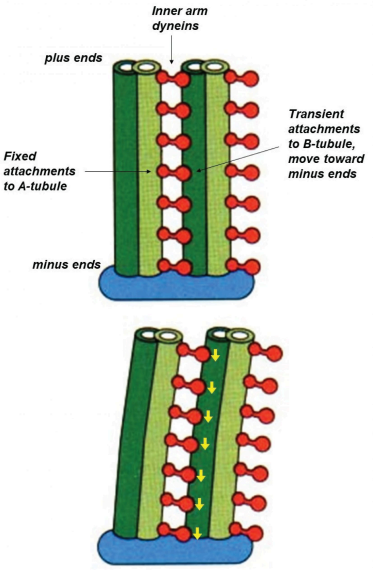

Figure 10. Dynein drives filament sliding, which causes bending of the axoneme due to minus-end anchorage at the base. (From Alberts, *Molecular Biology of the Cell*)

Dysfunction of cilia causes diseases known as 'ciliopathies'

The wide variety of problems that can result from impaired cilia is a testament to the vital importance of cilia for human health. Defects in non-motile sensory or primary cilia can cause kidney disease, blindness, obesity, abnormal heart formation, polydactyly (extra fingers or toes), cleft palate, and other developmental malformations (see Table 2). Defects in the movement of motile cilia can cause severe mucous buildup in the lungs, and infertility. A host of rare, congenital syndromes, each associated with a constellation of symptoms that naively seem unrelated, are now recognized as **ciliopathies**, caused by various defects in ciliary function. Three example ciliopathies, outlined in Table 2, are caused by impairment of molecules that control entry of cargoes into the cilium or regulate intra-flagellar transport within the cilium. The very broad range of defects can be explained by the central role that intra-flagellar transport plays in delivering many cargoes required for ciliary signaling in many different tissues.

Ciliopathy	Associated symptoms	Defective ciliary functions (and examples of molecules implicated)
Bardet-Biedl syndrome	Obesity Retinitis pigmentosa Polycystic kidney disease Polydactyly (extra fingers, toes) Hypogonadism Anosmia (inability to smell)	Motor-driven cargo transport inside cilium (BBS1, BBS2), Regulation of cargo entry at the base of the cilium
Joubert syndrome	Ataxia (lack of muscle control) Polydactyly Cleft lip or palate	Regulation of cargo entry at the base of the cilium (TMEM67)
Meckel syndrome	Cystic kidney disease Liver fibrosis and malformation Malformations in CNS Polydactyly Cleft palate or lip Cardiac abnormalities Incomplete gonads	Regulation of cargo transport inside cilium (MKS1), Regulation of cargo entry at the base of the cilium

Table 2. Some ciliopathies caused by impairment of signaling in primary, non-motile cilia.

Simple exercises to check what you recall

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Learning Objective #5. *Describe the dynamics of microtubules. Understand the importance of microtubule dynamics for mitosis, and why drugs that inhibit microtubule disassembly are used to treat cancer.*

The emphasis above on motor-based movements might give the impression that cytoskeletal filaments play a static role in cell motility, acting solely as tracks along which molecular motors move. While this is true in some contexts (e.g., nerve axons, motile cilia, and muscle sarcomeres), in other contexts, the filaments play a much more dynamic role. Below is a brief (7-second) movie, where you can see dynamic F-actin (tagged with a fluorescent marker) at the leading edge of a cell that is crawling upward in the image. The leading edge of the cell is pushed upward by the rapid growth of the F-actin filaments, which are simultaneously flowing backwards (*this movie has no sound*):

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In dividing cells, bundles of microtubules constantly grow and

shorten, pushing and pulling the chromosomes back-and-forth. To understand how filament dynamics can contribute to cell motility, it is important to understand the dynamics exhibited by the filaments alone. We will focus on microtubules.

The 'dynamic instability' of microtubule filaments

Microtubules spontaneously grow and shorten by addition and loss of tubulin subunits from their tips (Figure 11). They also exhibit a behavior called **dynamic instability**, that can be summarized as follows: In solutions containing free tubulin and GTP, growing and shortening microtubules coexist. When individual filaments are followed over time, they randomly switch back-and-forth between growth and shortening, exhibiting large fluctuations in length, as seen in this 32-second video (*includes narration*):

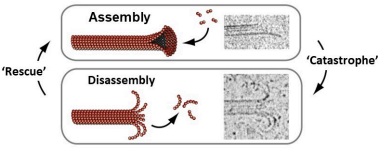

Figure 11: Microtubules grow and shorten by addition and loss of tubulin subunits from their tips. (*Electron micrographs from Chretien 1995 J Cell Biol 129:1311-28; and Mandelkow 1991 J Cell Biol 114:977-91.*)

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Each of the tubulin subunits that makes up a microtubule is an enzyme, containing a pocket that binds guanine nucleotides (see Figure 2) and catalyzes nucleotide hydrolysis. The dynamic instability of microtubule filaments is powered by this hydrolysis of GTP (Figure 12). Under physiological conditions, only GTP-containing tubulin is competent for assembly onto growing filament tips. GDP-containing tubulin cannot assemble. Thus, newly-added subunits at growing microtubule tips contain GTP (forming a 'cap' of GTP-tubulin). As more growth occurs these subunits become buried deeper in the filament wall, where they are stimulated to hydrolyze their GTP, releasing phosphate but retaining GDP. Hydrolysis causes a conformational change in the tubulin that weakens side-to-side interactions between neighboring subunits in the microtubule lattice, thereby destabilizing the GDP-containing portion of the filament. Despite this destabilization, the GDP lattice will remain intact and the filament will continue to grow, provided that a GTP cap exists at the tip. If the GTP cap is lost, however, the GDP lattice quickly disassembles. This explains why microtubules can suddenly switch from slow growth to rapid shortening, a transition colorfully named a 'catastrophe'. Shortening microtubules can also suddenly resume growth, a transition known as a 'rescue', which presumably occurs when the GTP cap spontaneously re-forms.

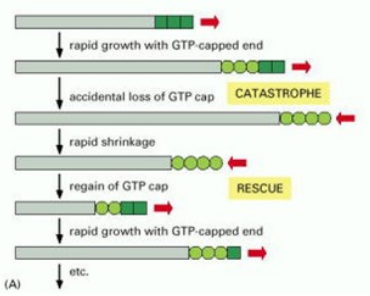


Figure 12. Microtubule dynamic instability. Dark green squares represent tubulin subunits carrying GTP, newly added to a growing microtubule tip. Light green circles represent buried subunits that have hydrolyzed their GTP, released phosphate, and retain only GDP. (From Alberts, Molecular Biology of the Cell)

F-actin dynamics and treadmilling

F-actin filaments exhibit a treadmilling behavior, which differs from the dynamic instability of microtubules but shares a dependence on nucleotide hydrolysis. G-actin is an enzyme with a pocket for binding and hydrolyzing ATP (see Figure 2). Its assembly onto filaments occurs predominantly by addition of ATP-containing monomers. Incorporation into a filament stimulates the monomers to hydrolyze ATP and release phosphate, so the filaments retain only ADP. Because the assembly and disassembly processes are not chemical reversals of one another, it is possible for the two ends to behave quite differently: For example, one end can be assembling while the other is disassembling. This is called treadmilling.

Common features of dynamic protein polymers

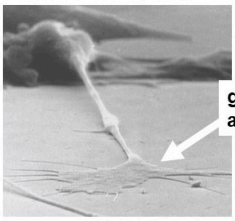
The following features are shared by the dynamic protein polymers, F-actin and microtubules:

(i) Filaments spontaneously grow by addition and loss of subunits from their tips.
(ii) Each subunit has a binding pocket for nucleotide (G-actin binds ATP, tubulin binds GTP).
(iii) Assembly occurs by addition of NTP-bound monomers to tips.
(iv) Assembly into filament promotes NTP hydrolysis. Thus, apart from the freshly-assembled cap, filaments retain only NDP-bound subunits.
(v) Disassembly occurs primarily by loss of NDP-bound forms from tips.

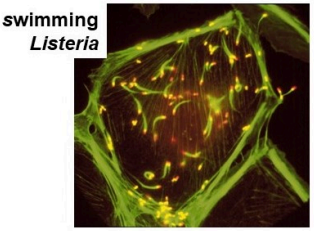
Microbiology note: Neither microtubules nor F-actin

are found inside bacterial cells. However, bacteria contain several ancient, evolutionarily related filaments with similar characteristics, based on the FtsZ, ParM and Min proteins. Like F-actin and microtubules, these bacterial filaments grow and shorten in a highly dynamic fashion, and their assembly is coupled to nucleotide hydrolysis.

Dynamic filaments drive many cellular movements



From Kathryn Tosney's webpage



From Julie Theriot's webpage

Dynamic microtubules	***Dynamic actin filaments***
Chromosome movement in mitosis	Contractile ring in cytokinesis
Mitotic spindle positioning	Lamellipodia and filopodia protruding from crawling cells
Steering of axon growth cones	*Listeria monocytogenes* swimming inside host cells

Figure 13. Dynamic cytoskeletal filaments drive many types of cell movement.

Actively growing/shortening actin and microtubule filaments drive many types of non-muscle cell motility. Examples of cell motility

that require *microtubule* growth and shortening are (a) positioning of the mitotic spindle within a dividing cell, and (b) steering of axon growth cones during neural development. Examples of motility driven by dynamic *actin* filaments are (i) the protrusion of lamellipodia and filopodia from the leading edge of crawling cells, and (ii) the swimming movement of the infectious bacterium, *Listeria monocytogenes*, in the cytoplasm of a host cell. (*Listeria* infection is the cause of **listeriosis**, a type of food poisoning. To see listeria swimming, check out: https://www.youtube.com/watch?v=sF4BeU60yT8.)

Mitosis: dynamic microtubules move chromosomes

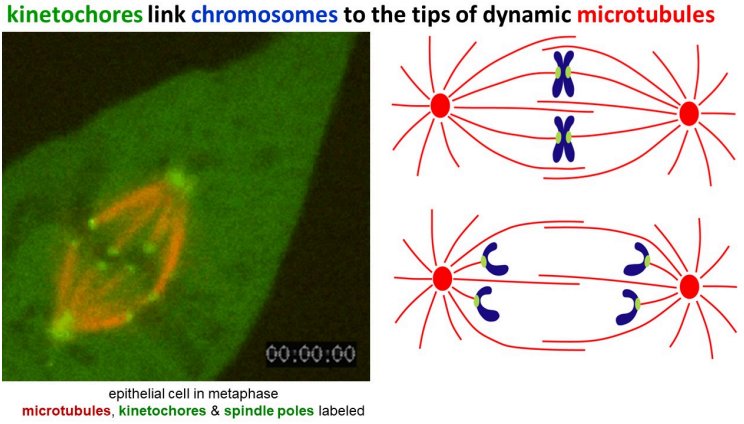


Figure 14. Microtubule polymerization and depolymerization drives chromosome movements during cell division.

Dynamic filaments are vital for **mitosis**, the process by which duplicated chromosomes are segregated during cell division. In cells preparing to divide, microtubules assemble into a bipolar array called the **mitotic spindle** (Figure 14). Spindle microtubules are highly dynamic – constantly growing and shortening. Their tips

capture the duplicated chromosome pairs. While the chromosomes are captured, the growing and shortening microtubules push and pull them to the equator of the spindle, and then separate each pair so that when cell cleavage occurs, each daughter cell gets a complete copy of the genome.

Accurate mitosis is essential for the development of a human baby, composed of trillions of (10^{12}) cells, from a single embryonic cell. Mitosis is also critical for maintenance of healthy tissues. Many tissues in our body contain populations of stem cells, which continually divide to replenish dead cells (e.g., in our blood stream, skin, and intestines). Mitosis also has a role in cancer: Abnormal mitosis, where the daughter cells inherit broken, extra or missing copies of a chromosome, contributes to the progression of certain types of cancer. Moreover, drugs that block the activity of the mitotic machinery are often used as chemotherapeutics (Table 3). Such **anti-mitotic drugs** act by inhibiting the rapid, out-of-control cell division of tumor cells. They also block division of healthy cells, and therefore cause other side-effects that limit the dose a patient can withstand.

Drug	**Colchicine**	**Taxol**
Source	Autumn Crocus 	Pacific Yew bark 
Used to treat	Gout	Cancer
Since	1500 BC (ancient Egypt)	1993 (Bristol-Myers Squibb)
Target molecule	**Tubulin** (K_D ~ 100 μM) Inhibits microtubule ***assembly***	**Tubulin** (K_D ~ 1 μM) Inhibits microtubule ***disassembly***
Therapeutic effect	Anti-inflammatory Blocks neutrophil motility	Anti-mitotic Selectively kills cancer cells
Notes	Anti-mitotic effects on overdose GI: nausea, vomiting, diarrhea Blood: low cell counts Hair loss	Environmental controversy over massive tree harvests for early trials

Table 3. Microtubule filaments are the targets of useful therapeutic drugs. You do not need to memorize this information, just know that tubulin-targeting drugs can inhibit various aspects of microtubule physiology, and generally have anti-mitotic effects.

Two excellent, short videos of mitosis

Mitosis in a lily plant cell, one minute (*includes narration*):

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Mitosis in a newt lung cell, one minute (*includes narration*):

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Simple exercises to check what you recall

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Extra Q&A from past students that might be helpful

Hi Dr. Asbury,

I have a question regarding Monday's IRAT. Question number 5 asked "How do the nucleotides ATP and GTP participate in non-muscle motility" and one of the options was "ATP is an energy source driving actin filament 'treadmilling', where one end assembles while the other simultaneously dissembles". I don't remember that being talked about in the syllabus and I am wondering if you can explain what this means, given it was a true statement? If maybe I overlooked it, can you direct me to which page this is talked about on?

Thanks so much for contacting me, and for your question about treadmilling of F-actin. I think there are perhaps three questions underlying your email, and I will try to address them all here:

First, what do I mean by "treadmilling"? The individual actin subunits can each bind and hydrolyze ATP — in other words, each one is an ATP-splitting enzyme, and like the other ATP-splitting enzymes discussed in our course (myosin, kinesin, dynein, SERCA...), they harness energy from the splitting reaction in order to drive dynamic processes. The ATP-splitting that occurs within F-actin drives treadmilling, which is when G-actin subunits are

being added to one end of a filament while they are simultaneously being lost from the other end. This happens because the subunits must contain ATP in order to bind onto the end of a filament, and because after they are bound into the filament, they are stimulated to split their ATP and release the phosphate, (retaining only ADP). The splitting reaction takes a little time, so the newly grown filament tip is composed of freshly added ATP-containing subunits, bound on top of ADP-containing subunits that have resided inside the filament for more time. We say the filament is "capped" with ATP-containing actin. The ADP-containing actin does not bind as tightly to its neighbors in the filament, so if it happens to be exposed at the opposite end of a filament, it typically falls off. The overall result is that you can have a growing end where ATP-containing actin is getting added, while simultaneously at the opposite end of the filament there is ADP-actin falling off. We call this "treadmilling" because it is like a treadmill, with the actin subunits moving through the filament from one end to the other. This movement is powered by ATP hydrolysis.

Your second question is whether and where this "treadmilling" was described in the syllabus. And underlying that question, I think you are probably wondering, will I be expected to know all this for my upcoming quiz? This Pressbook course pack only briefly mentions actin dynamics. It mentions briefly how the polarity of F-actin allows the two ends of the filaments (the "plus" and "minus" ends) to grow at different rates. It also mentions that dynamic F-actin pushes the leading edges of crawling cells forward, and that it drives movement of Lysteria, an infectious bacterium, inside host cells. I chose to focus this Pressbook course pack more on the very

similar dynamics of microtubules. For brevity, our coverage of F-actin dynamics is only very limited. This is also true during my in-class session, and in the pre-class movies that Dr. Jay Gatlin (at U Wyoming) prepared.

Thus, my expectation is for you to understand tubulin dynamics in a little bit more detail than actin dynamics, and I do not expect you to understand the details of actin treadmilling specifically. The two most important general concepts related to filament dynamics are that (1) energy supplied by the hydrolysis of nucleotides — GTP for tubulin, ATP for actin — is harnessed by these protein polymers to make them assemble and disassemble very dynamically in some situations, and that (2) these nucleotide hydrolysis-powered dynamics can generate pushing and pulling forces, for example to move the front of a crawling cell forward (an actin-driven process) and to separate chromosomes during cell division (a microtubule-driven process).

Cell Membranes and Transport

FRED RIEKE AND BERTIL HILLE

Session Learning Objectives and Quick Synopses

1. Describe the structure and topology of cellular membranes.

Cell membranes surround the cell and surround each organelle in the cell. They are formed by bilayers of phospholipids that form a hydrophobic core (phospholipid tails) between two hydrophilic layers (phospholipid headgroups). Molecules that are lipid soluble dissolve in and pass readily through the bilayer. In addition, integral and peripheral membrane proteins are embedded in the lipid bilayer. They confer additional membrane properties as receptors, enzymes, or permeation pathways.

2. Contrast ion channels and ion transporters and the forces that drive them.

Ion channel proteins are gated pores in the membrane that allow selected ions to flow down their electrochemical gradients. In ion channels, the ions are driven only by their

own concentration gradients and by the electric field acting on them. We call this flow passive. Other transport proteins act more like enzymes that couple the movement of one kind of ion or solute to energy derived either from the flow of another kind of ion or directly from the hydrolysis of ATP. Often these transporters can establish gradients of ion concentration. We call that active transport. It is primary active transport when the pump is driven by ATP, and secondary active transport when the energy comes from another ion gradient.

3. Know the normal balance of Na^+, K^+, Cl^- and Ca^{2+} with respect to the plasma membrane.

As a result of active transport, Na^+, Ca^{2+}, and Cl^- are more abundant outside of the cell in serum, whereas K^+ is more abundant inside the cell in cytoplasm (Table 2). These non-equilibrium distributions are essential for many cellular responses including electrical excitability.

Cell membranes

All cells are surrounded by a plasma membrane that forms the boundary between the living cytoplasm and the extracellular space. The membrane retains the cytoplasmic contents and prevents intrusion by most molecules outside the cell. The membrane is flexible and semi-fluid, conforming dynamically to the changing forces of intracellular cytoskeletal proteins and the extracellular matrix and substrate. Red blood cells, for example, take their biconcave disk shape from their specialized spring-like intracellular cytoskeletal network. Nevertheless, they readily bend and squeeze unharmed in transient contortions as they rush through narrow

capillaries. Inside nucleated cells, other membranes define organelles like the mitochondria, the nucleus, and the endoplasmic reticulum. The plasma membrane and the intracellular membranes share a common basic architecture and a common origin. They are made of a molecular bilayer of lipids peppered with membrane proteins. We start by discussing the lipid component and then return to the proteins.

Learning Objective #1. *Describe structure and topology of cellular membranes.*

Membranes form a hydrophobic lipid bilayer

Typical membrane lipids are phospholipids and cholesterol. These molecules are primarily hydrophobic ("water fearing") but bear a charged or polar group at one end that is hydrophilic ("water loving") (Figure 1). We call this dual nature, amphipathic ("both preferences"). Soaps and detergents are also amphipathic. This allows them to bring greasy substances into solution in water.

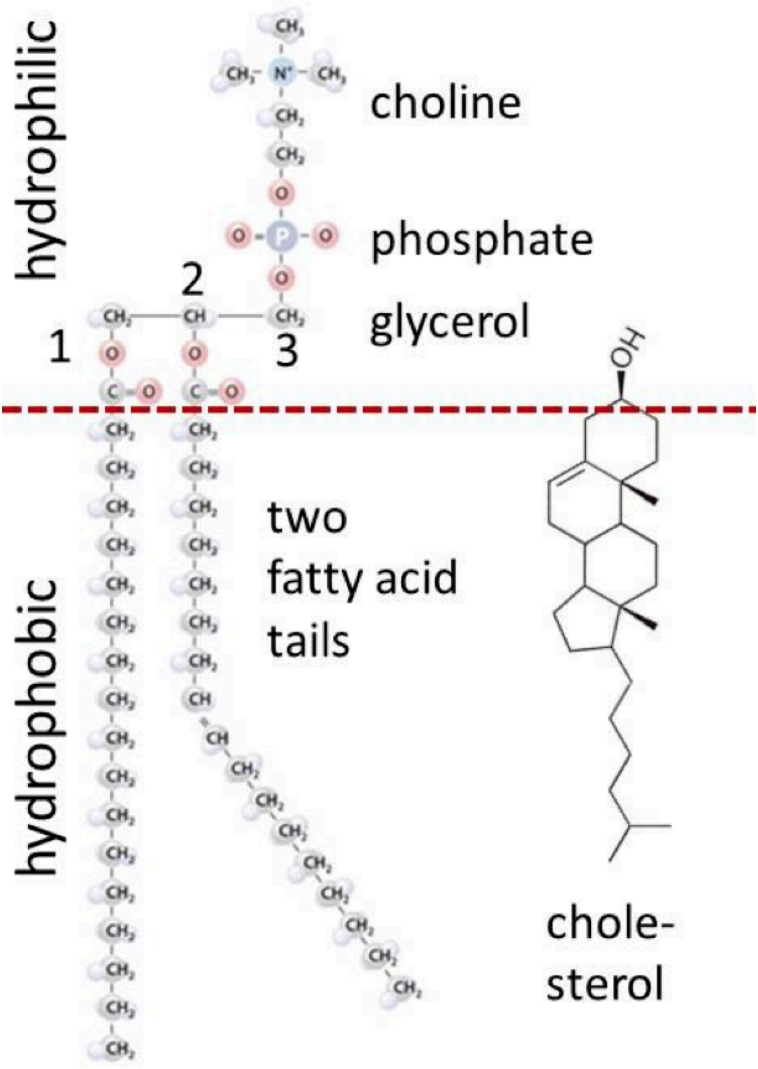

Figure 1: Structures of two membrane lipids. ***Left****: phosphatidylcholine.* ***Right:*** *cholesterol.*

In the structure of phospholipids, we see two hydrophobic fatty acid chains attached to a glycerol scaffold at the glycerol 1- and 2-positions (Figure 1). The glycerol 3-position carries the hydrophilic phosphate and head group. Common head groups include (Figure 2) serine, ethanolamine, choline, and inositol, each with its own special roles and forming phospholipids with names like phosphatidyl serine, phosphatidylinositol, and so forth. Amphipathic phospholipids spontaneously form a lipid bilayer made of two lipid leaflets as a thin flexible sheet (Figure 3). This is a minimum energy state for phospholipids in water because it maximizes the interactions of the hydrophobic fatty acid side chains with each other and away from water, and it maximizes the interaction of the hydrophilic head groups with water.

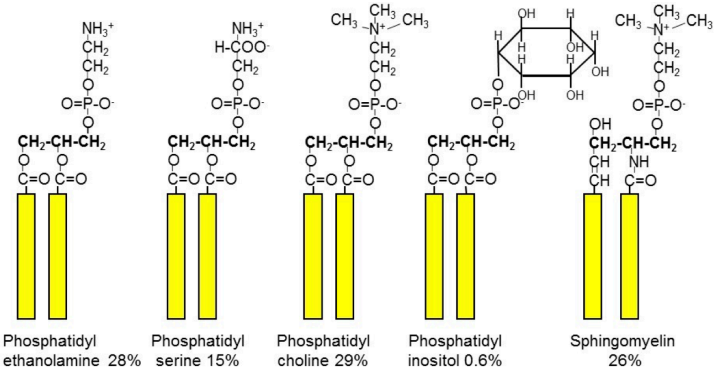

Figure 2: Structures of common membrane phospholipids. The glycerol-like moiety is shown in bold to emphasize similarity in structure. The fatty acyl chains are just shown as yellow boxes but represent a mixture of chain lengths and unsaturation. The percentage indicates the relative abundance in the plasma membrane of a red blood cell.

At physiological temperatures, the lipid side chains are free to undergo rapid spontaneous thermal motion, waving around in a fluid-like state while still remaining anchored to the glycerol, which carries the head group. In addition, each phospholipid molecule experiences spontaneous thermal rotation, stochastically spinning

around its long axis, as well as lateral diffusion by Brownian motion in the plane of the membrane. All these motions make the lipid bilayer an ultrathin flexible, oily fluid. The thickness is just twice that of the two opposing fatty acid leaflets, about 3 nm, far thinner than the wavelength of light and therefore resolvable only by electron microscopy.

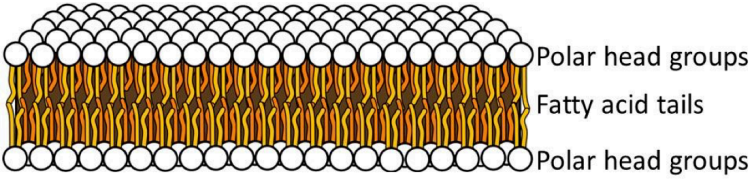

Figure 3: A bilayer of phospholipids forms a membrane.

Passive permeability of the bilayer

Some molecules easily cross the lipid bilayer membrane and some do not (Figure 4). Permeability is a measure of how easily molecules cross the membrane. Key permeability properties of cell membranes are well understood if we remember that the lipid bilayer is like a thin sheet of oil. Hydrophobic molecules presented in the aqueous phase, being lipid soluble, pass easily into the oily lipid bilayer and cross the membrane barrier. Illustrative examples would include aspirin, steroid hormones, O_2, CO_2, and NO (Box A). We say the membrane is permeable to these hydrophobic molecules, which is good in these cases since their physiological actions require them to enter cells from the outside. Thus, the steroid hormone estrogen is made in the ovaries but has to act on receptors in the nucleus of cells throughout the body. In all respiring cells, O_2 has to enter the cell continuously, and CO_2 has to leave. Similarly, O_2 has to enter red blood cells in the lungs and be delivered to tissues in the capillaries during every circuit of the circulation, and the reverse for CO_2. Each

of these steps requires the hydrophobic steroid or gas molecules to dissolve in the plasma membrane and to pass across it and emerge on the other side, a process that takes only a small fraction of a second. For the same reasons, some degree of hydrophobicity is essential in the design of new drugs that are intended to be taken orally so they can be absorbed across cells of the intestinal wall and cross into target tissues.

Box A: Membrane lipid bilayer permeability

Hydrophobic, lipid-loving, bilayer-permeant, small molecules

- O_2, CO_2
- steroids like estrogen & cortisol
- local and general anesthetics
- aspirin

Hydrophilic, water loving, bilayer-impermeant molecules

- ATP, DNA, RNA
- K^+, Na^+, Cl^-, Ca^{2+}, PO_4^{-3}
- neurotransmitters like acetylcholine, glutamate & GABA
- sugars like glucose

On the other hand, molecules that are polar (sugars) or charged (ions) are hydrophilic and do not enter into oil. They are reluctant to leave water and do not enter or cross the lipid bilayer easily. Examples include Na^+, ATP, and glucose (Box A). Cell membranes are relatively impermeable to them, which allows the cell to develop ion gradients across membranes and to retain its high-energy molecules, metabolites, and genetic material inside. Many important physiological responses involve changes of membrane permeability to charged and polar molecules. In electrophysiology we will see that the ion-impermeable lipid bilayer can be thought of as an electrical insulator. It allows a small charge imbalance across the membrane to generate an electrical potential difference across the membrane – the membrane potential. The electrical responses of cells involve changes of permeability to ions and ion movements through the opening and closing of ion channels in the plasma membrane. In this way, as we shall see later, the plasma membrane is the seat of all electrical signaling in nerve and muscle.

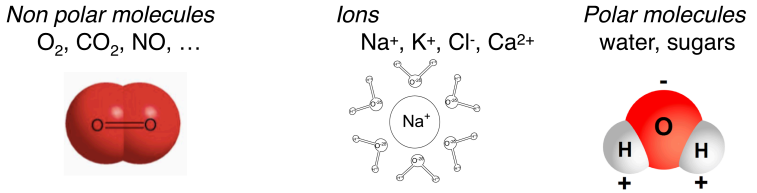

Figure 4: Different types of molecule differ in membrane permeability. *Non-polar molecules can cross membranes without help. Charged ions or polar molecules cannot.*

Proteins add functions to cell membranes

Box B. Integral membrane proteins

- many hormone receptors
- neurotransmitter receptors
- antibody receptors
- ion channels
- ion pumps--ATPases
- other transporters
- enzymes
- anchors and adhesion points for cytoplasmic and extracellular scaffolds
- electron transporters--cytochromes

A more complete view of biological membranes would show a thin lipid sea with many proteins inserted and floating in it (Figure 5). Some proteins, the integral membrane proteins, have one or more hydrophobic transmembrane segments and never leave the membrane (Box B). Others, the peripheral proteins, bind to other membrane proteins and lipid head groups but do not have any transmembrane segments. They may associate and dissociate dynamically from the membrane compartment depending on the state of the cell.

Almost all integral membrane proteins are initially inserted into membranes of the endoplasmic reticulum (ER) during protein synthesis as the nascent peptide chain is lengthened by protein translation. The ER membrane then buds off a membrane vesicle that passes to the Golgi with its new membrane proteins. Eventually, more budding from the Golgi sends a vesicle further to, e.g., the plasma membrane.

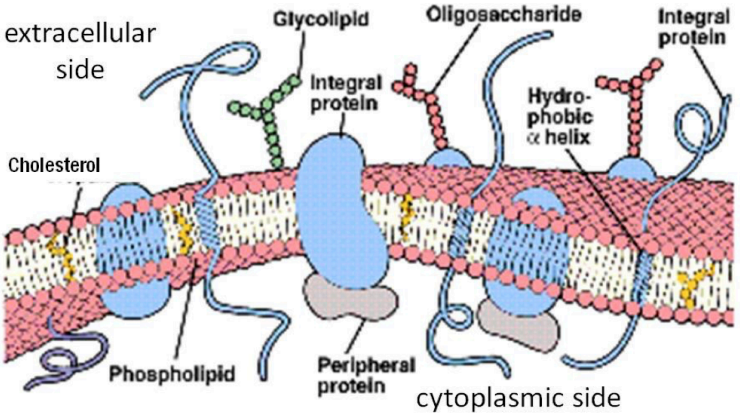

Figure 5: Membrane proteins in the lipid bilayer. Integral membrane proteins (blue) with extracellular glycosylation and peripheral membrane proteins (gray).

Details of membrane origin and traffic will be discussed in a separate session emphasizing cell biology of membrane budding and endo- and exocytosis. They explain how all membranes have a similar architecture since they derive from the same source. Membrane proteins contribute many biological functions to the membrane including transport and enzymatic catalysis (Box B). Although the basic architecture of the membrane is conserved, the different membranes of a cell are specialized by having different sets of functional proteins to allow them to perform different functions, so both at the level of gene expression and in the events of membrane traffic, decisions are made as to which protein belongs where.

Learning Objective #2. *Contrast ion channels and ion transporters and the forces that drive them.*

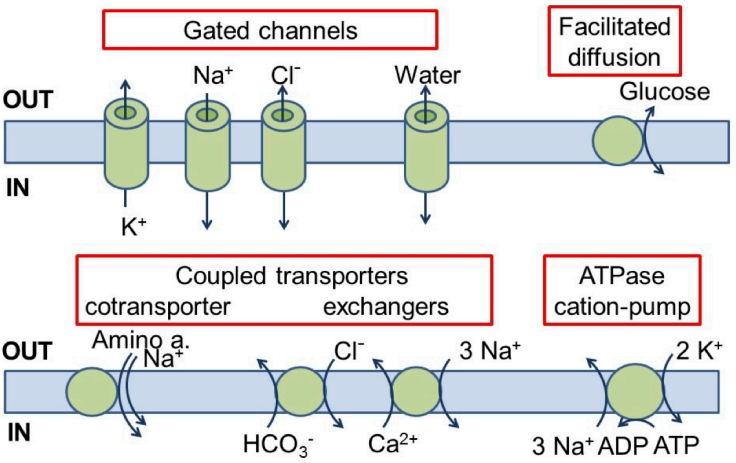

Figure 6: Three classes of ion transport. These cartoons are drawn in a conventional shorthand that indicates the net function but not the true structure of these proteins. Each diagram represents families of related proteins. Solute cotransporters exist for a variety of possible organic molecules including various sugars and amino acids. ATPase cation pumps exist for Na^+/K^+, H^+/K^+, and Ca^{2+} ions.

Now we will emphasize certain transmembrane proteins that facilitate movement of ions across the membrane, sometimes coupling with transport of other small molecules. One can consider them catalysts that enable regulated transmembrane movements of a small select number of molecules. They include ion channels, coupled transporters, and ion ATPase pumps (Figure 6). We introduce them superficially first.

(A) The ion channels are the simplest. When they are open, they act like a pore that allows ions to flow. They have ion selectivity – a preference for certain ions. The direction of ion flow is set entirely by two thermodynamic forces on the ions: (i) the ion concentration gradient across the membrane, and (ii) an electrical force if there is a difference in electrical potential across the membrane. We can say that the direction of ion flux is thermodynamically downhill, and,

more technically, we say it is down the electrochemical gradient. The ion channels in Figure 6 are selective for K^+, Na^+, or Cl^- and would be called K^+ channels, Na^+ channels, or Cl^- channels. Some ion channels are less selective, passing a range of small cations or a range of small anions. Ion channels have an additional feature: most of them open and close their pore in response to stimuli such as a neurotransmitter, a voltage change, heat, or an intracellular second messenger. We say they are gated. The figure also shows a water channel, a pore that allows osmotic flow of water across the membrane, e.g. in the kidney. As a peek forward to the next few weeks, Table 1 lists the properties of ion channels you will be encountering soon.

(B) The next category in Figure 6, the coupled transporters, couples the movement of one molecule (here an organic solute or bicarbonate or Ca^{2+}) to the downhill flow of another (Na^+ or Cl^-). The action is like that of an enzyme that in this case has two substrates. Nothing happens until both substrates are present. They bind and induce conformational changes of the protein that exposes each of them to the opposite side of the membrane. Such transporters are sometimes called "carriers." The first example shown is a Na^+-coupled solute transporter, of which there are many types (for sugars, amino acids, neurotransmitters, etc.). Here Na^+ and the coupled solute move in the same direction into the cell, so it is called a co-transporter. These cotransporters use the free energy of Na^+ allowed to flow downhill (down its electrochemical gradient) into the cell, to drive the cotransport of the other substrate. They can drive uphill transport of solutes, creating a concentration gradient. Such a process is called secondary active transport, an active transport that does not use ATP as its direct source of energy. It is analogous to using the cascading of water over a waterfall (Na^+ entry) to do some work (solute transport).

Table 1. Some ion channels you will meet in this course

Preferred Ion	Channel types	Location
Na^+	Voltage gated Na^+ channels	Nerve, muscle
	Epithelial Na^+ channels	Epithelia
K^+	Voltage-gated K^+ channels	Nerve, muscle
	Leak K^+ channels	Ubiquitous
Ca^{2+}	Voltage-gated Ca^{2+} channels	Nerve, muscle
	Ca^{2+}-release channels (IP_3R)	Ubiquitous (ER)
	Ca^{2+}-release channels (RyR)	Ubiquitous (ER and SR)
Cl^-	ClC channels	Muscle
	CFTR	Epithelia
	$GABA_AR$, GlycineR	Synapses
NS-cation	AChR, GluR	Synapses
	Mechano, CNG,TRP	Sensory organs

NS cation: nonselective cation channel (e.g. Na^+ and K^+ equally)

The second group of coupled transporters exchanges ions from opposite sides of the membrane. Shown are examples of chloride/-bicarbonate exchangers and Na^+/Ca^{2+} exchangers. All these coupled transporters are fully reversible, operating in whichever direction thermodynamics dictates, and are capable of secondary active transport. For completeness, we also mention a type of transporter that has only one substrate and no coupled ion flow (Figure 6). This transport process, called facilitated diffusion, is by necessity thermodynamically downhill for the single substrate. A notable example is the Glut4 glucose transporter inserted into the plasma membrane by the action of insulin to allow passage of glucose in either direction.

(C) The final example in Figure 6 is an ion pump that couples transport of Na^+ and K^+ to the free energy of hydrolysis of ATP, the metabolic dollar bill. Now both ions are moved uphill (in opposite directions) at the direct expense of metabolic energy. Such pumps that consume ATP directly are said to perform active transport. They generate ion gradients across the membrane important for ion movements in coupled transporters and in ion channels. Before considering how the pumps work, we will discuss the ion imbalances that these pumps set up.

Learning Objective #3. *Know the normal balance of Na^+, K^+, Cl^- and Ca^{2+} with respect to the plasma membrane.*

Four ion gradients

Table 2. Ion concentrations in serum and inside a neuron

Ion	Extracellular concentration U.S. clinical units	Extracellular concentration Scientific units	Intracellular concentration Scientific units
Na^+	142 mEq/L	142 mM	12 mM
K^+	4 mEq/L	4 mM	155 mM
Ca^{2+}	3.0 mEq/L*	1.5 mM	~100 nM
Cl^-	103 mEq/L	103 mM	7 mM

* Ca^{2+} is often reported as mg/dl: values for total Ca^{2+} are ~9 mg/dl or ~4.5 mg/dl ionized (or free) Ca^{2+}. Values in the table are for free Ca^{2+}.

Note on units: The table gives extracellular concentrations in two kinds of units. One is the familiar international scientific units of moles per liter (molar, M), here abbreviated as mM (millimolar = millimoles per liter). The other is the units used in U.S. clinical work, shown variously as mEq/L (millimoles per liter multiplied by the valence of the ion) or as mg/dl (milligrams per 100 ml). In discussing physiology and biochemistry, we use the scientific units as all scientists do in the world, but as clinicians you will always see the U.S. clinical units in any lab reports. The values for a healthy person are not as exact as the table implies. In medical practice, clinical lab reports you get also will indicate for your guidance the range of values that would be considered

Gradients of ions across the plasma membrane are key for physiology and medicine–indeed for life. The word gradient means a difference in concentration on the two sides of the membrane. Recall that salts like NaCl dissociate in water into the free cations and anions – Na^+ ("sodium ion") and Cl^- ("chloride ion") in the case of NaCl. This makes an electrolyte solution. With respect to ions, our plasma (the extracellular medium for cells) is like a dilute version of seawater, reflecting the origins of animals in early oceans. As in seawater, the concentrations of Na^+ and Cl^- are high and K^+ is low (Table 2). In contrast, inside the cell, the Na^+ is low, Cl^- is often low, and K^+ is high. Resting cellular free Ca^{2+} is extraordinarily low, making Ca^{2+} a good ion for intracellular signaling as we will see later. Note that the table has a footnote explaining two conventions for the units of extracellular concentration that will be important for you as a clinician. Here we will use international scientific units, but in the clinic you will use the other. Shortly we shall see that ion gradients allow cells to make electrical signals. Later curriculum blocks show how ion gradients help to regulate cellular and body volume and to transport essential metabolites into cells by Na^+-coupled transporters. Right now, it would be important to commit to memory the direction of these four important cellular ion gradients, remembering which ions are high outside, and which are high inside. These gradients are an essential concept for understanding electrical excitability and cell physiology.

Ion pumps make ion gradients

The existence of ion gradients tells us that the cell is not at equilibrium and that some work has been done to make such an imbalance. As is always the case, doing work requires expenditure of energy; this is the job of the ATPase ion pumps. In cell membranes, there are several pumps in this protein/gene family specialized for active transport of Na^+, K^+, Ca^{+2+}, and protons. We start with the Na^+/K^+-ATPase, sometimes just known as the Na^+-pump for short.

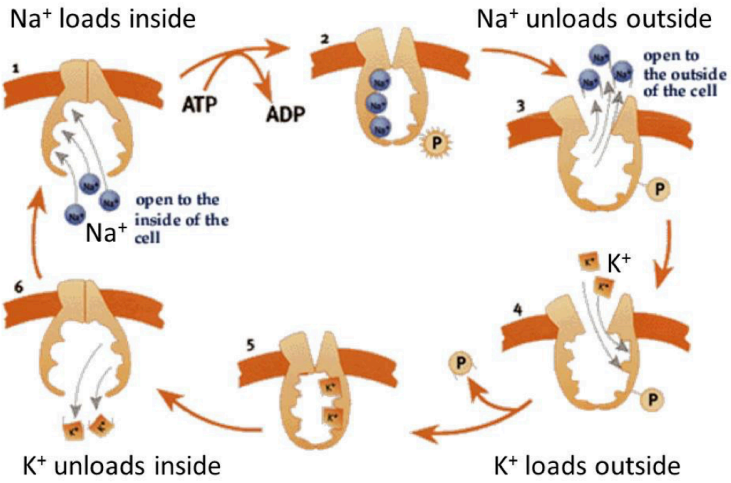

Figure 7: Cartoon of the Na^+/K^+ ATPase cycle. Starting from upper left and going clockwise around in one pump cycle, 3 Na^+ load, ATP is cleaved to ADP and a phosphate (P), Na^+ is unloaded, 2 K^+ are bound, and K^+ is unloaded. The pump itself alternately opens a gate to the outside and a gate to the inside driven by ATP so the ions are transported to the opposite side of the membrane, setting up a gradient.

Pumps are integral membrane enzymes in cell membranes. Like other enzymes, they have a reaction velocity that increases with substrate concentration and that saturates when the substrates are abundant. Also, their reaction is cyclic with substrates binding and

products unbinding in a fixed and cyclic stoichiometry. The unusual feature compared with most enzymes is that the substrate is picked up in one compartment and through a conformational change the product is released in a different compartment. They operate across membranes. The reaction for the Na^+/K^+-ATPase in the plasma membrane, already diagrammed in Figure 6, can be written as a chemical formula:

$$E + 3\ Na^+_i + 2\ K^+_o + ATP \quad -> \quad E + 3\ Na^+_o + 2\ K^+_i + ADP + P$$

where E stands for the enzyme, the numbers indicate the stoichiometry of the reaction, subscript "o" means outside the cell, and subscript "i" means inside the cell, except that P_i is the conventional abbreviation for "inorganic phosphate." Let's turn this into words: 3 Na^+ ions are moved from the inside to the outside, 2 K^+ ions are moved from the outside to the inside, and 1 ATP is broken down to ADP plus inorganic phosphate in the cytoplasm. Some known substeps of the pump cycle can be represented by a cyclic cartoon (Figure 7). Because this pump operates all the time in all our cells, the cytoplasmic Na^+ is removed and replaced by K^+, creating and maintaining the Na^+/K^+ gradients of Table 2. Some cells, like kidney cells and most excitable cells, must move ions rapidly to accomplish their transport and excitability functions. They have many copies of the Na^+-pump in their plasma membrane. Others, like red blood cells, have very few ion channels and can get by with a low density of Na^+-pumps. Red blood cells therefore do not need to consume glucose and produce ATP as rapidly as a kidney cell would.

This pump can be blocked by cardiac glycosides like digitalis (related to Digoxin used clinically) and ouabain. When this is done experimentally, the ion gradients of the cell gradually run down over hours. Such experiments also show that as much as 25-40% of the energy metabolism of a kidney cell is devoted to providing the ATP for the Na^+/K^+-ATPase. Clinically, digitalis analogs are used at very low concentration to reduce the Na^+ gradient just a little in the heart. As you will learn later in the Cardiovascular Block, a decreased Na^+ gradient reduces the activity of the Na^+/Ca^{2+} exchanger (see Figure 6), which is powered by the Na^+ gradient.

Therefore, some additional cytoplasmic Ca^{2+} accumulates inside the cardiac cells, boosting muscle contraction and helping to ameliorate congestive heart failure.

Practice questions and review topics:

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Optional digoxin practice case

Patient Bob Jones is a 68-year-old man who suffered a heart attack 6 months ago, and as a consequence now has severe systolic heart failure (weakened pumping function of the heart muscle). Initial medications prescribed for this have resulted in only slight improvement of the heart failure symptoms, so Bob's physician decides to add digoxin to the medication regimen.

Digoxin is a drug in the class called cardiac glycosides, which are derived from the foxglove plant and have great historic importance in medicine as they have been used for hundreds of years to treat heart failure. Newer drugs developed in recent decades have now become the mainstay of treatment for heart failure, but digoxin is still used in certain circumstances.

Among other effects, digoxin at optimal doses increases the force of contraction of heart muscle. The following questions will help you understand the physiology behind this therapeutic effect. Note: In a few weeks, you will be learning that intracellular Ca^{2+} triggers contraction of cardiac and skeletal muscle.

- Review the function of the Na^{+}-K^{+} ATPase pump.
- How would inhibiting this Na^{+}-K^{+} pump influence

ion distribution?

- How would inhibition of the Na^+/K^+ ATPase influence forces contributing to ion movement across the plasma membrane?
- How might this decreased Na^+ gradient influence function of a secondary active transport system?
- Review the mechanism of action of the Na^+-Ca^{2+} exchanger.
- What would be the result on intracellular Ca^{2+} concentrations if activity of the Na^+/K^+ ATPase pump is slowed?
- Predict how this change could be useful in the treatment of cardiac failure.
- Now put together the physiologic processes discussed in this case, to explain the mechanism by which digoxin augments the force of myocardial contraction.

G Protein Coupled Receptors

MARK BOTHWELL

Introduction: What is Cellular Signaling?

Signaling means communication of information. Familiar biological examples include actions of endocrine hormones and neurotransmitters.

Cellular signaling requires at least four steps.

1. creation or release of a chemical as a message,
2. binding of the message molecule to a receptor protein at the target,
3. initiation of a response at the target, and
4. removal of the message.

Often the response in the target cell is an intracellular message, sometimes called a second messenger, that is recognized by another internal receptor and initiates another response. Ultimately a cascade of such intracellular events may produce several biological outputs. Examples of intracellular second messengers are rises of cyclic AMP or of Ca^{2+}, and examples of responses are speeding of heart rate or inducing gene expression. Over two hours class hours we will discuss three classes of receptors: the G protein coupled receptors, the tyrosine kinase receptors, and the nuclear receptors.

This chapter focuses on G protein-coupled receptors (GPCRs), which represent the largest family of plasma membrane receptors. The human genome contains over 800 GPCR genes. Among the ligands for GPCRs are monoamine neurotransmitters (adrenaline, noradrenaline, serotonin, dopamine, histamine), many peptide hormones, light photons, odorants, and sweet, bitter, and "umami" tastes (Box A). For many of these extracellular stimuli there are

multiple different GPCRs, meaning that the same extracellular signal might give rise to different intracellular responses depending on which receptor subtype it encounters. For example, there are three major types of receptors for adrenaline and noradrenaline, called α1, α2, and β adrenergic receptors. In turn, each receptor type is further diversified by being encoded by three slightly different genes, making a total of nine adrenergic receptor genes altogether. The GPCR family is exceptionally important clinically, as demonstrated by the fact that 35% of FDA-approved drugs target GPCRs or elements of their down-stream signaling pathways.

Box A: Diversity of GPCR ligands

Neurotransmitters

- norepinephrine
- adenosine
- histamine
- serotonin
- dopamine
- cannabinoids
- opiates
- acetylcholine
- GABA
- glutamate

Peptide hormones

- endothelin

- glucagon
- angiotensin II
- GSH, FSH, CRH
- GnRH, ACTH

Tastants, oderants, light

Learning Objectives

1. Describe in detail the activation of GPCR signaling including receptors, ligands & heterotrimeric G-proteins.
2. Know different G-proteins coupling to different second messengers.
3. Describe two effector pathways: cAMP and PLC.
4. Know how GPCR signaling is terminated.

Learning Objective #1. *Describe in detail the activation of GPCR signaling including receptors, ligands & heterotrimeric G-proteins.*

GPCR Structure and Function.

GPCRs are integral plasma membrane proteins with 7 transmembrane segments (Figure 1). GPCRs transduce signals from extracellular ligands to signals in intracellular relay proteins, the hetero-trimeric GTP binding proteins (G proteins). By coupling to many downstream intracellular cascades and effectors, the G proteins initiate pleiotropic changes in many intracellular targets.

Ligand -> GPCR -> G protein -> 2nd messengers -> many intracellular effectors

Thus, extracellular signals are typically amplified to produce robust, varied, and cell-specific intracellular responses.

Signaling from GPCRs to G proteins.

GPCRs couple to intracellular, GTP-binding, **heterotrimeric G proteins** that consist of Gα, Gβ, and Gγ subunits (Figure 1). At rest these three G protein subunits are assembled into the heterotrimeric complex, Gαβγ. Since Gβ and Gγ are inseparable once co-assembled, it is customary to talk about a resting complex of Gα with Gβγ. Gα and Gβγ are not transmembrane proteins but they are tethered at the inner leaflet of the plasma membrane by hydrophobic lipid modifications.

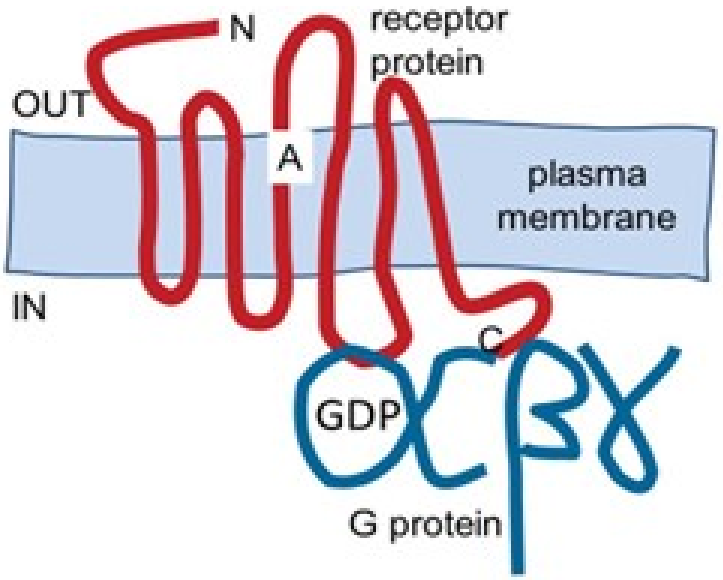

Figure. 1: Conceptual cartoon of a GPCR and a G protein. The receptor in the plasma membrane has seven α-helical trans-membrane segments. The coupled hetero-trimeric G-protein is represented schematically by the letters α, β, γ in the cytoplasm. Labels: A, *agonist binding pocket*; C, *receptor C-terminus in cytoplasm*; N, *receptor extracellular* N*-terminus.*

Gα subunits are powerful signaling proteins. In the inactive resting Gαβγ complex, the Gα subunit is bound to the guanine nucleotide, guanosine diphosphate (GDP), but when a GPCR is activated, the receptor can catalyze a "nucleotide exchange" reaction" on the Gα subunit (Figure 2).

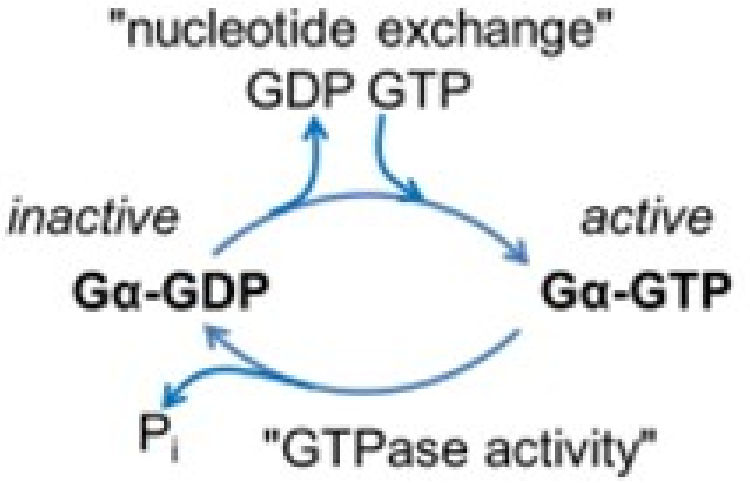

Figure 2. Cycle of activation and inactivation of α-subunit of G protein. Ligand-bound GPCR catalyzes nucleotide exchange on inactive Gα-GDP, forming active Gα-GTP. The GTPase activity of the α-subunit eventually restores Gα-GDP.

GDP leaves and guanosine triphosphate (GTP) binds instead. GTP binding activates the G protein (hence the name). GTP is a high-energy molecule similar to ATP except that is formed from guanosine rather than adenosine. Once formed, the Gα-GTP-Gβγ complex is unstable so that the active Gα-GTP and Gβγ separate from one another and from the receptor as well (Figure 3).

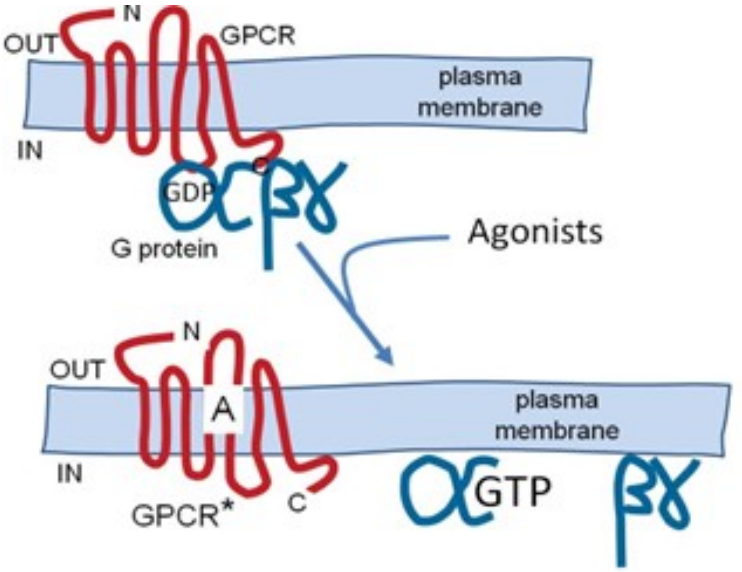

Figure 3. Activation of a G protein by ligand-bound GPCR. Agonist allows GPCR to catalyze nucleotide ex-change and dissociation of GPCR from α-GTP and βγ.*

However, the G protein subunits normally still remain attached to the plasma membrane by their lipid anchors. Thus there are now three active products that couple to downstream effectors: the activated receptor, the Gα-GTP subunit, and the Gβγ dimer. The activated receptor is again free to couple repeatedly with additional G proteins, amplifying the signal. Because the activated G proteins are membrane associated, the next step will be an interaction with membrane-associated effector proteins or recruitment of cytoplasmic effector proteins to the membrane. Gα is both a regulatory switch and an enzyme. It slowly cleaves the bound GTP to give GDP (Fig. 2). Gα-GDP is inactive, binds Gβγ again, and waits for a new round of activation. This exemplifies a typical role of GTP in biology–as a timer of switches and as an informational molecule.

Learning Objective #2. *Know different G-proteins coupling to different second messengers.*

Five families of Gα-GTP subunits.

As Gα-GTP and Gβγ each has the capacity to convey cellular signals we will discuss them separately, focusing primarily on Gα-GTP.

Since there are hundreds of types of GPCRs, one might expect hundreds of types of downstream signals. However, that is not the case. There are only five families of G protein α-subunits, giving five kinds of responses. Although we name the five G protein families below for completeness, we will focus discussion on only two of them (Gs and Gq). At least 50 types of GPCRs are specialized to signal through each of the first 4 families.

1. Gαs (s for "stimulatory") was the first G protein discovered. It stimulates plasma membrane adenylyl cyclases, increasing cellular cyclic AMP (cAMP), which, e.g., stimulates phosphorylation of target proteins by cAMP-dependent protein kinase. Gαs is the target of cholera toxin, which activates Gαs and its downstream signaling. In the gut, this irreversible activation is the signal inducing diarrhea in cholera.
2. Gαi/o (i for "inhibitory" and o for "other") G proteins inhibit most adenylyl cyclases, decreasing cellular cAMP responses. Gαi/o is the target of Pertussis toxin, which turns them off. Gαo is said to constitute 1% of brain proteins. Being so abundant, it is a major source for active Gβγ subunits.
3. Gαq activates phospholipase Cβ (PLCβ), a lipase enzyme that cleaves the signaling phosphoinositide lipids (PIP2) of the plasma membrane, generating several key 2nd messengers IP3

and diacylglycerol. We expand on these signals in a moment.
4. Gα12 enhances RhoA, Rho kinase, changes expression of some genes, and slows dephosphorylation of myosin light chains affecting smooth muscle tone.
5. Gαtransducin is found only in retinal photoreceptors. Transducin transduces the light response from **rhodopsin** by activating cyclic GMP (cGMP) phosphodiesterase that cleaves and depletes cytoplasmic cGMP. In vision, each photon depletes a small fraction of photoreceptor cGMP, generating a small electrical signal in cGMP-gated ion channels.

Each of these pathways involves second messengers and effector enzymes. The long cascade of signaling may take up to tens of seconds to be completed. However in a few cases, such as vision using rhodopsin and transducin, an extreme high density of the receptors and G proteins and miniaturization of the geometry have allowed responses that take only tens of milliseconds. When we watch the world, vision through rhodopsin and Gαtransducin, is so fast that we are not aware of any time delay. On the other hand, increases of heart rate during exercise, mediated by Gαs and cAMP, take minutes.

Downstream coupling of Gβγ

The Gβγ subunits are potent plasma membrane signals as well. They act on several ion channels in the membrane and on several signaling enzymes, but they will concern you only later and are not detailed here.

Learning Objective #3. *Describe two effector*

pathways: cAMP and PLC.

Signaling from Gαs to cAMP

As Figure 4 summarizes, the pathway from Gs is a long signaling cascade. Since there are at least three stages of amplification (catalysis by the GPCR and catalytic enzymatic activities of two enzymes), the signal from even a single molecule of ligand is greatly amplified and the response can be robust. Every cell in the body has specialized responses to cAMP signaling. Gαs, or Gs as it is called for short, activates the membrane enzyme **adenylyl cyclase**, which converts ATP into cytoplasmic 3′,5′-cyclic adenosine monophosphate (cAMP). This diffusible intracellular 2nd messenger in turn is an activating ligand for the cytoplasmic enzyme, cAMP-dependent protein kinase (PKA). As its name implies, PKA uses ATP to phosphorylate specific cellular proteins (on the hydroxyl groups of serine and threonine). This is a regulatory step. The number of target proteins regulated by PKA phosphorylation is large—dozens in a single cell. Three prominent examples include, (i) phosphorylation and recruitment of **voltage-gated Ca2+ channel**s in heart to increase contractility, (ii) phosphorylation of phosphorylase kinase, which in turn phosphorylates phosphorylase, which releases glucose-1-phosphate from glycogen and boosts energy metabolism in liver, and (iii) response to odorants by sensory neurons in the olfactory epithelium by direct action of cAMP on opening of cAMP-gated ion channels of the plasma membrane of olfactory neurons.

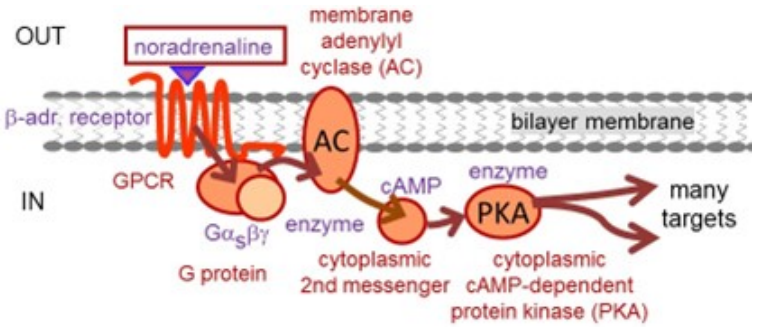

Figure 4. Signaling pathway from Gs-coupled receptors through cAMP and cAMP-dependent protein kinase. The cartoon uses β-adrenergic receptors as an example, but there several hundred GPCRs that couple to Gs.

Signaling from Gαq to PLC

Gq elicits very different responses that Gs. The Gq signaling branches at once into two pathways, shown as blue and green pathways in Fig. 5. Gq-GTP recruits the cytoplasmic enzyme phospho-lipase C (PLC) to the membrane where it cleaves the polar head group from the membrane phospholipid phosphatidylinosiitol 4,5-bisphosphate (PIP2). This yields (i) the membrane lipid component, diacylglycerol (DAG), and (ii) the soluble cytoplasmic component, inositol 1,4,5-tris phosphate (IP3). Each is a key 2nd messenger. In the blue pathway, DAG recruits and activates the cytoplasmic enzyme protein kinase C (PKC), and PKC phosphorylates specific target proteins (again on hydroxyls of serine and threonine). The end results are analogous to phosphorylations by PKA, but the targets and responses are not identical. In the green pathway, the diffusible intracellular IP3 messenger is a ligand for a Ca^{2+}-permeable ion channel, the IP3 receptor (IP3R), on the endoplasmic reticulum (ER) (Figure 5). When the channel opens, Ca^{2+} flows from the ER to the cytoplasm. The resulting Ca^{2+} rise triggers many Ca^{2+}-sensitive cellular processes, such as, secretion, contraction, or altered gene expression.

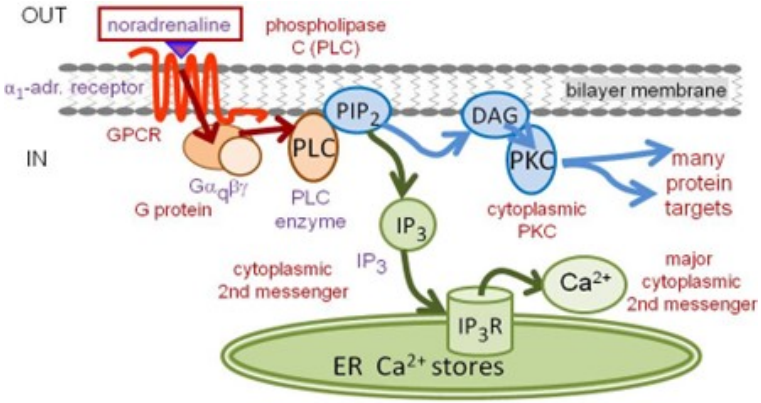

Figure 5. Two signaling pathways activate by Gq-coupled receptors and phospholipase C (PLC). One activates protein kinase C (PKC) via diacyl-glycerol (DAG) (blue). The other activates IP3 receptors (IP3R) to release Ca^{2+} from intracellular stores in the endoplasmic reticulum (ER) (green). Here α1 adrenergic receptor is used as an example.

Learning Objective #4. *Know how GPCR signaling is terminated.*

Termination of GPCR signaling

Termination of signaling requires turning off activated receptors, turning off activated G-proteins, and return of second messenger levels, protein phosphorylation levels, and other changed metabolites to their original values. Consider the first two. Receptors quickly deactivate upon unbinding and washing away of the agonist ligand. They are also inactivated by other processes even while

ligand is still present. Such mechanisms that prevent over-stimulation are usually called receptor desensitization. In one canonical shutdown pathway, activated receptors are recognized and phosphorylated by G-protein coupled receptor kinases (GRKs) (Figure 6). Phosphorylated receptors may have reduced activity, and they can be turned off fully by binding of **arrestins** at the plasma membrane. The arrestin-receptor complex may be unable to couple to downstream G proteins, but it can mediate other signaling and it also may be removed from the plasma membrane by clathrin-mediated endocytosis, a true down regulation of receptor protein. A loss of function mutation in the GRK1 gene leads to stationary night blindness Oguchi type-2 where a severe retardation of dark adaptation of rhodopsin (the GPCR) in rod photoreceptors impairs vision at low light levels.

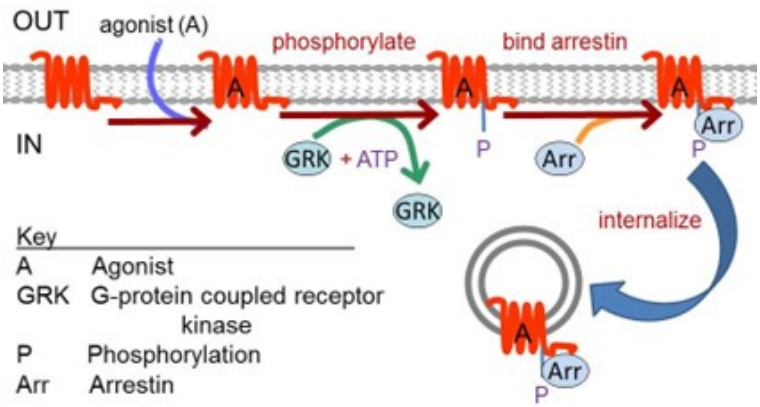

Figure 6. Homologous desensitization of a GPCR. This termination patheway recognizes active GPCRs with agonist and through several steps shuts them off selectively.

As we mentioned already, signaling by activated Gα-GTP is terminated by GTP hydrolysis, a reaction catalyzed by the Gα subunit itself that yields the inactive form, Gα-GDP (Figure 2). Thus

activated G-proteins have an intrinsic self-timer function that terminates their activity. The resulting Gα-GDP in turn is a scavenger that binds any free Gβγ dimers, re-forming the inactive heterotrimeric G protein GαβγGDP.

Note: The heterotrimeric G proteins discussed here should be distinguished from the small, low molecular weight, monomeric G proteins of the Ras, Rab, Rho, etc. families with which the Gα subunit does share some homology (Box B).

Box B: Heterotrimeric versus monomeric G proteins

Heterotrimeric G proteins

- Have three subunits: αβγ
- Activated by GPCRs
- Active when GTP is bound to α
- Turned off when α cleaves GTP to GDP
- The α subunit has much homology with the monomeric G proteins

Monomeric G proteins

- Have only one subunit
- Low molecular weight
- Active when GTP is bound
- Turned off when they cleave GTP to GDP

Signaling by Ras will be discussed in the section on signaling by

receptor tyrosine kinases. The monomeric G proteins have the same GTP-binding and GTPase properties as Gα subunits of heterotrimeric G proteins (Figure 2), and they both use the bound GTP to time their active signaling life time. But the monomeric G proteins do not couple to GPCRs or to Gβγ, and they signal to quite different downstream effectors.

Test your knowledge

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Receptor tyrosine kinases and nuclear hormone receptors

MARK BOTHWELL

Introduction

After G protein-coupled receptors, receptor tyrosine kinases and nuclear hormone receptors are the two largest families of receptors mediating cell-cell communication. Receptor tyrosine kinases (RTKs) are receptors for a variety of polypeptide hormones and growth factors. Nuclear hormone receptors are receptors for steroids and other non-peptide hormones. RTKs and nuclear hormone receptors often mediate effects that are slower in onset but more lasting in duration than GPCRs, but like GPCRs they typically initiate cascades of events that change the physiological state and patterns of gene expression of cells.

Session Learning Objectives

1. Describe the structure and function of RTKs.
2. Describe signaling mechanisms used by RTK signaling (MAPK cascades).
3. Describe crosstalk and interactions between pathways.

4. Describe steroid hormone receptors.

5. Describe steroid hormone receptor mechanisms of activity.

Learning Objective #1. *Describe the structure and function of RTKs.*

RTK Structure and Function

Receptor tyrosine kinases (RTKs) signal across the plasma membrane in response to binding of growth factors, hormones such as insulin, and other secreted polypeptides. RTK proteins are large intrinsic membrane proteins possessing a single membrane-spanning segment. The receptors function as dimers of two identical subunits.

Protein kinases represent one of the most important classes of proteins engaged in cellular regulation. Over 500 human genes encode protein kinases. While the majority of protein kinases phosphorylate the hydroxyl groups of protein serine or threonine residues (Fig. 1), about 90 human genes encode protein kinases known as tyrosine kinases because they phosphorylate the hydroxyl groups of protein tyrosine residues. More than half of tyrosine kinases are referred to as receptor tyrosine kinases (RTKs) because their tyrosine kinase enzymatic activity is activated by binding of extracellular peptide hormones such as insulin or of growth factors

such as nerve growth factor (NGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF).

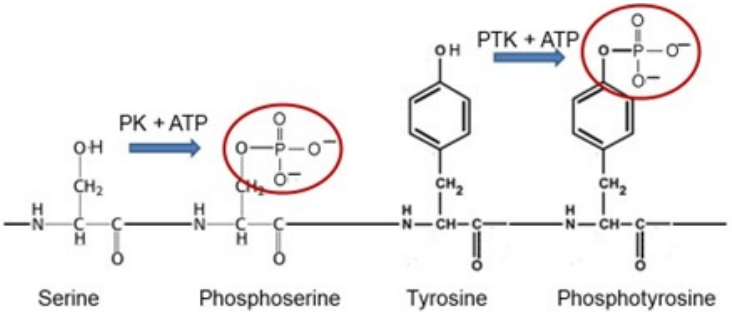

Figure 1: Phosphorylation of serine and tyrosine residues. PK stands for protein serine/threonine kinase, and PTK, for protein tyrosine kinase.

RTKs span the plasma membrane, so that hormone or growth factor binding to the portion of the receptor exposed to the extracellular environment stimulates the tyrosine kinase activity of the portion of the receptor exposed to the cytoplasm (Fig. 2A).

Some RTKs exist constitutively as dimeric aggregates, while others form such dimers in response to ligand binding (Fig. 2B). In the ligand-activated dimer, each subunit phosphorylates tyrosine residues in its partner subunit, a process known as auto-phosphorylation. In contrast to the serine-threonine kinase activity of PKA or PKC, the tyrosine kinase activity of RTKs is directed against a relatively smaller number of substrates, most notably the protein chains of the receptor itself.

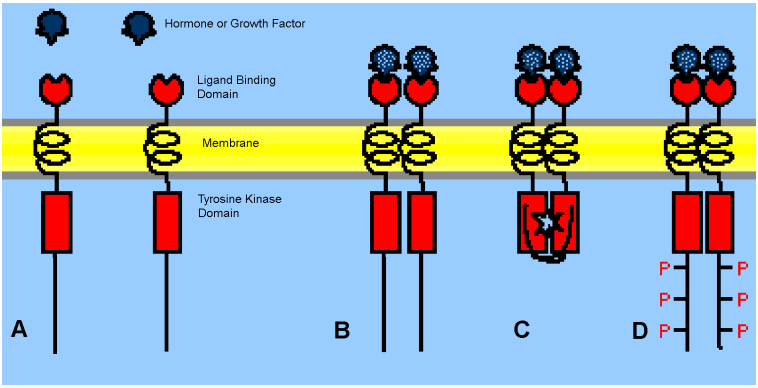

Figure 2. Ligand induced auto-phosphorylation of Receptor Tyrosine Kinase. Time advances left to right. (A) Quiescent receptors. (B) Ligand binds. (C) Receptor conformation changes. (D) Auto-phosphorylation makes a scaffold of phospho-tyrosine residues ready to bind signaling proteins.

A simple exercise to check what you recall

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https://uw.pressbooks.pub/physiology/?p=253#h5p-36

Learning Objective #2. *Describe signaling mechanisms used by RTK signaling (MAPK cascades).*

Most signaling from RTKs occurs by nucleation of protein complexes scaffolded on the phosphorylated tyrosine residues

(phosphotyrosines) of activated RTKs (Fig. 1 and 2D). The phosphotyrosines are recognized as docking sites by different cytoplasmic signaling proteins, initiating several parallel signaling pathways.

Ras/ERK Pathway

One important signaling pathway initiated by RTK autophosphorylation employs the monomeric G protein, Ras. Like heterotrimeric G proteins, monomeric G proteins cycle between an activated state, with GTP bound, and an inactive state, with GDP bound (Fig. 3), and the activated form binds effector proteins to cause downstream events. The activity of monomeric G proteins is controlled by (i) GTP Exchange Proteins (GEFs) that promote exchange of bound GDP with GTP, increasing Ras signaling activity, and (ii) GTPase Activating Proteins (GAPs) that promote the intrinsic GTPase activity of Ras, converting bound GTP to GDP and turning off Ras signaling activity. Unfortunately, a variety of human Ras gene mutations dysregulate Ras. Elevated cell proliferation associated with activating mutations of Ras drives one third of human cancers.

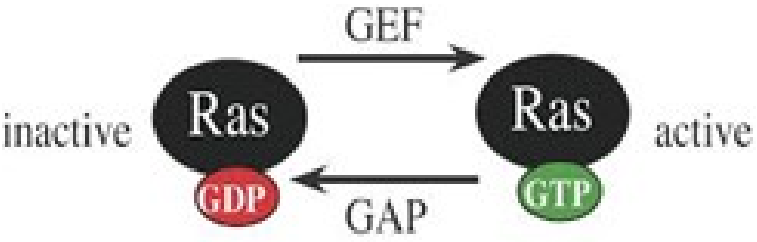

Figure. 3. A GTP/GDP cycle controls Ras activity.

A key GEF controlling Ras activity is the protein called SOS. SOS is turned on by binding to an adaptor protein Grb2 that associates with particular phospho-tyrosine residues of activated RTKs (see

Fig. 4). Thus, activation of RTKs can promote activation of Ras through Grb2 and SOS acting as adaptors. This is an example of formation of a larger intracellular signaling complex.

A common functional theme among monomeric G proteins is cascading activation of a series of three serine/threonine-directed protein kinases (Fig. 4). The final protein kinase in the series is a Mitogen Activated Protein Kinase (MAPK). MAPK becomes active as the result of phosphorylation by a MAPK kinase (MAPKK) and the MAPKK becomes active following phosphorylation by a MAPKK kinase (MAPKKK). MAPKKK is activated by binding to a monomeric G protein such as Ras.

In the case of Ras, the MAPKKK is a protein named Raf, the MAPKK is MEK1 or MEK2, and the MAPK is ERK1 or ERK2. ERK1 and ERK2 typically function redundantly so they are often referred to jointly as ERK1/2. ERK1/2 signals in both the cytoplasm and nucleus. Among other important functions, ERK1/2 is a master regulator of the cell cycle. Thus ERK1/2 activity resulting from growth factor activation of RTKs promotes cell proliferation and tissue growth and repair. Constitutive activation of ERK1/2 is the primary driver of cell proliferation in most cancers. ERK1/2 activity also reduces cell death. Reduced cell death enhances the ability of increased cell proliferation to increase tissue mass, contributing to the tumorigenic effect of constitutively activated ERK1/2. Constitutive activation of ERK1/2 in cancer usually results from activating mutations of RTKs and/or Ras rather than mutations of ERK1/2 themselves.

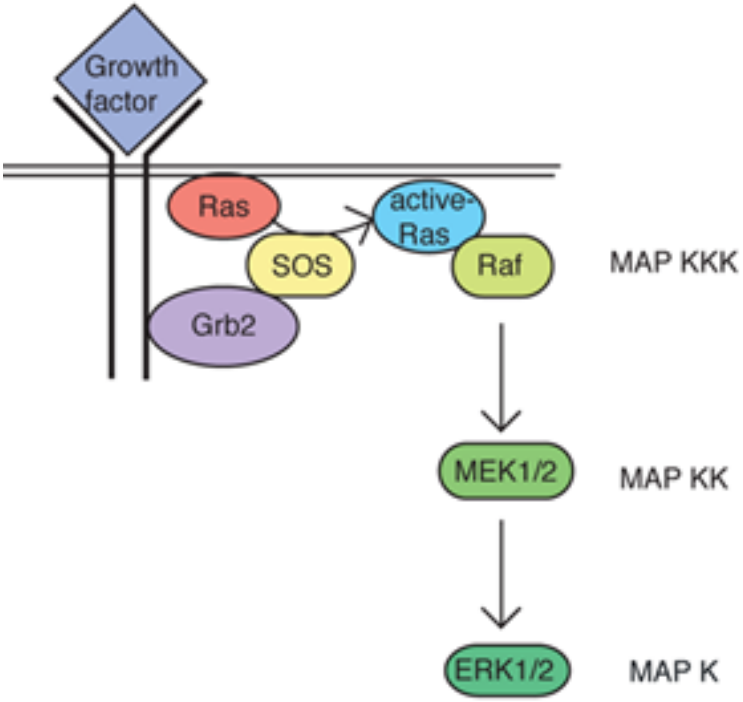

Figure. 4. The Ras/ERK signaling pathway of receptor tyrosine kinases. This exemplifies typical signaling cascades from RTKs. All these sequential amplification steps culminate in an activated MAP K that will phosphorylate many specific downstream targets.

Two simple exercises to check what you recall

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PI3K/Akt Pathway of RTK Signaling

Some phosphotyrosine residues of RTKs bind and activate a phospholipid-modifying enzyme, phosphatidylinositol 3-kinase (PI3K), triggering another cascading sequence of kinases. For example, insulin receptor signaling employs a variant version of this mode of signaling (Fig 5). A phosphotyrosine residue of the activated insulin receptor binds a protein called IRS1. The insulin receptor then phosphorylates tyrosine residues of IRS1, causing IRS1 to bind and activate PI3K. The PI3K enzyme adds an additional phosphate group to phosphatidylinositol-4,5-bis-phosphate (PIP2), producing phosphatidylinositol-3,4,5-trisphosphate (PIP3). A protein kinase, Akt, is recruited to the plasma membrane by binding to PIP3, allowing Akt to be phosphorylated and activated by several different membrane-associated protein kinases. Activated Akt controls both cytoplasmic and nuclear functions. For example, insulin receptor-mediated activation of Akt acts in the cytoplasm to cause plasma membrane insertion of the glucose transporter, Glut4, allowing cellular uptake of glucose, while Akt acts in the nucleus to activate the transcription factor Fox01, which controls enzymes for hepatic glucose synthesis.

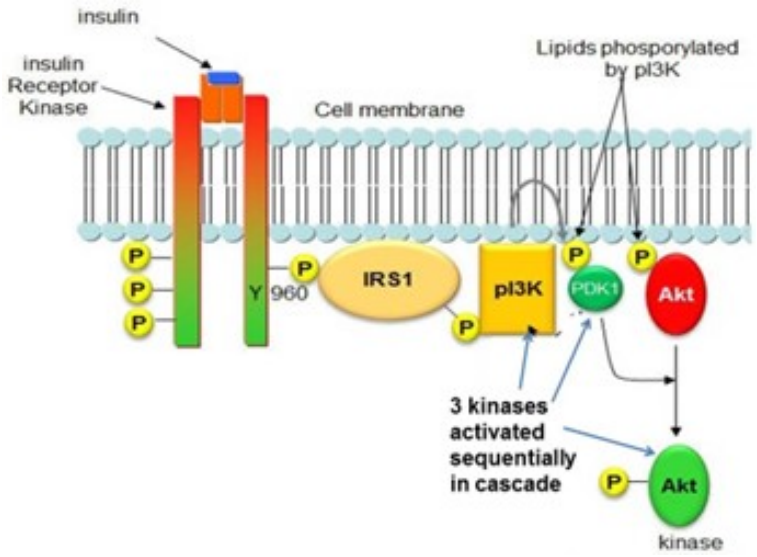

Figure 5. PI3K/PIP3/Akt signaling pathway of the insulin receptor on the plasma membrane. Phospho-Akt phosphorylates many targets to carry the insulin signal forward.

Learning Objective #3. *Describe crosstalk and interactions between pathways.*

Phospholipase C (PLC) Pathway

Phosphotyrosine residues of some RTKs bind and activate [the gamma isoform of] Phospholipase C (PLC). The PLC enzyme activated by RTKs and the PLC activated by GPCR signaling are products of different genes, but they catalyze the same reaction,

cleaving the lipid PIP2 to produce IP3 and DAG second messengers, increasing cytoplasmic calcium ion concentration, and activating protein kinase C (PKC), respectively. This pathway represents an important mode of convergence of RTK and GPCR signaling pathways.

Convergence and Cross-talk between RTK and GPCR pathways

An important feature of regulatory biology is that distinct pathways controlling similar functions may be linked in a manner that coordinates their functions. Convergence, as seen for PLC in GPCR and RTK pathways, represents one mode of linkage of RTK and GPCR signaling. Cross-talk represents another. The concept of cross-talk is illustrated by two mechanistically different examples.

Example 1: The epidermal growth factor (EGF) receptor is an RTK that is activated by a variety of different growth factors (Fig. 6, green). Some EGF receptor ligands are released from cells by vesicular secretion, but others, such as HB-EGF (orange), are initially expressed as intrinsic membrane proteins that must be proteolytically cleaved by ADAM17 to release the active extracellular ligand peptide. Both Gi and Gq GPCR pathways activate ADAM17. Thus, GPCR ligands often cause EGF receptor activation. This type of crosstalk is known as transactivation.

Example 2: A second type of crosstalk/transactivation mechanism is illustrated by the Trk family of RTKs (Fig. 6, blue) that function as receptors for the nerve growth factor family of growth factors [known as neurotrophins]. GPCRs that activate the cAMP/PKA signaling pathway cause phosphorylation and activation of Fyn, a protein kinase. Activated Fyn binds and induces signaling by the TrkB neurotrophin receptor, even in the absence of neurotrophins. Thus, GPCR ligands such as adenosine can cause transactivation of TrkB signaling in neurons.

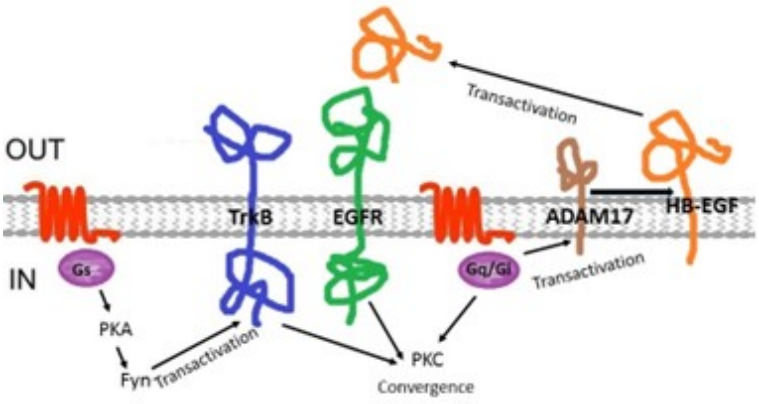

Figure. 6. Examples of convergence and cross-talk between GPCRs and RTKs.

Learning Objective #4. *Describe steroid hormone receptors.*

Steroid receptors are a subfamily of a larger group of receptors known as nuclear hormone receptors. The ligands for nuclear hormone receptors are structurally and functionally diverse, as illustrated in Figure 7, including hormones such as steroid hormones, thyroid hormone, and the hormonal derivatives of vitamin D and vitamin A, but also including aromatic hydrocarbons including the potent environmental toxin, dioxin. By exerting hormone-like effects, environmental dioxin pollutants cause reproductive and developmental problems, and cancer.

Ligands for nuclear hormone receptors all have one important property in common; they are chemically hydrophobic. This allows them to pass easily across cellular membranes. Their receptors

reside in the interior of cells, shuttling between the cytoplasm and nucleus. The various steroid hormone receptors and the other nuclear hormone receptors are closely similar in structure, and they all signal in much the same way.

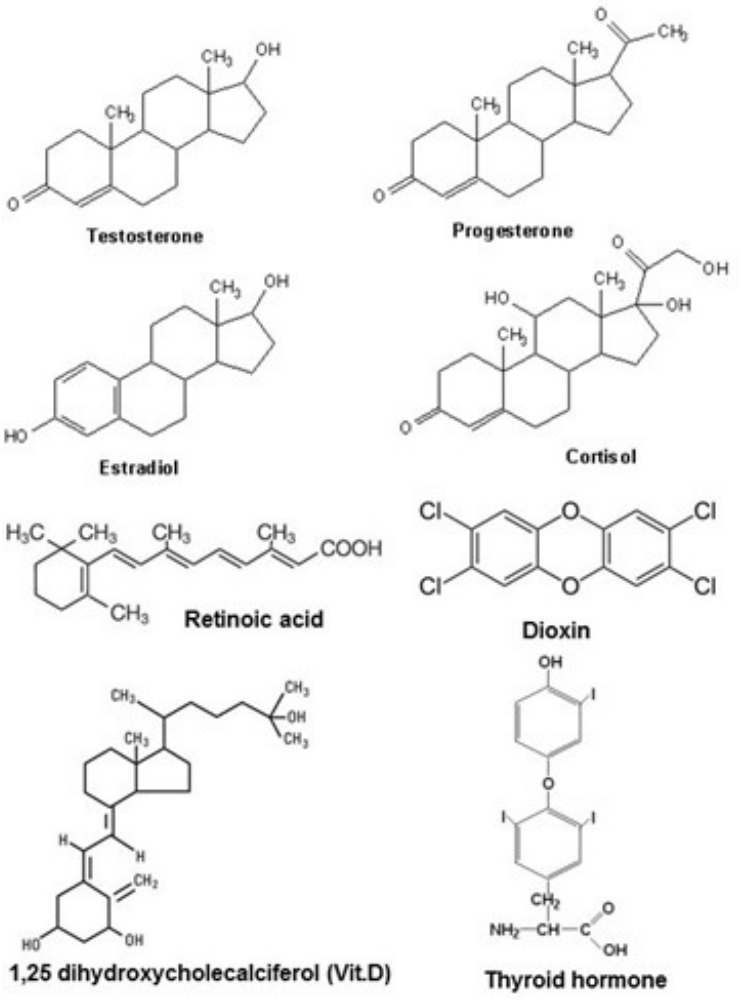

Figure. 7. Steroids and other ligands of nuclear hormone receptors. All are hydrophobic.

A simple exercise to check what you recall

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Learning Objective #5. *Describe mechanisms of activity of steroid hormone receptors*

Unlike GPCRs and RTKs, nuclear hormone receptors are not associated with membranes. Steroid receptors and other nuclear hormone receptors signal by acting as transcription factors that regulate expression of specific genes (Fig. 8). They bind directly to short (6-nucleotide) DNA sequence motifs in the promoters of steroid-regulated genes. These sequence motifs are known as hormone response elements, abbreviated as HRE in the figure. [More specifically, scientists distinguish retinoic acid response elements (RRE), steroid response elements (SRE) and glucocorticoid steroid response elements (GRE), for example].

Binding of steroids to their receptors increases the affinity of the receptors for the cognate DNA sequence motifs in the gene promoter. The promoter-receptor-steroid complex promotes expression of the regulated gene. Following binding of the hormone/nuclear hormone receptor complex to DNA, the receptor recruits other transcriptional regulatory proteins, including RNA Polymerase II, allowing transcription of the gene to commence (Fig. 8). However, in some cases steroid receptors bind the promoter in

the absence of steroid. In such cases the bound receptor actively represses gene expression, until binding of steroid to the receptor switches it from a repressive to an activating mode of action.

Nuclear hormone receptors function as dimers. Their dimeric structure allows them to bind optimally to sequence elements in both strands of the DNA double helix. Steroid receptors generally function as homodimers (two identical subunits in the complex). On the other hand, some nuclear hormone receptors function as heterodimers (two non-identical subunits). Figure 8 illustrates such a scenario for the retinoic acid receptor (RAR), which functions as a heterodimer with a second subunit known as retinoic X receptor (RXR). RXR also acts as the heterodimeric partner of other nuclear hormone receptors, including thyroid hormone receptor and vitamin D receptor.

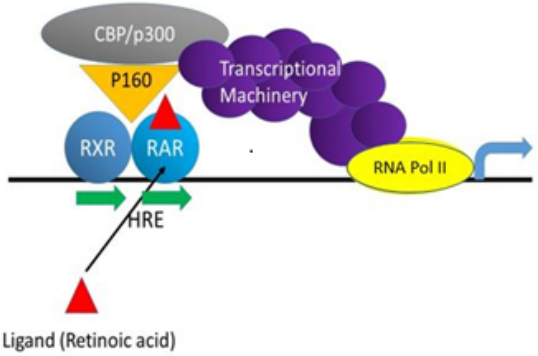

Figure 8. Steroid and other nuclear hormone receptors engage the transcriptional at gene promoters. Here, the heterodimeric retinoic acid receptor binds HRE DNA elements in a gene promoter, recruiting transcriptional adapter proteins and RNA polymerase II.

Conclusion

An important feature of signaling by steroid receptors and other

nuclear hormone receptors is that binding sites on DNA for each receptor appear in dozens or even hundreds of different genes. Thus, hormones such as estrogen and thyroid hormone coordinately change expression of many different genes and change many aspects of cell function in many different tissues.

Membrane Potentials

FRED RIEKE AND BERTIL HILLE

Learning Objectives

1. Describe the elements of ionic electricity: ions, charge, potential gradients, forces, current, and conductance.

Dissolved salts dissociate into small ions. These are charged particles with typical charges of –2, –1, +1, or +2. The ions are moved across membranes by two forces: concentration gradients and electric fields acting on the charge of the ion. Any net movement of an ion across the membrane is by definition an electric current. Ion currents are conducted through the membrane by ion channels.

2. Know how ion diffusion in ion channels establishes membrane potentials.

When an ion-selective ion channel opens in the membrane, specific ions can move down their electrochemical gradient (this gradient is a combination of electrical and concentration gradients). By moving charges from one side to the other, the resulting ion currents change the total charge in the cell and the electrical potential across the membrane. Without charge movement, there can be no membrane potential change.

3. Recognize that fluxes needed to make typical membrane potentials are extremely small: membrane capacitance.

Membrane capacitance is by definition the amount of charge you have to move across the membrane to change the membrane potential a certain amount. In cells, the membrane capacitance is small, so the quantity of ions that has to move for electrical signaling is tiny, depleting the existing gradient by only a little.

4. Explain with Na^+, K^+, Cl^-, and Ca^{2+} what contribution each could make individually to electrical potential changes in the plasma membrane of excitable cells.

Na^+, K^+, Cl^- and Ca^{2+} ions have different ion gradients and charge. Therefore, opening selective ion channels for each of them pulls the membrane potential towards a different final value. For example, open K^+ channels produce a negative inside potential, and open Na^+ channels produce a positive inside potential. Therefore electrical responses of cells depend on opening the right ion channels in the right sequence to shape the signal.

Standard laws of electricity govern how cells make electrical signals across their membranes. This chapter reviews these laws. The need to think about electricity may seem daunting to some biologists, but really it is not particularly complicated. Electricity obeys a few simple physical rules in a logical manner. You have studied them before. Comfort with the key concepts in this chapter will be very helpful in subsequent more specific and detailed study of electrophysiology. As we will see, if you have both (i) gradients of ions across a membrane and (ii) a membrane permeability to some of these ions by open ion channels, the necessary net flow of the

charged ions in these channels forms an electric current and can change the membrane potential.

To motivate our study of principles, we look ahead at a nerve cell sending an electrical message, the spike-like action potential (we will examine this process in more detail later in the course). The action potential is a stereotyped, efficient, brief, electrical signal that can travel regeneratively at high speed along an axon. It is the long-distance, electrical message of the nervous system, the unit of information like the information bits in the wires of a computer. Figure 1 is an electrical recording from the inside of an active nerve cell. We will note just three typical properties for now. (i) This neuron starts with a NEGATIVE inside resting potential (-60 mV). (ii) During the action potential, the inside of the cell becomes briefly POSITIVE. And (iii) the whole signal is finished in <1 ms (0.001 s). Think of this signal as a brief membrane potential excursion of about 100 mV, the pulse-like unit of propagated information. We will begin to explain in physical terms how cells make electrical signals like this and how they propagate.

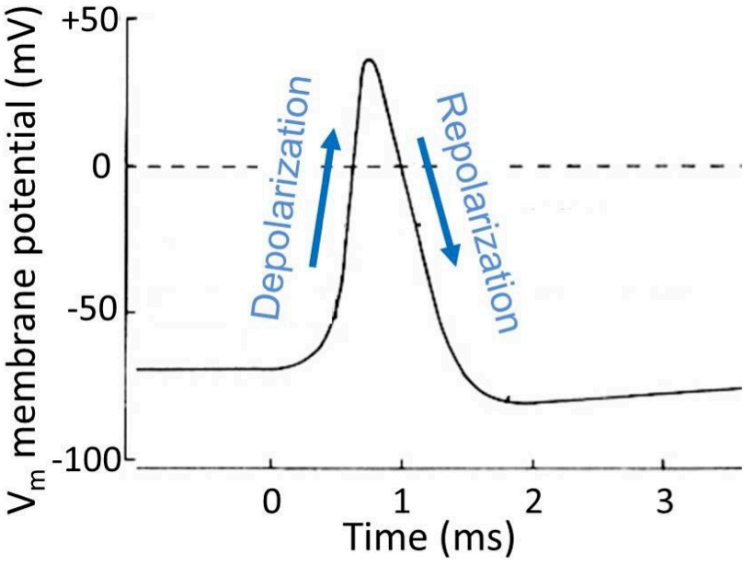

Figure 1: A preview of the action potential. An electrical recording showing the time course of the membrane potential changing in a nerve axon during passage of an action potential (spike). Note negative resting potential and positive-going spike-like electrical signal. The action potential depolarizes (positive-going rising phase) and repolarizes (negative-going falling phase) the membrane.

Important convention: *Note that when we speak of membrane potentials of the plasma membrane, we consider the inside potential relative to the outside. In simple terms you can think of this as the potential* INSIDE *the cell. The whole potential drop occurs in a region of <10 nm as you cross through the insulating membrane.*

Learning Objective #1. *Describe elements of ionic electricity: ions, charge, potential gradients, forces, current, conductance, and membrane capacitance.*

An Analogy

If it is helpful, you can make an analogy between familiar gravitational rules and less familiar electrical rules. The mass of an object is analogous to electrical charge. The gravitational potential is analogous to electrical potential (voltage). You have to do work to lift a mass to higher gravitational potential, and similarly you have to do work to move a charge to a region of higher electrical potential. One real difference is that charges, voltages, and forces can be either positive or negative, attractive or repulsive, but masses, gravitational potential energy, and gravity are always positive and attractive.

First rules of ionic electricity

These rules are important to understand physiology. They are also summarized in a separate one-page file "Electric Keywords" that we hope you will keep referring to as you study electrophysiology. Please make sure that you are comfortable with them!

1. *Charge*: Ions have a charge given in multiples of one elementary electron charge. Cations (Na^+, K^+, Ca^{2+}) are positively charged and anions (Cl^-) are negatively charged.
2. *Two forces that move ions*: (i) Charges are moved by the force of electric fields: Opposites attract, so cations (positively

charged) move towards a negative pole, and anions towards a positive pole. (ii) In addition, ions are moved by diffusion (thermal agitation) down their concentration gradients–even in the absence of electric fields.

3. *Current*: A net movement of charge (flow of ions) is an electric current (an ion current) measured in amperes (A) and symbolized by I. The direction of current is defined by convention as the direction of positive charge movement. Hence, if only positive K^+ ions are moving OUT of a cell, then electric current is also moving OUT of the cell; however, if only negative Cl^- ions are moving OUT of the cell, we use this convention to say that electric current is flowing INTO the cell.
4. *Voltage*: If cations are removed from a compartment (the cell), the compartment becomes more negative, i.e, a negative electrical potential or negative voltage will be set up inside the cell. The voltage arises because there is a charge imbalance. In electrophysiology, the words voltage and electrical potential will mean the same thing. A voltage is measured in units of volts (V) and symbolized by V. It is defined as the amount of electrical work it would take to remove one more cation. [Aside: When you buy a flashlight battery, it is typically a 1.5 volt battery. We could write $V_{battery} = 1.5$ V. The bigger the battery (AAA,AA,A,B,C,D, etc.), the more current and charge it can provide, but the alkaline battery chemistry always makes a 1.5 V potential. In biology, the membrane potentials are much smaller, within the range from -100 mV to +50 mV. All our electrical signaling occurs within this small voltage range as in Figure 1.]
5. *Conductance*: Conductance is a measure of how easily ions can cross the membrane (it is the inverse of the resistance). A membrane with many open ion channels has a high conductance (low resistance), and one with no open ion channels has a conductance that is almost too low to measure since the lipid bilayer is very impermeable to charged ions. Thus conductance, symbolized G, is proportional to the

number of ion channels open. We now show how to measure it.

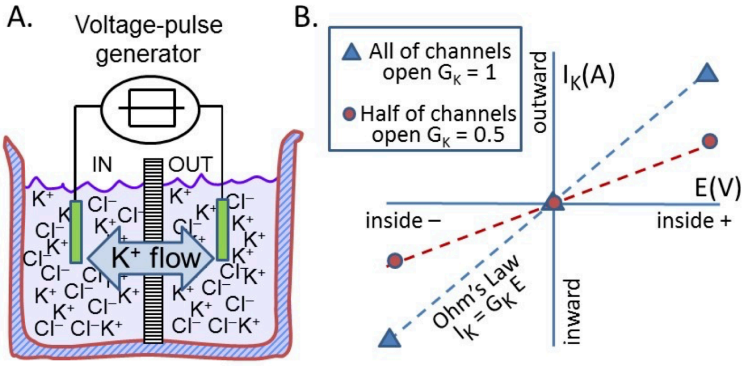

Figure 2: Measuring the electrical conductance GK of a mem–brane containing K^+-selective ion channels. **A.** *A voltage pulse generator changes the voltage across the membrane.* **B.** *The resulting K+ currents are plotted against the applied voltage. By physiological convention, outward currents are called positive and inward currents are called negative as the plot shows. In this example there are no ion gradients across the membrane.*

An electrical experiment to measure conductance of a membrane (Figure 2A)

A beaker with electrolyte solutions is divided into two compartments called IN and OUT by a membrane containing only K^+ channels. The IN and OUT compartments contain equal concentrations of dissolved KCl and each has an immersed electrode (e.g. a wire, green) connected to a voltage-pulse generator. The pulse generator changes the potential on the wire for brief periods and can make the IN side briefly negative, positive, or unchanged. Figure 2B plots the results as blue triangles. These triangles show the measured current I_K carried by K^+ ions versus the applied voltage. (i) When the transmembrane voltage is kept at 0 mV, no net K^+ current flows (symbols at the origin). There is no

force on the K^+ ions. (ii) When the IN voltage is made negative, there is an inward K^+ current, the negative electrical potential pulls K^+ ions towards the IN compartment. (iii) when the IN voltage is made positive, there is outward K^+ current.

The blue triangles in Figure 2B describe a straight line through the origin. The conductance is the slope of this line. This illustrates Ohm's law

$$I_K = G_K E$$

which says that K^+ current I_K is linearly proportional to G_K (the conductance to K^+) and the electrical driving force E. Remember that the conductance is the inverse of the resistance ($G = 1/R$), which connects the equation above to the more familiar form of Ohm's law ($E = I R$). Ohm's law tells us that more force (more E) means more current.

The blue line and blue symbols in Figure 2B describe the current when all the K^+ channels are open. If we close half of them, we get the red line. As you might expect, the flow of K^+ is reduced to half, the slope is only half, and the conductance of the membrane is half. Electrical conductance is easy for electrophysiologists to measure and conveniently tells us how many ion channels are open. This concept is used repeatedly when we start with electrophysiology.

Learning Objective #2. *Know how ion diffusion in ion channels establishes membrane potentials.*

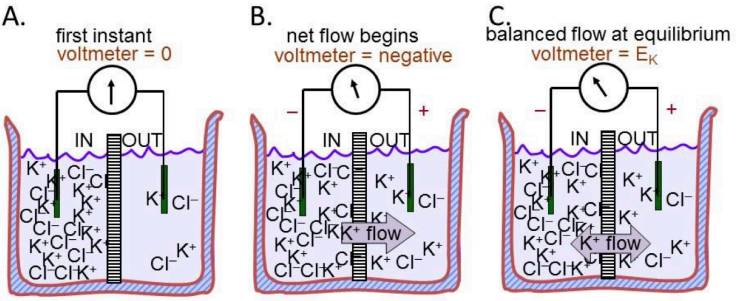

Figure 3: A thought experiment to explain how diffusing ions make a membrane potential in an ion-selective membrane.

A thought experiment on membrane potentials

We now use these rules of electricity to describe the origin of membrane potentials (Figure 3). Again electrolyte in a beaker is divided by a membrane containing only K^+ channels. But now there is no pulse generator, just a recording amplifier that reports any potential difference that might appear spontaneously across the membrane. These compartments, for example, could represent the inside and the outside of a cell with a K^+-permeable membrane since this time, more like a real cell, the IN compartment contains high K^+, and the OUT compartment, low K^+ (Figure 3A). Each solution is initially electrically neutral with an equal number of anions and cations, and the voltmeter cartoon indicates that there is initially no potential difference between them. Rule 2 and your intuition say that K^+ ions will start to flow towards the OUT compartment because they are free to diffuse down their concentration gradient (Fig. 3B). Since there are no Cl^- channels in the membrane, there is no movement of Cl^- ions across this membrane. Thus, there is a net movement only of positive charges, and Rule 3 says that the outward K^+ flow carries an outward electric current I_K. Rule 4 says that since positive charge is being removed from the IN compartment, that compartment starts to become more nega-

tive–as indicated by the second voltmeter cartoon. A membrane potential has been generated!

However, rule 2 says that the net flow of K^+ cations will soon slow down as they experience an electrical retarding force from the growing membrane potential that makes it progressively harder for them to leave the negative IN compartment. An equilibrium is reached as the opposing electrical force pulling K^+ ions back becomes equal to the diffusion force pushing them out (Fig. 3C). The forces have become balanced. Now the membrane has established a stable negative internal membrane potential all by itself! This whole process takes only a millisecond or two. The potential difference arose because (i) there is a K^+ gradient, and (ii) the membrane has K^+-selective ion channels. This thought experiment corresponds very closely to the situation in real cells. They have K^+ inside and they have a negative resting potential inside (Fig. 1) because of a predominance of open K^+ channels at rest. We have applied Rule 4 to understand the negative resting potential and now generalize with the most important concept to understand electrical signaling:

Take-home concept 1: To create or change the cell membrane potential requires a net movement of charge (ions) across the membrane. This concept means that the action potential of Figure 1 must be produced by net movements of ions (charge) across the membrane.

Learning Objective #3. *Recognize that fluxes needed to make typical membrane potentials are extremely small.*

Our last rule of electricity says that very few ions need to move to produce sizable changes in membrane potential:

6. *Capacitance*: Electrical capacitors store charge. Recall that a substance like the lipid bilayer that does not conduct electricity is called an insulator. Two conductors separated by a thin insulator form an electrical capacitor. Therefore, the thin plasma membrane bathed by inside and outside conducting electrolyte solutions has the property of an electrical capacitor. The magnitude of a capacitance (symbolized C) is defined as the number of charges that you have to put on the capacitor to achieve a certain potential change. Therefore, the capacitance of cell membranes tells us how many ions have to be moved across the membrane to change the membrane potential during signaling. For a spherical neuron 20 m in diameter, a net movement of only ~10^{-17} moles of cations into the cell will be enough to make the inside 100 mV more positive–equivalent to one action potential. This movement amounts to only 0.002% of the number of cations already in the cell (~6×10^{-13} moles). Therefore, the ion fluxes needed to make signals in biological membranes are tiny, and the charge movement and potential changes can be completed quickly. However, after repeated bouts of signaling it becomes essential to accelerate ion pumps to restore the gradually declining ion gradients. This need for accelerated pumping is accentuated in fine nerve processes where a large surface-to-volume ratio means that the reserve pool of ions inside is smaller.

> **Take-home concept 2:** Although the membrane capacitance does require that some ions move to generate electrical signals (concept 1), the quantity can be quite small so that a single action potential does not disturb the existing ion gradients very much.

Learning Objective #4. *Explain with Na+, K+, Cl- and Ca2+ what contribution each could make individually to potential changes in the plasma membrane of excitable cells.*

The three questions approach

We begin with three questions that will allow us to determine what each ion can do to the membrane potential. We have just applied these questions to the K^+ ion in Figure 3. Now we generalize. No equations, just simple logic that goes a long way.

Question 1: Which ion is permeable?

Question 2: Which direction does it want to flow?

Question 3: What membrane potential would that induce?

For Figure 3, the three answers were:

- K^+ is permeable;
- It would flow OUT;
- That would make the voltage of the IN side negative.

Now you can go through each of the ions in Table 1, supposing that the membrane is selectively permeable only to K^+, Na^+, Ca^{2+}, or Cl^- one at a time, using the three questions to decide what sign of membrane potential would result. You should have found two ions that could make the membrane potential negative and two that could make it positive. Excitable cells open and close ion channels to each of these ions to generate their electrical signals. In particular, you will learn shortly that for the nerve cell in Figure 1, the resting membrane is K^+ permeable and therefore negative

inside; the membrane during the action potential becomes Na^+ permeable (Na^+ channels open) and therefore a few Na^+ ions move in and the potential becomes positive; and then the Na^+ channels close, and K^+ channels bring the membrane back to the negative resting potential.

Recap

Let us apply our learning to the action potential (AP) rising phase in anticipation of your next session on electrical excitability. The AP rising phase requires net entry of Na^+ ions (Rule 3). The number of ions moving is modest (Rule 6). Nevertheless, repetitive firing of APs will lead to intracellular Na^+ accumulation, so, since its Na^+ substrate increases, the Na^+/K^+-ATPase pump speeds up and pumps the Na^+ out again more vigorously. Local glucose metabolism speeds up a bit to make the needed ATP. A brain imaging technique called PET scan measures glucose consumption and allows radiologists to observe which brain regions become active during specific mental activities. Soon, you will also learn that our larger nerve fibers often use myelin as a way to reduce the electrical capacitance of sections of axon. In myelinated nerve, Schwann cell membranes are wrapped on top of each other in many tight layers around the axon, reducing the effective capacitance and reducing the amount of ion movement needed to make an action potential. This permits faster depolarizations with smaller Na^+ flow (Rule 6) and faster propagation of the action potential message. We will return to this later.

The Nernst Equation

So far we have been thinking qualitatively about the sign of the membrane potential, and that suffices for basic understanding.

However, sometimes we want to say how large the membrane potential will be rather than just working out its sign. Scientists need to do this to check that they really understand what is going on. For this, we return to the thought experiment of Figure 3. In the right panel, the membrane potential comes to an equilibrium value where the concentration force pushing K^+ ions out of the IN compartment is exactly matched by the electrical force pulling them back. This is an electrochemical equilibrium and therefore thermodynamics can give us an expression for the equilibrium potential. Since the problem involves a membrane selectively permeable only to K^+ ions, we call this the "potassium equilibrium potential" symbolized by E_K. For completeness we write the answer called the Nernst Equation first in its general thermodynamic form for any ion with charge z, but then follow by writing a much more practical version for our body temperature in millivolts. If you do need to calculate equilibrium potentials, use the practical form. You should understand the significance of the Nernst Equation as a beautiful and compact explanation, but in general practice, you would rarely have to use it.

Nernst Equation $$E_{ion} = \frac{RT}{zF} \ln \left(\frac{[Ion]_o}{[Ion]_i}\right)$$

Practical form $$E_{ion} = \frac{61}{z} \text{ mV } \log_{10}\left(\frac{[Ion]_o}{[Ion]_i}\right)$$

In the formal first form, there are thermodynamic constants: R (gas constant), T (temperature), and F (Faraday's number). In the practical form, their values have already been factored in for 37°C. Notice that the equilibrium potential depends on the ratio of the ion concentration outside and inside. The ratio appears inside a logarithm. Box C reminds us that $\log_{10}$ (x) equals the power of 10 that would give the value x. It is also a button on your calculator!

Box C. the logarithm

x	$\log_{10}(x)$
10	+1
1	0
1/10	-1
1/100	-2

With the practical form of the Nernst equation, we can plug in the ion concentrations listed in the chapter on Cell Membranes and Transporters to get the equilibrium potentials of each of the four important ions (Table 1). These values define the range of plasma membrane potentials that cells are able to achieve. As expected from the three-questions approach, E_K is a negative number, -97 mV. That is as far negative as the plasma membrane potential ever gets. To do so, the membrane would have to be exclusively permeable to K^+ ions; that is, the only ion channels open would be K^+ channels. In resting axons, open K^+ channels greatly outnumber open Na^+, Cl^-, and Ca^{2+} channels so the plasma membrane is primarily permeable to K^+, and the resting potential approaches E_K. By contrast, but as expected from the three-question approach, E_{Na} is a positive number, +65 mV. As you will see shortly, E_{Na} sets the positive limit of the nerve action potential (Figure 1) when mostly Na^+ channels are open. Remember that at the equilibrium potential for an ion, the diffusion force and the electrical force are exactly balanced (they cancel each other) and there would be no further net flow of that ion. In several disease or nutritional conditions the ion gradients across our cell membranes change. This can severely impact the resting potential and the electrical signaling of nerve and muscle.

Key concept

Table 1. Ion equilibrium potentials in a neuron

Ion	Extracellular concentration Scientific units	Intracellular concentration Scientific units	$[Ion]_o/[Ion]_i$	z	Equilibrium potential
Na^+	142 mM	12 mM	11.8	+1	**+65 mV**
K^+	4 mM	155 mM	0.026	+1	**-97 mV**
Ca^{2+}	1.5 mM	~100 nM*	15,000	+2	**+127 mV**
Cl^-	103 mM	7 mM	15	-1	**-71 mV**

* Notice that here the concentration units for Ca^{2+} inside are nanomolar. Nano means 10^{-9}, very small.

Real membranes have several types of open channels. Although we say the resting potential is negative because K^+ channels are open there, the resting potential is never as negative as the ideal E_K (-97 mV). This is because even at rest, there are some open Na^+ and Cl^- channels whose equilibrium potentials are more positive than E_K (+65 and -71 mV in Table 1 for neurons). When several different channels are open simultaneously, the membrane potential (V_m) will lie somewhere between the equilibrium potentials of each of those channels. Thus, if the conductance to K^+ is the largest at rest, V_m will be brought closer to E_K, and if the conductance to Na^+ rises, V_m will depolarize, eventually approaching E_{Na} if the Na^+ conductance becomes the largest. This important concept will explain how excitable cells make electrical signals (membrane potential changes) in the nervous system by opening and closing ion-selective channels.

Aside 1: ***Mitochondrial membrane potential.*** When you study mitochondrial bioenergetics, you may be curious how the inner mitochondrial membrane makes a large inside-negative membrane potential in the matrix of the mitochondrion during electron transport. The answer is that unlike the plasma membrane, the mitochondrial inner membrane has almost no open ion channels and instead it has powerful proton transporters (the electron transport chain) driven by metabolism that remove protons actively from the matrix interior, leaving the inside lumen negative. The

negative matrix potential and the proton gradient are used as the electrochemical energy source to phosphorylate ATP during oxidative phosphorylation.

Review Questions

The membrane potential is symbolized by V_M in these review questions.

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Practice Questions

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Action Potential, Threshold, Refractory Period

FRED RIEKE

Learning Objectives and Quick Synopses

1. Explain the ionic basis of the action potential.

The action potential is produced by the opening of voltage-activated Na^+ and K^+ channels. Na^+ channel opening creates the positive change in voltage of the initial upswing of the action potential. K^+ channel opening helps the voltage repolarize and return to its resting value.

2. Explain the concept of threshold in terms of the underlying ion channel activity.

As the cell membrane is depolarized from the resting potential, threshold is the voltage at which Na+ channels begin to dominate the membrane potential. Their opening makes the voltage more positive, which then causes more channels to open, which makes the voltage yet more positive … . This positive feedback cycle creates the threshold.

3. Describe the refractory period and how it is produced mechanistically.

The refractory period refers to the time following an action potential that a cell is unable to generate another action potential. This is produced by the time required for Na^+ and K^+ channels to return to their normal resting states following activation during the action potential.

Overview

This chapter: (1) introduces the action potential and some of its key properties; (2) reviews the key aspects of electrical signaling that provide the mechanistic basis of the action potential; and (3) explains the action potential by stepping through the learning objectives. The Action Potential, Propagation chapter builds on these ideas to understand how action potentials propagate down axons to carry signals long distances.

Key properties of action potentials

A nerve cell has a negative membrane potential at rest – meaning the inside of the cell has an excess of negative charge. If we inject a weak, brief depolarizing current (i.e. an inward movement of positive charge, making the membrane potential less negative) into such a cell, we don't see much interesting; specifically, the voltage changes smoothly in response and generally follows the shape of the injected current (Figure 1, far left trace). As we increase the magnitude of the injected current, at some point something very different happens (Figure 1, second trace from left): the membrane voltage makes a large and rapid excursion in response to injected current. This is an action potential – a large, rapid and discrete

(i.e. stereotyped) change in membrane voltage. Action potentials are the basic unit of signaling in the central nervous system. All the information our brain receives about the sensory world, all the thoughts and dreams generated in the brain, and all the signals sent from the brain to muscles to generate motor outputs are conveyed via action potentials.

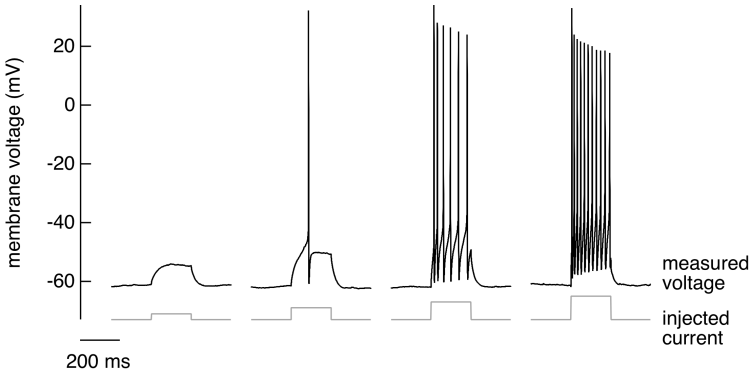

Figure 1: Action potentials. The figure shows the voltage response of a neuron to injected current, with the magnitude of the injected current progressively increasing from left to right (gray traces at bottom). Note that weak currents (far left) fail to elicit an action potential, a critical current provides just enough depolarization to generate a single discrete action potential (second from left), and that the number of action potentials generated increases as the magnitude of the current increases beyond that critical level. This critical level is the threshold for action potential generation.

A key role of action potentials is transmitting signals over long distances with minimal delay. The sensory signals that initiate a spinal reflex, for example, can travel the ~1 m distance from your finger tips to the spinal cord in ~10 ms; likewise, a signal from the spinal cord can travel to a muscle in the arm to elicit a reaction to a sensory input in ~10 ms. Propagation of these signals without action potentials would take seconds rather than milliseconds, and hence spinal reflexes would be slowed tremendously without action potentials. Even for cells without long axons, action potentials can

provide the voltage change needed to control other voltage-dependent cellular processes, such as the opening of voltage-gated calcium channels and associated events like exocytosis.

We will focus on several key properties of action potentials:

1. Individual action potentials are all-or-none events — they occur once the voltage exceeds a threshold level, and once triggered they are stereotyped — i.e. their shape does not depend on the stimulus that generated them (Figure 1).
2. The number of action potentials generated scales with the strength of the stimulus (the current injected into the cell in Figure 1 increases from left to right).
3. Action potentials propagate rapidly down axons, at speeds up to 100 m/s (see also Action Potential, Propagation).
4. Action potentials are generated by the opening and closing of ion channels in the cell membrane, and hence our understanding will be based in the properties of these channels. Mutations in these same channels cause of a number of inherited diseases. This should not be surprising given the importance of action potentials in signaling.

Quick review of membranes, ions and channels

(see also the chapters on Membrane Transport and Membrane Potentials)

Electrical signaling relies on concentration gradients of charged ions across the cell membrane. The membrane itself is impermeable to these charged ions. Ion channels — integral membrane proteins with a water-filled pore — provide the primary route for ions to cross the membrane. Channels are typically opened by intracellular or extracellular ligands. The opening of some ion channels — and in particular the Na^+ and K^+ channels that produce the action potential — depends on the membrane voltage. Most ion channel types do not exhibit such voltage dependence, but as we will see below it

is absolutely essential for action potential generation. Finally, the selectivity of channels for specific ions — e.g. Na^+ or K^+ — together with differences in the distribution of these ions across the membrane accounts for differences in how the membrane voltage reacts to the opening of these channels (and hence to stimuli that cause the channels to open). The distribution of ions is captured by the equilibrium potential for that ion: key approximate values for our discussion are E_K ~ -100 mV, E_{Cl} ~ -90 mV, E_{Na} ~ +70 mV.

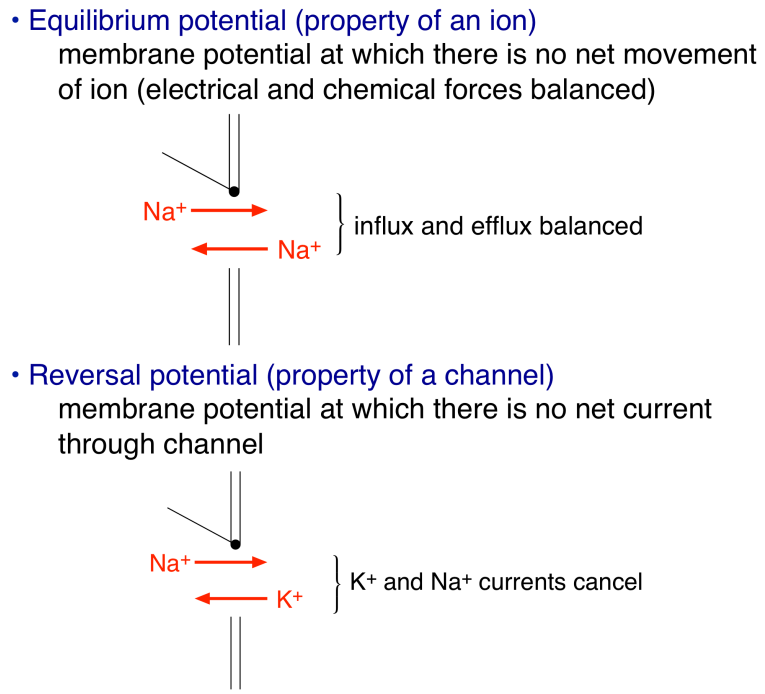

Figure 2: Equilibrium and reversal potentials. See text for details.

The amount of current flowing through an open ion channel is proportional to the difference between the membrane voltage and the reversal potential determined by the ion selectivity of the

channel (no net current flows when the voltage is equal to the reversal potential, see Figure 2). The proportionality between the current and the voltage "driving force" is given by the channel's conductance – i.e. $I = G(V-V_{rev})$ where I is the current, G is the conductance, V is the voltage and V_{rev} is the reversal potential, and the difference between the voltage and reversal potential, $V-V_{rev}$, is the driving force. This is Ohm's law applied to an ion channel. Ohm's law is probably more familiar as $V = IR$, where R is the resistance. Ohm's law can be rewritten as $V = I/G$, from which you see that the conductance is simply the inverse of the resistance (i.e. if current flows easily, the resistance is low and the conductance is high). If it helps, you can think of an analogy with a water pipe: a large pipe that lets water flow easily has high conductance and low resistance, and the driving force is analogous to the water pressure – i.e. how hard are ions being pushed through the channel. The number of open channels, the voltage driving force, and the single channel conductance determine the magnitude of the total current flowing into or out of a cell through a specific channel type. Thus, these are the key parameters that will control electrical signaling.

How do ion channels influence membrane voltage? The simple rule is that the flow of ions through a channel changes the voltage towards the reversal potential for that channel — i.e. the voltage at which there is no net movement of ions (Figure 2). For perfectly selective channels, this reversal potential occurs at the ion's equilibrium potential. This description alone is enough to get us to a simple qualitative description of the action potential. The depolarizing "upswing" of the action potential is due to the opening of Na^+ channels. Recall that Na^+ has a positive equilibrium potential, so when Na^+ channels open the voltage shifts towards the Na^+ equilibrium potential. The repolarization back to a negative membrane potential is due to closure of those Na^+ channels, with help from K^+ channels that open somewhat more slowly than the Na^+ channels. Recall K^+ has a negative equilibrium potential, so opening of K^+ channels will help with repolarization. Essential for action potential generation is the difference in equilibrium

potentials of Na^+ and K^+, which causes the voltage to shift in opposite directions when Na^+ vs K^+ channels open.

For channels through which more than one ion type can permeate — e.g. non-selective cation channels — the reversal potential is the voltage at which there is no net current through the channel (0 mV for a non-selective cation channel). Opening of channels with a reversal potential more positive than the membrane potential will produce inward current and make the membrane potential more positive (as for Na^+ channels); conversely opening channels with a reversal potential more negative than the membrane potential will produce outward current and make the membrane potential more negative (as for K^+ channels). Another way to remember this is that inward movement of positive charge makes the inside of the cell and membrane potential more positive, and outward movement of positive charge makes the inside of the cell and membrane potential more negative.

These issues form the core of how action potentials are generated. Before reading the rest of the material below, make sure you are comfortable with the description above and the Membrane Potential chapter, which describes these properties of ions and membrane in more detail.

Resting potential

The resting potential refers to the voltage across the cell membrane in the absence of external stimuli (i.e. at "rest"). In other words, the resting potential is the difference between the voltage inside and outside the cell in the absence of external stimuli. The resting potential is dominated by open K^+ channels, and hence is near the K^+ equilibrium potential. Resting potential is not exactly equal to E_K because some other channels with different ion selectivity are also open when the cell is at rest. When multiple channels with different ion selectivities are open, the cell voltage reflects a weighted average of the associated reversal potentials. This is

directly analogous to the concept of a reversal potential for a channel, now applied to an entire cell: the resting potential is the voltage at which there is no net current into or out of the cell, just as the reversal potential is the voltage at which there is no net current through a channel. This no net current situation is created when currents through all the open channels sum to 0. Since there is no net current into a cell at the resting potential, there is no net movement of charge into or out of the cell, and the voltage does not change. Typical resting potentials are near -70 mV.

Consider a simple example: a cell that contains only K^+ and Na^+ channels. If only K^+ channels are open, the membrane potential will be equal to the K^+ equilibrium potential, E_K. If only Na^+ channels are open, the membrane potential will be equal to the Na^+ equilibrium potential, E_{Na}. If an equal number of Na^+ and K^+ channels are open, and the conductance of each type of channel is the same, then the membrane voltage will be halfway between the K^+ and Na^+ equilibrium potentials – at this voltage current from K^+ flowing out of the cell and Na^+ flowing in will cancel such that there is no net current flow.

These same concepts determine both the resting potential and the change in voltage in response to a stimulus that alters the set of open channels – i.e. a stimulus can alter the membrane voltage by changing the relative number of open K^+ and Na^+ channels. In the example here, if we start with a cell with only K^+ channels open and a stimulus opens Na^+ channels such that an equal number of Na^+ and K^+ channels are open, the voltage will change from E_K to a voltage half way between E_K and E_{Na}. (This discussion neglects how quickly the voltage gets to the new value, so for now assume we are talking about a long-lasting stimulus and slow changes in voltage).

An important point here is that two distinct types of K^+ channels contribute to the control of the action potential. "Leak" K^+ channels, which do not show strong voltage dependence in their opening, are key determinants of the resting potential; as their name implies, these channels show a high level of constitutive activity (i.e. many are open when the cell is "at rest"). These are distinct from voltage-

activated K^+ channels that open during the action potential (see below) and close following return of the membrane potential to near its resting value.

We will now apply the concepts introduced above to understand how the key properties of action potentials listed earlier can be explained in terms of the underlying ion channels. Make sure you are comfortable with the material up to here before proceeding!

> **Learning Objective #1:** *Explain the ionic basis of the action potential.*

How is the action potential produced? An early idea due to Bernstein was that action potentials reflected a momentary breakdown in the membrane, such that for a brief moment the membrane became permeable to all ions. Such a breakdown in membrane permeability would permit all charged ions to cross the membrane, and thus create a current with a reversal potential of 0 mV. This current, in turn, would depolarize the cell from its normal resting potential towards (but not past) 0 mV. This hypothesis, however, is inconsistent with the observation that the peak voltage reached during the action potential can exceed 0 mV (e.g. Figure 1). For the membrane to reach positive potentials, the permeability to ions with positive equilibrium potentials (Na^+ and/or Ca^{2+}) must be higher than the permeability to ions with negative equilibrium potentials (K^+ and Cl^-). Thus, action potentials must reflect a selective increase in permeability (via opening of channels) to ions with positive equilibrium potentials.

Our modern understanding of the action potential comes from beautiful work by Alan Hodgkin and Andrew Huxley. They used the squid giant axon — which is about the size of a cocktail straw — to control (rather than only measure) the membrane voltage and to

replace permeable ions, such as Na^+, with impermeable ions such as choline. This combination of voltage control and ion replacement allowed them to directly observe how voltage altered the flow of Na^+ and K^+ across the membrane. These experiments identified Na^+ and K^+ as the key ions responsible for the action potential and provided a detailed biophysical explanation for how action potentials are generated. The key role of Na^+ explains why action potentials reach positive potentials: a sufficient number of Na^+ channels open to drive the membrane potential close to the Na^+ equilibrium potential.

Learning Objective #2: *Explain the concept of threshold in terms of the underlying ion channel activity.*

How do we explain the abrupt threshold for action potential generation as in Figure 1? The opening of both the voltage-activated Na^+ and K^+ channels that dominate the action potential increases with depolarization. Na^+ and K^+ channels differ in two critical ways, however: the direction of current flow when the channels open, and the speed with which the channels open following a change in voltage.

The Hodgkin cycle

Depolarization and opening of K^+ channels at voltages near action potential threshold leads to outward movement of K^+ (since the voltage is depolarized relative to the K^+ equilibrium potential). This outward K^+ current repolarizes the cell — i.e. it counteracts the initial depolarization (Figure 3). This is a form of negative feedback because the effect of an external stimulus on voltage is opposed by

K^+ channel opening and the resulting current. The control of blood sugar by insulin release is another example of negative feedback: increased blood sugar leads to insulin release, which leads to movement of sugar from the blood into stores.

In contrast to the behavior of K^+ channels, depolarization and opening of Na^+ channels leads to an inward Na^+ current, which leads to more depolarization (Figure 3). This is a form of positive feedback — which serves to amplify the initial depolarization. A microphone and connected speaker placed too close to each other provide a familiar example of positive feedback: the microphone detects sound from the speaker, amplifies it, plays it back through the speaker, detects it, amplifies it, ... We refer to this positive feedback behavior of Na^+ channels as the Hodgkin cycle.

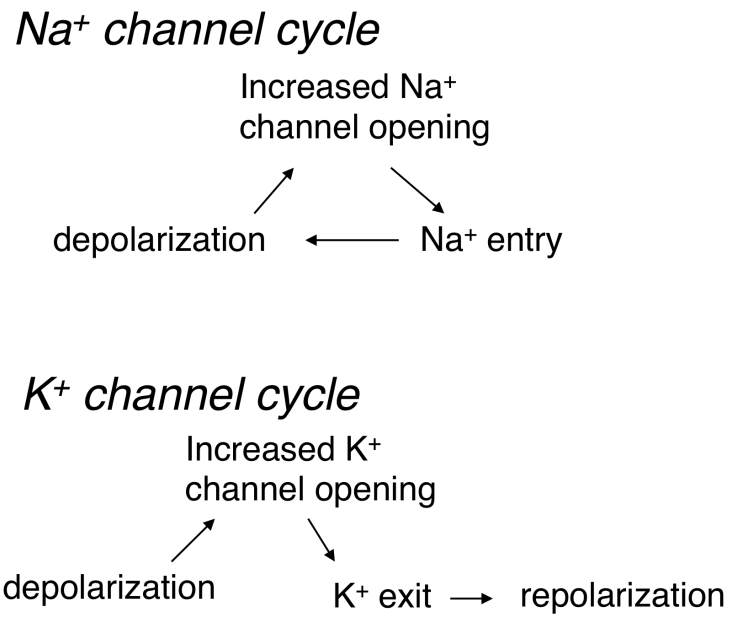

Figure 3: Impact of membrane depolarization on activity of voltage-gated Na^+ and K^+ channels. The difference between the impact of channel opening on current flow is central to the different roles of these channels in action potential generation.

Practice Question

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The threshold for action potential generation occurs when a depolarizing stimulus opens a sufficient number of Na^+ channels such that the net current flowing into the cell is inward (i.e. the inward current through Na^+ channels exceeds the outward current through K^+ and Cl^- channels). At this critical voltage, the depolarization produced by the initial stimulus begins to be amplified through the positive feedback of the Hodgkin cycle. The inward current produced causes more Na^+ channels to open, more inward current, more depolarization, ... Once the cell enters this positive feedback cycle, there is no turning back until all available Na^+ channels have opened and the membrane potential approaches the Na^+ equilibrium potential. This sequence of events is what underlies the discrete nature of the action potential.

Practice Questions

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Repolarization and channel inactivation

The description above is missing a key step — getting the voltage back down to its resting value so that the cell can respond to a

subsequent stimulus and generate another action potential. Two mechanisms allow the voltage to recover:

Na^+ channels transition from the open state to an inactivated state. In the inactivated state the channels are effectively out of commission: they no longer permit Na^+ ions into the cell, and they cannot reopen when subject to depolarization (Figure 4). Recovery from the inactivated state to the closed state (a state from which they can reopen) occurs very slowly at depolarized voltages and much more rapidly at substantially negative voltages – e.g. those close to the resting potential. This is a safety mechanism that effectively insures that Na^+ channels do not continue to permit inward depolarizing current after the peak of the action potential has been reached. Efficient recovery of Na^+ channels to the closed state occurs only when the membrane has been repolarized to near the resting potential – and hence only then are channels available for opening.

K^+ channels open more slowly than Na^+ channels, and this slowed opening means that K^+ channel opening occurs at an ideal time to help the voltage recover back to the resting potential.

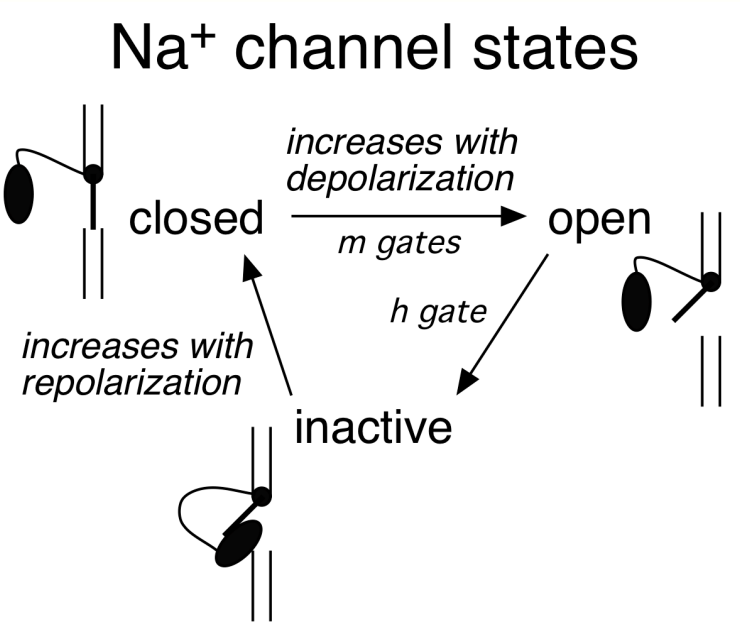

Figure 4: Functional states of a voltage-activated Na^+ channel.
Depolarization leads to closed channels opening. Open channels go through a channel-intrinsic transition to an inactivated state. Recovery to the closed state from the inactivated state occurs rapidly only when the membrane voltage is substantially negative – e.g. near the resting potential.

Figure 5 summarizes the time course of the opening of Na^+ and K^+ channels during the action potential.

Aside 1: *Hodgkin and Huxley predicted the mechanistic details of the channels underlying the action potentials decades before we even knew what an ion channel was or how it worked. They in fact predicted several key channel*

properties that served as a guide for subsequent work, such as the presence of an inactivated channel state.

One of the key contributions Hodgkin and Huxley made was to develop a simple model that captured the kinetics of channel opening and closing. This model relies on several "gates" that account for the transitions of Na^+ and K^+ channels between different states. The importance of this description was that it made clear predictions about the structure of ion channels – predictions that turned out to be quite accurate:

m: Na^+ channel activation gate (see Figure 4)

- opens by depolarization
- resets to "ready" position rapidly by repolarization after the h gate is reset
- Aside: opening of several m gates is required for channel opening. We now know those gates represent different channel subunits

h: Na^+ channel inactivation gate

- causes channel to cease to permit Na^+ entry into the cell
- inactivation develops with time after activation gates open – i.e. activation of h gate is triggered by activation of m gates, not by voltage directly.
- h gate is reset by membrane repolarization
- Aside: we now know that the h gate is produced by a globular protein moiety attached to a flexible tether; this protein moiety can plug the channel pore (the "ball-and-chain" model; see elliptical black ball in Figure 4 schematics)

n: K channel activation gate

- activates in response to depolarization
- resets in response to repolarization

Alterations in the properties of these gates are important clinically. Amazingly, the empirical description Hodgkin and Huxley came up with to describe their data has proven to be an accurate description of how ion channels work at a molecular level (remember next to nothing was known about channels when they did their work).

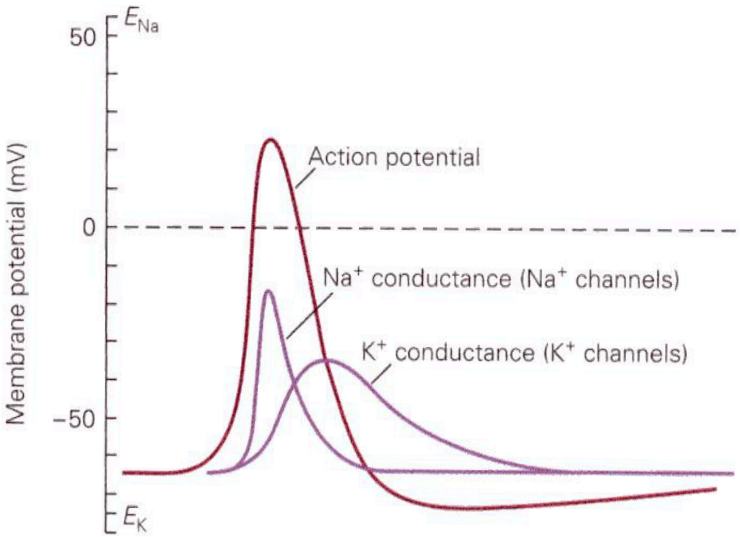

Figure 5: Time course of Na^+ and K^+ conductances (or equivalently, of the number of open channels of each type) during the action potential. Key features are that Na^+ channels open rapidly and briefly compared to K^+ channels.

Aside 2: *Local anesthetics (e.g. lidocaine) work by blocking Na+ channels, so that sensory nerve fibers and motor nerve fibers are blocked. Na+ channels are also blocked by the toxin in puffer fish (tetrodotoxin); the tingly sensation from puffer fish sushi comes from a just-right suppression of Na+ channels!*

Learning Objective #3: *Describe the refractory period and how it is produced mechanistically.*

The state of the cell's ion channels immediately following an action potential is very different than it was prior to the action potential. Many Na^+ channels are still in the inactive state, since recovery from inactivation occurs rapidly only at negative voltages and then takes some time (a few ms) to complete. And extra voltage-gated K^+ channels are open, thus causing the membrane potential to be more tightly tied to the K^+ equilibrium potential than normal. These two effects create the refractory period — a period following an action potential during which the cell is either completely unable (absolute refractory period, typically lasting a few ms) or resistant (relative refractory period, often lasting tens of ms) to generating another action potential (Figure 6). The absolute refractory period occurs when there are not enough Na^+ channels available for opening to enter the positive feedback Hodgkin cycle; in this period, it is impossible for the cell to generate an action potential no matter how strongly it is stimulated. During the relative refractory period,

enough Na^+ channels have recovered from inactivation to produce an action potential, but a larger stimulus is required to reach action potential threshold because many Na^+ channels still are in the inactivated state and some voltage-activated K^+ channels remain open and hence make it harder for a stimulus to change the voltage and get to threshold.

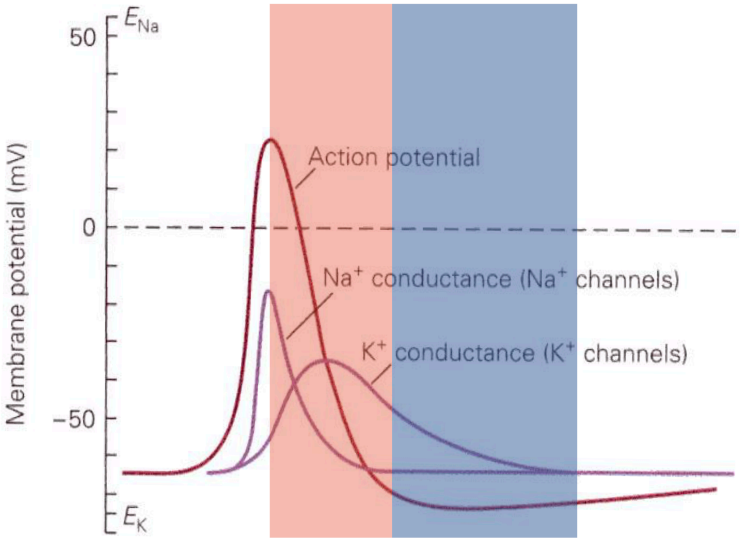

Figure 6: Absolute and relative refractory periods. During the absolute refractory period (red shaded region) the cell is unable to generate a second action potential because there are not enough Na^+ channels ready to open. During the relative refractory period (blue shaded region), the cell can generate a second action potential, but a stronger stimulus is required because some Na^+ channels may remain inactivated and because the stimulus is opposed by K^+ channels that remain open.

Practice Questions

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Action Potential Propagation

FRED RIEKE

Note: *Please make sure you are comfortable with material for the Action Potential, Threshold, Refractory Period chapter before starting this one.*

Learning Objectives and Quick Synopses

1. Describe how changes in resting membrane potential affect the action potential.

Changes in resting potential impact action potential generation in two ways: (1) manipulations that make the resting potential farther from threshold can mean that a larger stimulus is required to reach threshold; (2) manipulations that make the resting potential considerably more positive can cause failure of Na^+ channels to recover from inactivation.

2. Explain the propagation of an action potential

down an axon and describe how the refractory period contributes to this.

The propagating action potential depolarizes the axon in front of it, bring it to threshold so it too can generate an action potential. The propagating action potential leaves in its wake an area of membrane that is still refractory, and this prevents the action potential from propagating in both directions along the axon.

3. Describe the importance of myelination for action potential propagation.

Myelin decreases the membrane capacitance and increases its resistance. Both of these mean that less current is lost as the action potential propagates. This enables "saltatory conduction" wherein the action potential is regenerated at specific sites along the axon that lack myelin; transmission between these sites is passive (without regeneration).

Overview

In the Action Potential, Threshold, Refractory Period Chapter we studied how the gating of Na^+ and K^+ channels produces the action potential. Here we study two additional aspects of action potentials: (1) how changes in resting potential alter excitability – i.e. the number of action potentials that result from a given input to a cell, or alternatively how likely a small input is to generate an action potential; and, (2) how action potentials propagate down axons. Both of these are implicated in disease.

Learning Objective #1: *Describe how changes in resting membrane potential affect the action potential.*

A key fact about the threshold for action potential generation is that it is not determined as a fixed voltage relative to the resting potential, but is instead determined by the voltage at which enough Na^+ channels open to enter the positive feedback Hodgkin cycle. Thus threshold is determined by the number of available Na^+ channels and their voltage dependence. Manipulations that change the resting potential can also change the voltage difference between it and action potential threshold; this in turn changes excitability by either bringing the voltage closer to threshold or moving it farther from threshold. Manipulations that cause a cell's resting potential to be farther from the threshold (i.e. that make the resting potential more negative) decrease excitability – i.e. the cell is less likely to generate an action potential for a given stimulus. Manipulations that produce modest depolarization cause a cell's resting potential to be closer to threshold for action potential generation and hence increase excitability — i.e. the cell is more likely to generate an action potential for a given stimulus.

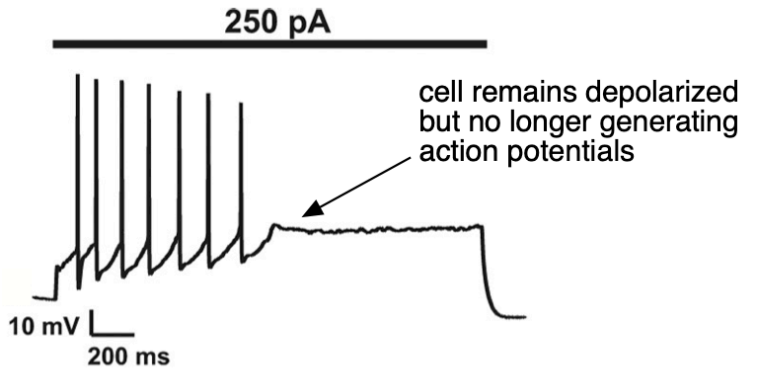

Figure 1: Depolarization block. Injection of a current step (top) initially leads to depolarization and action potential generation, but some cells are unable to maintain action potentials due to accumulation of inactivated Na^+ channels. From Tucker et al. (2012).

More extreme depolarization can completely suppress action potential generation as a cell goes into "depolarization block" (Figure 1) – in this case excitability goes to zero since the cell cannot generate an action potential. Depolarization block occurs when the voltage is sufficiently depolarized to suppress recovery of Na^+ channels from inactivation (remember that the h or inactivation gate resets very slowly at potentials positive to the resting potential, see the Action Potential, Threshold, Refractory Period chapter, Figure 4). This means that there are not a sufficient number of Na^+ channels available for opening for a cell to enter the Hodgkin cycle and generate an action potential. Such depolarization block persists until the voltage hyperpolarizes sufficiently for recovery of Na^+ channel availability.

Ion channel mutations are another important cause of changes in excitability. Some of these mutations directly alter key components of the machinery described above for action potential generation. For example, pacemaking in the heart depends on the duration of the cardiac action potential, and mutations that alter inactivation

of Na^+ channels can disrupt pacemaking (e.g. in some forms of long QT syndrome). Other mutations can alter the resting potential. Cl^- channels, along with the usual K^+ channels, play an important role in setting resting potential in muscle. A clinically important example is loss-of-function mutations in chloride channels in myotonia congenita, which can depolarize the muscle resting potential and increase excitability. As a result, muscle contraction can be substantially prolonged, causing transient paralysis (google 'myotonic goats' for some examples). Mutations in "leak" K^+ channels (remember from the Action Potential, Threshold, Refractory Period Chapter that these are distinct from the voltage-activated K^+ channels activated during the action potential) similarly can alter resting potential and excitability.

Changes in the distribution of ions across the membrane can also change excitability. A clinically important example is hyperkalemia — an elevation in extracellular K^+ concentration. Since K^+ plays a key role in setting the resting potential, and since E_K depends strongly on the external K^+ concentration, relatively small increases in extracellular K^+ can alter the K^+ equilibrium potential and depolarize the membrane sufficiently to have critical effects on excitability — including causing alterations and even failure of action potential generation in the heart or other muscles. The sensitivity to resting potential to changes in extracellular K^+ is one reason you will want to watch it closely when you get lab results back on your patients!

A simple exercise to check your understanding

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Learning Objective #2: *Explain the propagation of an action potential down an axon and describe how the refractory period contributes to this.*

The mechanisms we discussed in the Action Potential, Threshold, Refractory Period chapter account for the generation of an action potential, but not how action potentials are propagated in one direction down an axon. Action potentials are initiated at the axon hillock, a region of the axon near the soma with a high density of Na^+ channels and hence a low threshold for generating action potentials (Figure 2); a higher density of Na^+ channels means more channels are available to open, and as a result the positive-feedback Hodgkin cycle is entered at more hyperpolarized voltages. Action potentials then propagate down the axon as a wave of depolarization (Figure 3).

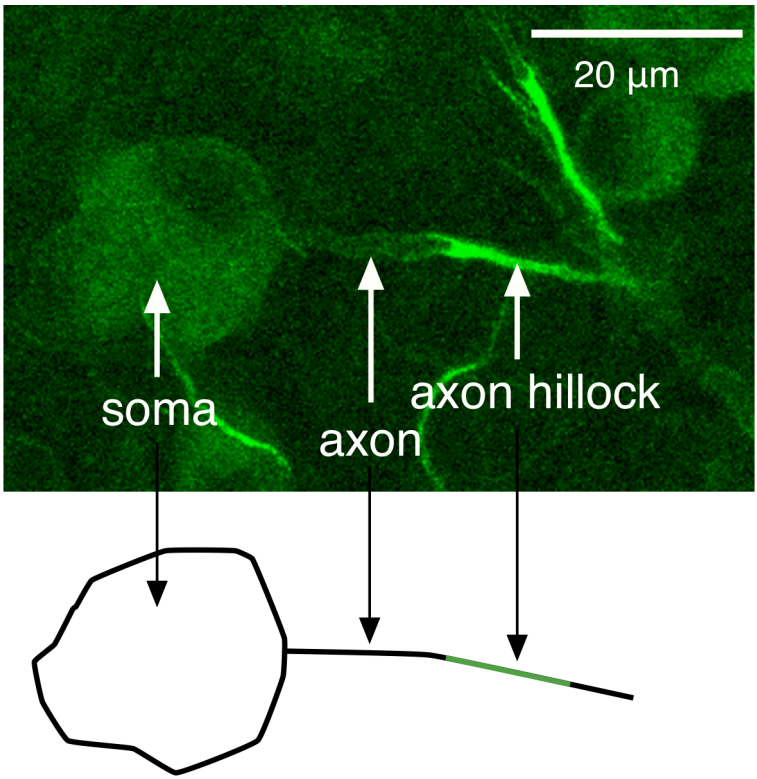

Figure 2: High density of Na^+ channels at the axon hillock – here in a retinal ganglion cell. The green label highlights Na+ channels, which are expressed at lower density in the soma and initial segment of the axon than in the axon hillock (modified from van Wart and Matthews, J Neurosci, 2006).

The propagating action potential sets up a spatial profile of depolarization along the axon since different parts of the membrane are at different stages of action potential generation (Figure 4). A region of membrane just in front of the action potential has not yet reached threshold, and a region behind is still depolarized from the falling edge of the action potential. The different states of these regions of membrane creates a fundamental asymmetry in the state of the ion channels in the regions flanking the location of the

propagating action potential: Na^+ channels are fully available in the region ahead of the action potential (yellow in Figure 4); Na^+ channels far behind the action potential are also closed and hence ready to open (also yellow in Figure 4); Na^+ channels in the region immediately trailing the action potential will be in the inactivated state (green in Figure 4), and hence unavailable for producing a subsequent action potential — thus this region of membrane is briefly refractory.

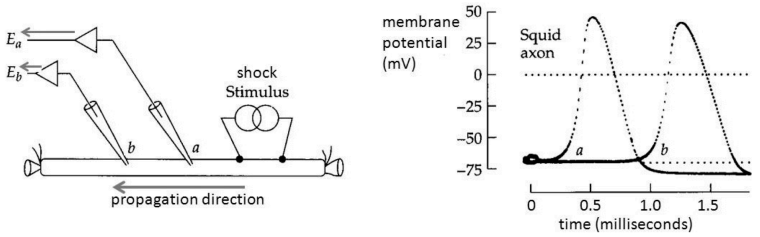

Figure 3: Action potential propagation. The left panel shows the experimental setup: an action potential is triggered by delivering an electrical shock to the axon, and the resulting voltage changes are measured at two locations along the axon. The right panel shows the resulting action potentials. They are displaced in time due to the time required for propagation from a to b. From Hille, Ion channels of excitable membranes.

Depolarization of the membrane at the leading edge of the action potential opens Na^+ channels (purple in Figure 4), and once a sufficient number have opened, the Hodgkin cycle is engaged and the action potential is regenerated. This sequential process causes the action potential to move down the axon. Propagation in the opposite direction is prevented due to the region of refractoriness (i.e. the green region of inactive Na^+ channels) in the wake of the action potential. Thus, the directionality of propagation (from the soma to the axon terminal) down the axon is generated by the biophysical properties of Na^+ channels (Action Potential, Threshold, Refractory Period Chapter, Figure 4) and the site of initiation at the axon hillock, not some intrinsic directionality of the axon itself.

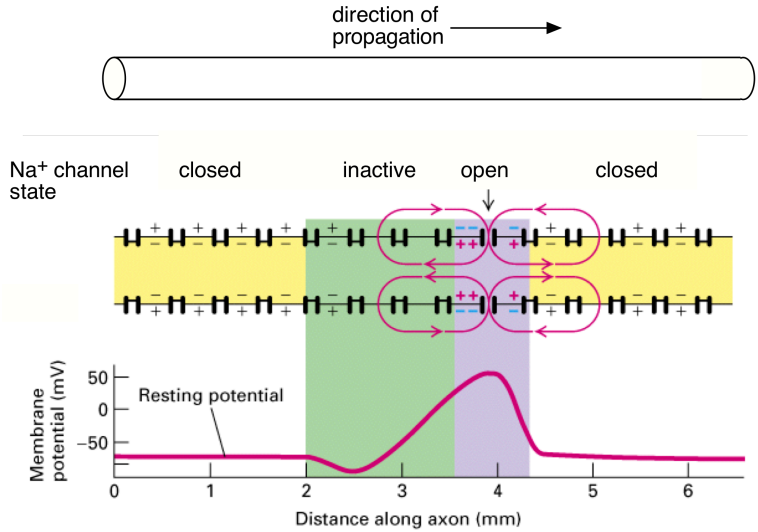

Figure 4: A propagating action potential leaves a wake of refractory Na^+ channels. Bottom panel shows spatial profile of voltage at one specific instant as an action potential propagates down the axon. Above shows the state of the Na^+ channels at different locations relative to the action potential. (Modified from Lodish, Molecular Cell Biology.)

A simple exercise to check your understanding

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Learning Objective #3: *Describe the importance of myelination for action potential propagation.*

The basic mechanism for propagation described above turns out to be much to slow to explain the speed of signaling in situations such as spinal reflexes. Two factors control the speed of propagation. One is the axon diameter: larger diameter axons have higher internal conductance and higher propagation speeds. Another key factor is the capacitance of the axon membrane.

What is capacitance and why does it slow action potential propagation? You have already encountered capacitance in the discussion of membranes (Membrane Potentials Chapter), but we review it here because it is a key factor controlling action potential propagation. For the membrane voltage to change, the electrical charge on the membrane must change. The relationship between charge and voltage is determined by the membrane capacitance: the greater the membrane capacitance, the more charge is required for a given voltage change (this is quantified as $Q = CV$, where Q is the charge on the membrane, C the capacitance and V is the membrane voltage). The charge comes from the current flowing into the cell (current is just charge per unit time). For a given amount of current, it takes longer to accumulate the larger charge required to change the voltage of a membrane with high capacitance than one with low capacitance. Think of a capacitor like a sponge in a cup. As you pour water into the cup, the sponge initially soaks it up. Only when the sponge is saturated does the water level in the cup rise. With a larger sponge, it will take more time to reach this point. Similarly, membrane capacitance "soaks up" incoming charge and slows changes in voltage.

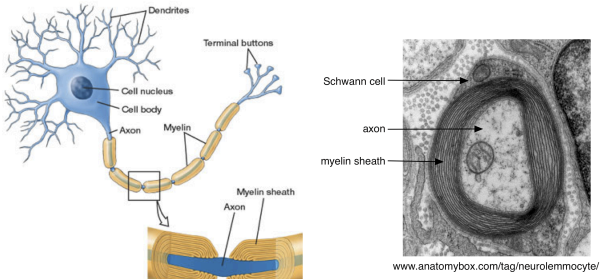

Figure 5: Myelin. Myelin produced by glial cells (Schwann cells here) wraps myelinated axons and hence decreases their capacitance and increases their membrane resistance (and hence decreases the leak of ions across the cell membrane). This is depicted schematically on the left and shown in an electron microscope image taken in cross-section on the right.

The need to charge the membrane capacitance poses a substantial limitation on the speed of action potential propagation. The solution that biology has reached to this problem is to wrap many axons — particularly those involved in long distance and rapid signal transmission — in myelin (Figure 5). Myelin is produced by glial cells (Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system). Myelin is an

electrical insulator — meaning that, like the cell membrane, charged ions cannot flow through it. The membrane capacitance is inversely proportional to the distance between the inner and outer membrane surfaces, and effectively thickening the membrane by wrapping it in myelin will decrease the capacitance. Myelin can decrease the capacitance by a factor of up to 1000. That means 1000 times less charge is needed to change the voltage!

> **Aside #1:** *The myelin sheath is compromised in diseases such as multiple sclerosis and Guillain-Barré, which can slow action potential propagation or cause it to fail.*

In the absence of myelin, action potentials propagate by successively depolarizing each section of an axon, as in Figure 4. In myelinated axons, action potentials propagate by jumping between specific regions of the axons that lack myelin. These regions are called nodes of Ranvier (Figure 6), and Na^+ channels are restricted to these nodes. Thus, the action potential is generated at one node and then propagates *without being regenerated* between the nodes. This is called "saltatory" conduction. The myelin sheath decreases the loss of current during propagation (because it decreases the membrane capacitance) between nodes and thus ensures that the depolarization at one node will be effective in depolarizing the next node and opening Na^+ channels. Action potential propagation in myelinated axons can be more than 100 times faster than that in unmyelinated axons. Generation of the action potential at a node of Ranvier follows all the rules we discussed in the Action Potential, Threshold, Refractory Period Chapter and above (e.g. you can think of Figure 4 as referring to the status of Na^+ channels at nodes ahead of and behind the action potential).

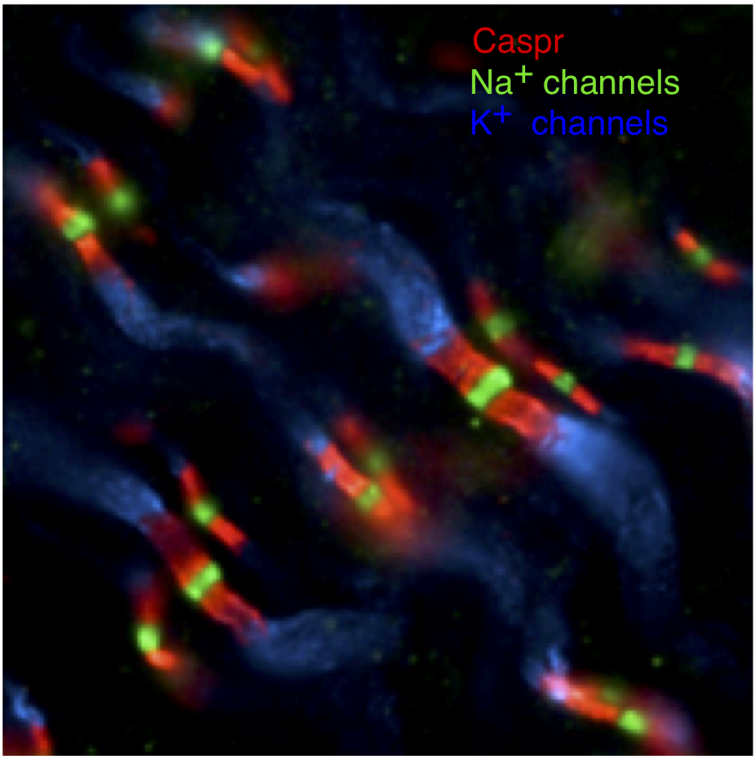

Figure 6: Nodes of Ranvier. *Na^+ channels (green) are at high density at the nodes. K^+ channels (blue), in contrast, are expressed at high density in a region of the axon largely covered in myelin. The red is a label for the molecular "glue" that binds the myelin to the axon. (www.urmc.rochester.edu/labs/shrager-lab.aspx)*

Simple exercise to check your understanding

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Aside #2: *The ability of neural axons to regenerate is strongly influence by the glial sheath through which the axon regrows. Peripheral axon sheaths formed by Schwann cells are a permissive environment and severed peripheral nerves are often able to grow and reinnervate their targets. Indeed, axon regeneration occurs constantly in the olfactory system due to constant turnover of olfactory neurons. Central axon tracts are not a permissive environment to support axon regrowth, and this impedes regeneration of severed axon bundles. Replacing the normal glial sheaths of severed central nerves, e.g. in the spinal cord, with glial sheaths from peripheral nerves can enhance regrowth and offers a potential approach to reconnect central neurons severed from their targets.*

Practice Questions

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Synapses and Neurotransmitter Receptors

JOHN TUTHILL

Learning Objectives

1. Describe how neurotransmitter is released at chemical synapses, including the role of calcium.

The neurotransmitter at chemical synapses is packaged in intracellular vesicles. A presynaptic action potential causes presynaptic calcium to rise. Calcium is the trigger to release the contents of synaptic vesicles by the process of exocytosis.

2. Compare excitatory and inhibitory neurotransmitters and identify the major examples of each neurotransmitter type in the CNS.

The 'workhorse' neurotransmitters in the CNS are glutamate, which is excitatory and GABA and glycine, which are inhibitory. Outside the CNS, acetylcholine is excitatory.

3. Compare ionotropic and metabotropic receptors.

Ionotropic receptors are ion channels that open rapidly to change membrane potential. Metabotropic receptors are GPCRs that initiate second messenger signaling and hence

produce slower onset and longer lasting changes in membrane potential.

4. Describe the mechanism by which neurotransmitter is cleared at chemical synapses.

Membrane transporters clear glutamate, GABA, and glycine from the extracellular space into glia or back into neurons. Acetylcholine is destroyed by the extracellular enzyme acetylcholinesterase.

5. Describe how temporal and spatial summation of synaptic potentials affect postsynaptic responses.

Postsynaptic electrical responses are augmented by summation when one presynaptic nerve terminal fires rapidly in succession or when several nearby nerve terminals fire nearly synchronously.

6. Outline the key differences between chemical and electrical synapses.

Overall Summary

Chemical synapses use neurotransmittters to activate postsynaptic receptors and deliver excitatory or inhibitory messages. The receptors amplify the message. In contrast, electrical synapses transmit by electric currents flowing through gap junctions between two cells without amplification and without the possibility of sign inversion.

Synapses are the communication junctions formed by neurons to each other and to the cells of their target tissues. The word synapse comes from Greek, meaning joining together, and the process of communication is called synaptic transmission. This is a

fundamentally important property; a nervous system without synaptic transmission would be like a society without language or any other form of communication. In addition to transmitting signals from one cell to another, synapses are the likely sites for many key brain functions such as decision making, learning, and memory.

Chemical synapses are the predominant form of synaptic transmission in the brain. Transmission at these synapses is mediated by a diffusible chemical transmitter released by the presynaptic cell in response to a change in voltage. Electrical synapses, in contrast, work through the direct flow of ions and other small molecules from one cell to another. They do not involve a chemical transmitter. Electrical synaptic transmission is relatively uncommon in the brain, but such electrical communication is important in sensory systems (e.g. retina), the liver, and cardiac muscle among other places. Electrical transmission is particularly effective at synchronizing the electrical signals in collections of cells – e.g. causing muscle cells to contract at the same time.

(Unless otherwise noted, all figures are from: Kandel ER, Schwartz JH, Jessell TM 2012, Siegelbaum SA, Hudspeth AJ. 'Principles of Neural Science', 5th ed. *McGraw-Hill, New York.*)

Learning Objective #1: *Describe how neurotransmitter is released at chemical synapses, including the role of calcium.*

Basic sequence of events

The mechanisms governing transmitter release were first revealed in studies of neuromuscular junction. The neuromuscular junction

is the chemical synapse formed by a motoneuron innervating a skeletal muscle fiber (Figure 1). While this synapse has several unique features, it also exhibits many general features of chemical synapses, and indeed was the place where many of these general features were first discovered. The basic sequence of events at neuromuscular junctions is as follows (Figure 2):

1. A nerve action potential – a large, rapid and discrete change in voltage – propagates down the axon of the motoneuron and depolarizes its synaptic terminal.
2. The presynaptic depolarization due to the action potential causes voltage-gated Ca2+ channels to open and to permit Ca2+ to enter the synaptic terminal.
3. Ca2+ entry is the signal to trigger exocytosis of presynaptic vesicles, which in motoneurons are filled with acetylcholine (ACh).
4. Released ACh quickly diffuses across the narrow synaptic cleft to the skeletal muscle fiber where it binds to nicotinic ACh receptors on the muscle membrane. These receptors are non-selective cation channels having about equal permeability to K^+ and Na^+. Binding of ACh opens these ACh receptor channels, leading to a local depolarization of the muscle called the end-plate potential. Under normal circumstances, the large synaptic depolarization always reaches firing threshold and gives rise to a muscle membrane action potential that propagates in both directions in the muscle.

Each action potential causes the muscle fiber to contract. Diseases that target the neuromuscular junction, for example myasthenia gravis, can reduce the end-plate potential so it sometimes fails to elicit an action potential in the muscle. Such failures manifest as muscle weakness.

5. ACh is removed from the synaptic cleft both by enzymatic degradation (by acetylcholinesterase) and by diffusion.

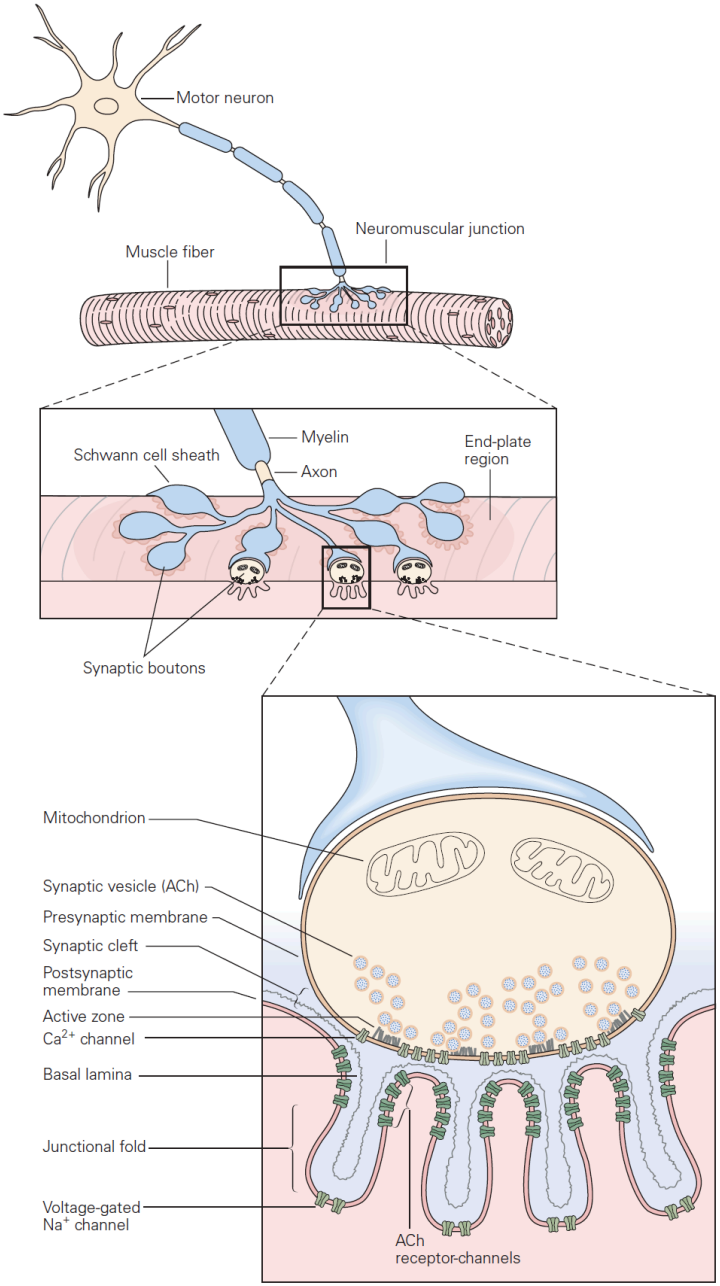
Motor neuron
Neuromuscular junction
Muscle fiber
Myelin
Schwann cell sheath
Axon
End-plate region
Synaptic boutons
Mitochondrion
Synaptic vesicle (ACh)
Presynaptic membrane
Synaptic cleft
Postsynaptic membrane
Active zone
Ca^{2+} channel
Basal lamina
Junctional fold
Voltage-gated Na^{+} channel
ACh receptor-channels

Figure 1: The neuromuscular junction is an ideal site for studying chemical synaptic signaling. At the muscle the motor axon ramifies into several fine branches approximately 2 μm thick. Each branch forms multiple swellings called synaptic boutons, which are covered by a thin layer of Schwann cells. The boutons lie over a specialized region of the muscle fiber membrane, the end-plate, and are separated from the muscle membrane by a 100-nm synaptic cleft. Each bouton contains mitochondria and synaptic vesicles clustered around active zones, where the neurotransmitter acetylcholine (ACh) is released. Immediately under each bouton in the end-plate are several junctional folds, the crests of which contain a high density of ACh receptors. The muscle fiber and nerve terminal are covered by a layer of connective tissue, the basal lamina, consisting of collagen and glycoproteins. Unlike the cell membrane, the basal lamina is freely permeable to ions and small organic compounds, including the transmitter. Both the presynaptic terminal and the muscle fiber secrete proteins into the basal lamina, including the enzyme acetylcholinesterase, which inactivates the ACh released from the presynaptic terminal by breaking it down into acetate and choline. The basal lamina also organizes the synapse by aligning the presynaptic boutons with the postsynaptic junctional folds.

Neurotransmitter release

Classic studies at the neuromuscular junction by Bernard Katz and colleagues revealed two features of neurotransmitter release that are shared by all chemical synapses (Figure 2):

(1) The chemical neurotransmitter is stored in presynaptic vesicles.

(2) Intracellular Ca^{2+} is the trigger signal for neurotransmitter release.

When an action potential propagates into the synaptic terminal, the resulting depolarization leads to the opening of voltage-gated Ca^{2+} channels and Ca^{2+} entry into the synaptic terminal. Like voltage-gated Na^{+} and K^{+} channels that produce action potentials, these voltage-gated Ca^{2+} channels open in direct response to depolarization. They contrast with ligand-gated channels that open due to the binding of a chemical ligand (e.g. the ACh channels expressed on the muscle at neuromuscular junction). The influx of Ca^{2+} and the resulting increase in internal Ca^{2+} concentration acts on proteins that control exocytosis. You have heard about these

SNARE proteins already (see the class on Cell biology of secretion); they are a key part of conserved machinery involved in the release of chemical neurotransmitters. Ca^{2+}-induced conformational changes in these proteins lead to the fusion of synaptic vesicles with the presynaptic cell membrane; neurotransmitter stored in the vesicles can then diffuse across the synaptic cleft to act on receptors in the postsynaptic cell (see below). The increase in internal Ca^{2+} is a requisite signal to initiate neurotransmitter release.

These properties — storage of transmitter in vesicles and the requirement for Ca^{2+} in triggering vesicle fusion — are conserved across all chemical synapses. Further, the protein machinery involved in exocytosis is largely conserved across synapses and is the target of several toxins such as botulinum and tetanus toxins. Botulinum toxin causes flaccid muscle paralysis by cleaving SNARE proteins and hence rendering presynaptic motoneurons incapable of transmitter release. In cosmetic medicine, this toxin under the name Botox is used to relax wrinkles caused by contraction of facial muscles.

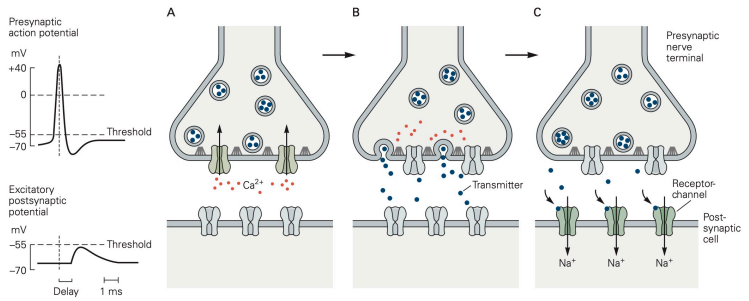

Figure 2: Synaptic transmission at chemical synapses involves several steps. *The complex process of chemical synaptic transmission accounts for the delay between an action potential in the presynaptic cell and the synaptic potential in the postsynaptic cell compared with the virtually instantaneous transmission of signals at electrical synapses.* **A.** *An action potential arriving at the terminal of a presynaptic axon causes voltage-gated* Ca^{2+} *channels at the active zone to open. The gray filaments represent the docking and release sites of the active zone.* **B.** *The* Ca^{2+} *channel opening produces a high concentration of intracellular* Ca^{2+} *near the active zone, causing vesicles containing neurotransmitter to fuse with the presynaptic cell membrane and release their contents into the synaptic cleft (a process termed exocytosis).* **C.** *The released neurotransmitter molecules then diffuse across the synaptic cleft and bind specific receptors on the postsynaptic membrane. These receptors cause ion channels to open (or close), thereby changing the membrane conductance and membrane potential of the postsynaptic cell.*

Diversity of central synapses

The neuromuscular junction is a highly specialized giant synapse — a one-to-one contact in which each action potential in the nerve is faithfully transmitted to the muscle under normal circumstances. Synapses between neurons in the nervous system show considerably more diversity. This diversity arises from differences in the identity of the released neurotransmitters, differences in the type of receptors that the neurotransmitters act on, and differences in how multiple synaptic inputs are combined or integrated by the postsynaptic cell. Below we consider each of these issues in more detail.

Postsynaptic receptors at central synapses

At all chemical synapses, as at the neuromuscular junction, released chemical transmitter diffuses across the synaptic cleft and binds to and activates receptors on the post-synaptic cell. Receptor activation leads to a conductance change (almost always a conductance increase, due to opening of ion channels), and this in turn leads to a synaptic potential in the postsynaptic cell. The synaptic potential is a local, graded change in membrane voltage analogous to the end-plate potential at neuromuscular junctions. In the CNS, the synaptic potential from a single excitatory nerve synapse is typically quite small and would not by itself initiate an action potential in the postsynaptic cell (see discussion of integration below). In this respect most synaptic potentials are quite different from single end-plate potentials, which in normal muscle always lead to an action potential. As described below, synaptic potentials in the CNS can be depolarizing (excitatory postsynaptic potentials or EPSPs) or hyperpolarizing (inhibitory postsynaptic potentials or IPSPs). Key types of receptors mediating these different synaptic potentials are summarized in the following table.

Synaptic potential	***transmitter***	***Receptor type***	***Predominant location***
Fast EPSP	*glutamate* *ACh*	NMDA, *kainate*, AMPA *Nicotinic ACh receptor*	*Central nervous system* *Skeletal muscle autonomic ganglia*
Fast IPSP	GABA *glycine*	GABAA *receptor* *Glycine receptor*	*Central nervous system* *Spinal cord*
Slow EPSP	*ACh*	*Muscarinic ACh*	*Smooth muscle*
Slow IPSP	GABA *ACh*	GABAB *receptor* *Muscarinic ACh receptor*	*Central nervous system* *heart*

Table 1: Neurotransmitters mediating several key types of synaptic potentials. Fast synaptic potentials are mediated by ionotropic

receptors and slow synaptic potentials by GPCR metabotropic receptors (see Learning Objective #3).

> **Learning Objective #2:** *Compare excitatory and inhibitory neurotransmitters and identify the major examples of each neurotransmitter type in the CNS.*

Synaptic potentials can be depolarizing (excitatory) or hyperpolarizing (inhibitory). The main ions involved in the generation of different synaptic potentials are Na^+, K^+ and Cl^-. Their equilibrium potentials are approximately E_{Cl} ~ -90 mV, E_K ~ -100 mV, and E_{Na} ~ +70 mV. These values should be familiar! The rules for how changes in the permeability of the membrane to these ions affects the membrane potential are the same as those you learned in the chapter on membrane electricity. Review those rules before proceeding if you are not comfortable with them. (You can also find most of them summarized in a separate section at the end of this chapter "*Terms used in ion electricity.*")

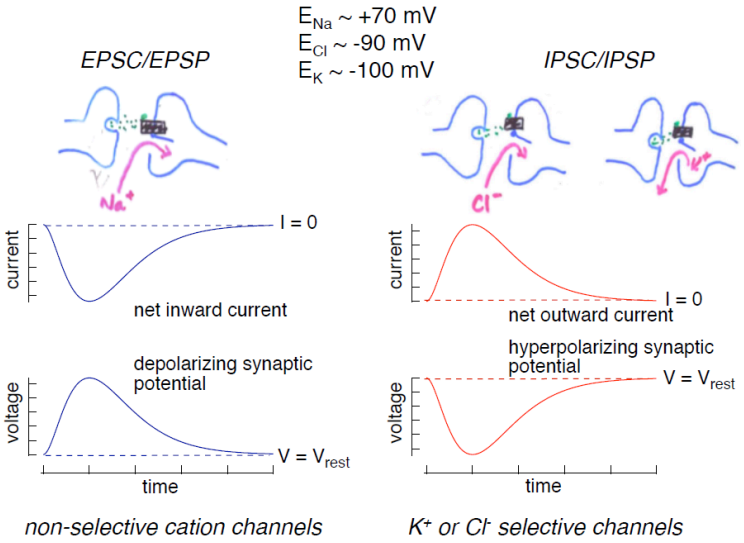

Figure 3: Generation of excitatory and inhibitory postsynaptic potentials. ***Left:*** *Activation of excitatory postsynaptic receptors leads to an inward current (negative by convention, carried primarily by Na+ at physiological voltages) in the postsynaptic cell, and this inward current depolarizes the cell, the EPSP.* ***Right:*** *Activation of inhibitory receptors leads to an outward current (K^+ moving out or Cl^- moving in) and hyper- polarization, the IPSP.*

1. *Excitatory postsynaptic potentials* (EPSPs; Figure 3, left): Depolarizing excitatory postsynaptic potentials are generated by the opening of non-selective cation channels; current through these channels reverses at a potential of 0 mV, significantly more depolarized than the cell's resting potential. Hence opening of these channels produces an inward current (referred to as an excitatory postsynaptic current or EPSC). This inward current produces the depolarizing excitatory synaptic potential, the EPSP.

2. *Inhibitory postsynaptic potentials* (IPSPs; Figure 3, right): Hyperpolarizing inhibitory postsynaptic potentials are generated by transmitter-gated ion channels permeable to either K^+ or Cl^-. Since the normal resting potential is more positive than the equilibrium potentials for K^+ and Cl^-, opening either of these types of channels

will produce an outward current (an IPSC) generated by either outward K^+ or inward Cl^- flow, and this outward current will hyperpolarize the cell.

Excitatory and inhibitory synapses in action

The knee jerk reflex provides a nice example of the importance of EPSPs and IPSPs. You will learn about these spinal reflex pathways in more detail later; here we focus on the role of EPSPs and IPSPs. Extension of the lower leg involves increasing activity in the quadriceps muscles and decreasing activity in the hamstring muscles. The knee jerk reflex can be triggered when a reflex hammer imparts a small stretch to the quadriceps muscles; the stretch activates attached stretch receptors, producing action potentials in the sensory afferent nerve fibers innervating the stretch receptor. These afferent nerve fibers make excitatory synapses in the spinal cord both onto extensor motoneurons innervating the quadriceps and onto inhibitory interneurons in the spinal cord. Each of these afferent synapses produces EPSPs resulting in propagated action potentials. In the extensor motoneurons, APs lead to contraction of the quadriceps.

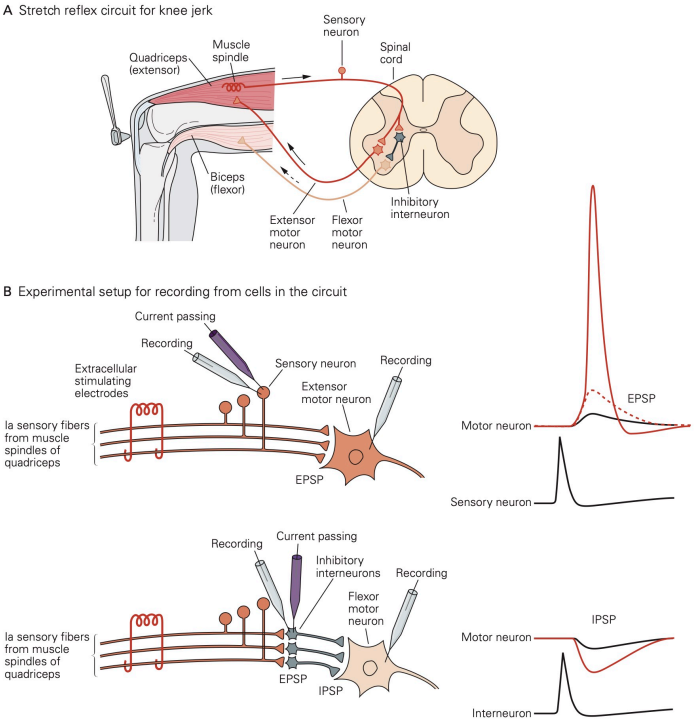

Figure 4: The combination of excitatory and inhibitory synaptic connections mediating the stretch reflex of the quadriceps muscle is typical of circuits in the central nervous system. **A.** *The stretch-receptor sensory neuron at the extensor (quadriceps) muscle makes an excitatory connection with an extensor motor neuron that innervates this same muscle group. It also makes an excitatory connection with an interneuron, which in turn makes an inhibitory connection with a flexor motor neuron that innervates the antagonist biceps femoris muscle group. Conversely, an afferent fiber from the biceps (not shown) excites an interneuron that makes an inhibitory synapse on the extensor motor neuron.* **B.** *This idealized experimental setup shows the approaches to studying the inhibition and excitation of motor neurons in the pathway illustrated in part* A. ***Above:*** *Two alternatives for eliciting excitatory postsynaptic potentials (EPSPs) in the extensor motor neuron. A single presynaptic axon can be stimulated by inserting a current-passing electrode into the sensory neuron cell body. An action potential in the sensory neuron stimulated in this way triggers a small EPSP in the extensor motor neuron (black trace). Alternatively, the whole afferent nerve from the quadriceps can be stimulated electrically with extracellular electrodes. The excitation of many afferent neurons through the extracellular electrode generates a synaptic potential (dashed trace) large enough to initiate an action potential (red trace).* ***Below:*** *The setup for eliciting and measuring inhibitory potentials in the flexor motor neuron. Intracellular stimulation of a single inhibitory*

interneuron receiving input from the quadriceps pathway produces a small inhibitory (hyperpolarizing) postsynaptic potential (IPSP) in the flexor motor neuron (black trace). Extracellular stimulation recruits numerous inhibitory neurons and generates a larger postsynaptic IPSP (red trace). (Action potentials from the sensory neuron and interneuron appear smaller because they were recorded at lower amplification than the motor neuron action potentials).

EPSPs in the inhibitory interneurons increase the rate at which they generate action potentials. The inhibitory interneurons make inhibitory synapses with the flexor motoneurons innervating the hamstrings. Thus, action potentials in the inhibitory interneurons lead to IPSPs that will decrease activity in the flexor motoneurons (the motoneurons exhibit some spontaneous activity). The decreased flexor motoneuron activity decreases activity of the hamstrings.

Together, the increase in activity of the quadriceps and decrease in activity of the hamstrings leads to extension of the knee. The opposing changes in activity in the two muscle types in response to activation of the same stretch receptors illustrates the importance of having both excitatory and inhibitory synapses.

Learning Objective #3: *Compare ionotropic and metabotropic receptors.*
Learning Objective #4: *Describe the mechanism by which neurotransmitter is cleared at chemical synapses.*

Synaptic potentials vary widely in time course. The speed of synaptic transmission is influenced by: (1) the time required for transmitter to be released and diffuse across the synaptic cleft—which is typically extremely short, and (2) the time required to generate the postsynaptic conductance change—which is quite

variable. As described above, rapid release of transmitter relies on the close proximity of the vesicle release sites and the Ca^{2+} channels that permit Ca^{2+} to enter the nerve terminal and trigger exocytosis. The time for diffusion across the synaptic cleft is minimized by the close proximity of pre and post-synaptic membranes.

Postsynaptic events substantially shape the time course of the synaptic potentials and in doing so introduce diversity in the time course of synaptic potentials. An important division is into fast versus slow chemical synaptic transmission:

Fast synaptic transmission

Neurotransmitter binds to and activates receptors that are ligand-gated ion channels – these are referred to as ionotropic receptors (Learning Objective #3). The lack of intermediate steps between transmitter binding and the conductance change leads to a brief synaptic potential (1–20 ms in duration typically).

Recovery of the synaptic potential is mediated by receptor desensitization and by clearance of released transmitter. Receptor desensitization occurs when the channel associated with the receptor closes even though transmitter is still bound to the receptor – a situation a bit like inactivation of voltage-gated Na^+ channels. Transmitter clearance at neuromuscular junctions is achieved via degradation of ACh by acetylcholinesterase; this is atypical, however, and other types of released neurotransmitter are cleared by transporters. Transporters located on glial cells near the synapse play a central role in "reuptake" or clearance of released transmitter (Learning Objective #4). These transport proteins do not use ATP but instead obtain the energy they need to move neurotransmitter out of the synaptic cleft by allowing ions such as Na^+ to flow down their electrochemical gradient; as a result, they are Na^+-coupled cotransporters (see also the chapter on membrane

transport). Several therapeutic drugs target these transporters (see below).

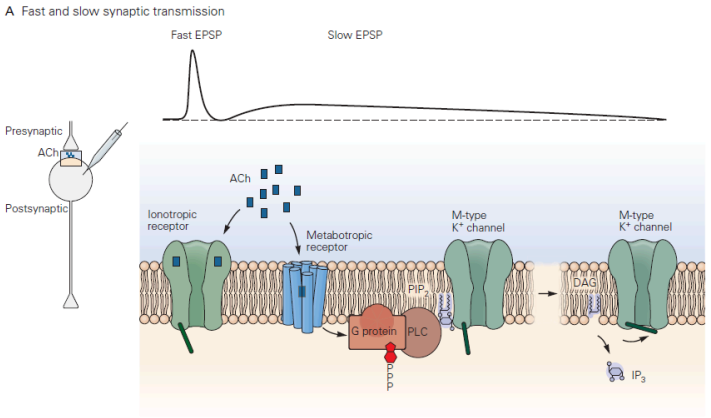

Figure 5: The release of ACh onto a postsynaptic neuron produces a fast excitatory postsynaptic potential (EPSP) followed by a slow EPSP. The fast EPSP is produced by activation of ionotropic nicotinic ACh receptors. The slow EPSP is produced by activation of metabotropic muscarinic ACh receptors. This receptor is a G-protein-coupled receptor that stimulates PLC to hydrolyze PIP2, yielding IP3 and DAG. The decrease in PIP2 causes the closure of the M-type delayed-rectifier K+ channel. (See also the chapter on G-protein-coupled receptors.)

Slow synaptic transmission

This type of postsynaptic response mechanism differs radically from that in fast chemical transmission. Neurotransmitter binds to and activates a G-protein- coupled receptor (GPCR) – referred to by neurobiologists as a metabotropic receptor (Learning Objective #3). This leads to G-protein signaling and a cascade of biochemical events that may include opening or closing of ion channels, producing a slow synaptic potentials. The electrical response, as you would guess, takes longer to initiate and terminate than fast synaptic potentials due to the delays introduced by the chemical

reactions comprising the G-protein cascade. Typical durations can be several to many seconds. A reason for the longer lasting synaptic potentials is that the biochemical events leading to conductance change can persist well after the transmitter has been removed from the synaptic cleft. The duration of slow synaptic potentials is determined by the time to shut off the activated G-protein signaling components. The G-protein needs to hydrolyze GTP to GDP, and downstream biochemical processes and second messengers need to return to the resting state. For more information about signaling through GPCRs, see also the chapter on G-protein Coupled Receptors.

Aside: ***Drugs acting on synaptic transmission.*** Many therapeutic and recreational drugs act on synaptic transmission. These have also been key tools in furthering our understanding of how synaptic transmission works. Essentially every component of the synapse is a target:

- *Receptor enhancement*: Benzodiazepines enhance effects of GABA. These drugs strengthen GABA synapses by causing the postsynaptic receptors to be more sensitive to GABA released by the presynaptic cell. These drugs are tranquilizers and may induce sleep.
- *Receptor agonists*: LSD mimics serotonin at serotonin receptors. The result may be hallucinations. Receptor agonists severely alter synaptic transmission by directly binding to and activating neurotransmitter receptors and

bypassing transmitter release from the presynaptic cell.

- *Receptor antagonists*: Antipsychotics block dopamine receptors (e.g. haloperidol). Curare blocks nicotinic ACh receptor leading to muscle paralysis. These drugs suppress synaptic transmission by partially or completely blocking neurotransmitter receptors.
- *Reuptake blockers*: Most antidepressants inhibit serotonin uptake (e.g. Prozac); cocaine blocks norepinephrine, serotonin and dopamine uptake. These drugs permit released transmitter to remain in the synaptic cleft for a longer period of time and thus to elicit a stronger postsynaptic response. Unlike for receptor agonists, here the generation of a signal in the postsynaptic cell still relies on release of a chemical neurotransmitter.
- *Block enzymatic degradation*: Nerve gases, some insecticides, and research and clinical anticholinesterases inhibit degradation of ACh by acetylcholinesterase at the cholinergic junction. Like reuptake blockers, these drugs permit transmitter to remain in the synaptic cleft for a longer period of time. Low doses of such drugs may have therapeutic effects by enhancing the response to released transmitter (much like drugs that block reuptake mechanisms at other synapses). Nerve gases typically work by asphyxia as the muscles controlling breathing cease to be controlled.

Learning Objective #5: *Describe how temporal and spatial summation of synaptic potentials affect postsynaptic responses.*

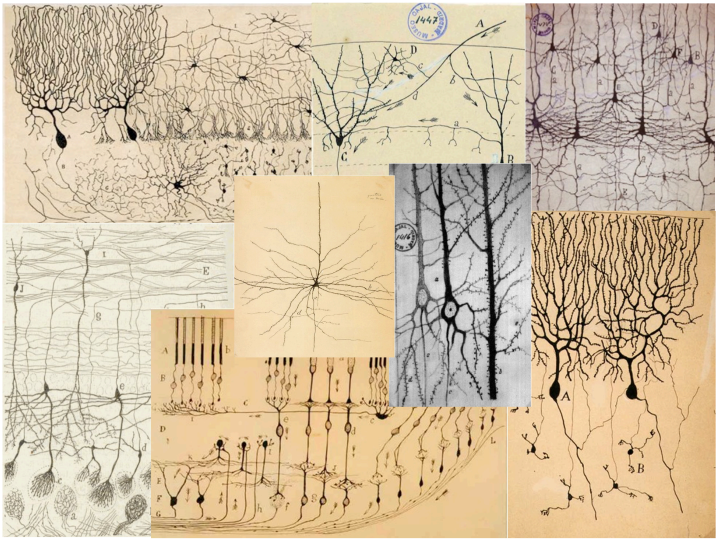

Figure 6: A gallery of neuron silhouettes.

An important difference between synapses in the central nervous system and those at the neuromuscular junction is the number of excitatory inputs required to generate an action potential in the postsynaptic cell. At a healthy neuromuscular junction, a single presynaptic action potential reliably leads to an action potential in the muscle. A typical central nervous system neuron receives input

from several thousand different neurons (convergence) and sends its outputs to several thousand neurons (divergence). These inputs are spread across the dendrites of the cells. You can appreciate the remarkable range of dendritic structures in Figure 6; these different structures are specialized for the precise function of the cell. Because the amplitude of a single synaptic input is typically less than 1 mV, multiple excitatory inputs must be summed or integrated to depolarize a neuron in the central nervous system to the firing threshold for generating an action potential. The term synaptic integration refers to the process by which multiple inputs are combined to control activity in the postsynaptic cell. This integration takes two forms:

- *Temporal integration*: integration of synaptic potentials from repetitive activation of one synaptic input. If the time between action potentials in the presynaptic cell is shorter than the duration of the synaptic potential produced in response to a single presynaptic action potential, the synaptic potentials produced by each action potential will begin to pile up, resulting in a larger response than that produced by a single presynaptic action potential. This process is referred to as temporal integration inputs originating at different times are combined or integrated.
- *Spatial integration*: integration of synaptic potentials from different synaptic inputs. Synaptic potentials produced at similar times from different synapses will be combined in the dendrites or at the soma of the postsynaptic cell and thus produce a larger response than that produced by activation of a single synapse. This is referred to as spatial integration because the inputs occur at similar times but at different spatial locations.

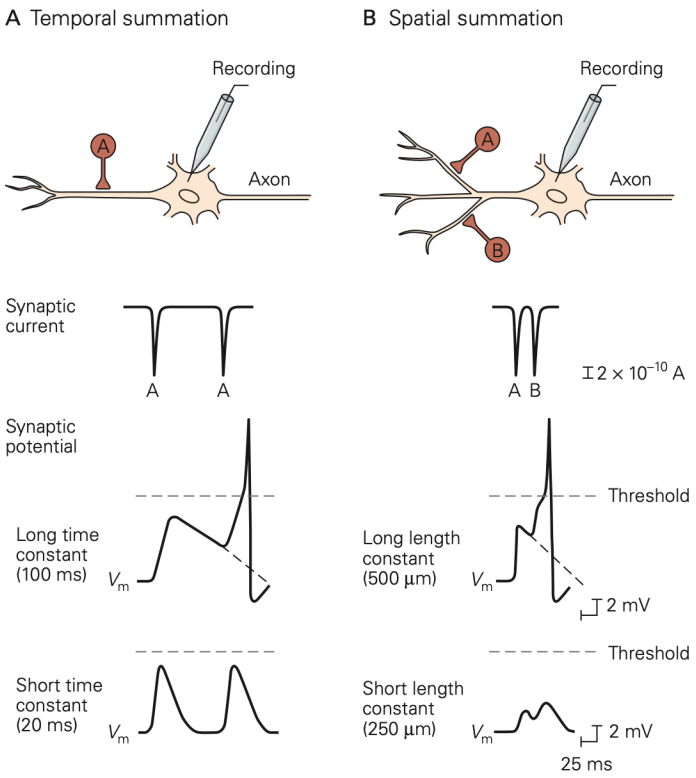

Figure 7: Central neurons are able to integrate a variety of synaptic inputs through temporal and spatial summation of synaptic potentials. **A.** *The time constant of a postsynaptic cell affects the amplitude of the depolarization caused by consecutive EPSPs produced by a single presynaptic neuron (A). Here the synaptic current generated by the presynaptic neuron is nearly the same for both EPSPs. In a cell with a long time constant the first EPSP does not fully decay by the time the second EPSP is triggered. Therefore, the depolarizing effects of both potentials are additive, bringing the membrane potential above the threshold and triggering an action potential. In a cell with a short time constant the first EPSP decays to the resting potential before the second EPSP is triggered. The second EPSP alone does not cause enough depolarization to trigger an action potential.* **B.** *The length constant of a postsynaptic cell affects the amplitudes of two excitatory postsynaptic potentials produced by two presynaptic neurons (A and B). For illustrative purposes, both synapses are the same (500 µm) distance from the postsynaptic cell's trigger zone at the axon initial segment, and the current produced by each synaptic contact is the same. If the distance between the site of synaptic input and the trigger zone in the postsynaptic cell is only one length constant (that is, the postsynaptic cell has a length constant of 500 µm), the synaptic*

potentials produced by each of the two presynaptic neurons will decrease to 37% of their original amplitude by the time they reach the trigger zone. Summation of the two potentials results in enough depolarization to exceed threshold, triggering an action potential. If the distance between the synapse and the trigger zone is equal to two length constants (ie, the postsynaptic cell has a length constant of 250 μm), each synaptic potential will be less than 15% of its initial amplitude, and summation will not be sufficient to trigger an action potential.

Aside: ***Active properties of dendrites.*** The discussion of temporal and spatial integration above focuses on dendrites as passive structures — i.e. the dendrites collect synaptic inputs and the resulting voltage changes spread passively to the soma where they are integrated. Although this is the classic view of dendrites, voltage-dependent ion channels in the dendrites can amplify or attenuate synaptic signals. Dendrites of some cells even express a sufficient number of voltage-activated Na^+ channels to generate action potentials. It is unclear presently how generally important active properties of dendrites are for synaptic integration, though there are now a few examples in which they have a clear impact on neural function. This is a very active research area.

Pre- and post-synaptic inhibition

The massive convergence of inputs to a typical central nervous system neuron raises a conundrum: how does a cell receiving thousands of inputs (even if those inputs produce synaptic potentials less than 1 mV) maintain a reasonable firing rate?

Fortunately, not all the inputs are excitatory, and indeed balancing excitatory and inhibitory inputs is critical to normal neural function. You can consider the neuron as a polling station where many voters are casting Yea and Nay votes to control action potential firing. The inhibitory signals that prevent runaway excitation are provided by inhibitory interneurons. These cells influence synaptic integration in two main ways.

- *Presynaptic inhibition* (Figure 8): In this case, activity in an inhibitory neuron (green in Figure 8) leads to activation of inhibitory synapses (which release GABA in the figure) made onto the presynaptic terminal (yellow). This activity reduces the amount of transmitter released by an action potential that subsequently propagates down the axon of the yellow cell and invades its synaptic terminal. A common mode of action is that the released inhibitory transmitter activates GPCRs, which initiate a G-protein cascade that inhibits presynaptic Ca^{2+} channels, causing fewer Ca^{2+} channels to open when the terminal is depolarized. This mode of inhibition requires that the (green) inhibitory neuron be active (i.e. generates an action potential) first, so that the green inhibitory synapse can be activated and exert its effect before the action potential arrives in the yellow synaptic terminal.
- *Postsynaptic inhibition*: Postsynaptic inhibition is a form of spatial integration. IPSPs generated by inhibitory inputs to a cell will fully or partially cancel EPSPs generated by excitatory inputs when they are combined in the dendrites or at the soma. Balancing of excitatory and inhibitory inputs in this context means that the average of all the EPSPs received by the cell tends to be cancelled by the average of all the IPSPs. Appropriate stimuli can disrupt this cancellation and cause either the EPSPs to win out, and the firing rate to increase, or the IPSPs to win out, and the firing rate to decrease. Thus, firing patterns of many neurons in the brain reflect changes in the balance of an ongoing barrage of excitatory and inhibitory synaptic inputs. As in a voting booth, the YES (excitatory) votes have to exceed the NO (inhibitory) votes for activity to

increase. These balanced choices underlie the decisions made by your brain.

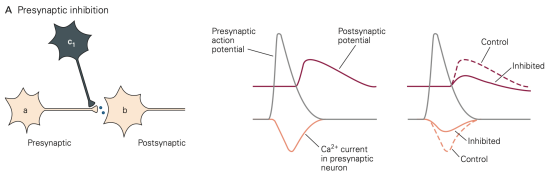

Figure 8: An inhibitory neuron (c1) forms a synapse on the axon terminal of neuron a. Release of transmitter by cell c1 activates a metabotropic receptor on the terminals of cell a, which inhibits the Ca^{2+} current in these terminals, thereby reducing the amount of transmitter released by cell a onto cell b. The reduction of transmitter release from cell a in turn reduces the amplitude of the excitatory postsynaptic potential in cell b, a process termed presynaptic inhibition.

Aside: Disruption of the balance between excitatory and inhibitory inputs is one likely cause of diseases marked by strong bouts of abnormal neural activity, such as epilepsy. One possible treatment for such conditions is an increase in the strength of inhibitory inputs — for example by drugs such as benzodiazepines. Other approaches to achieve an increase in inhibitory input and restore a balance of excitation and inhibition are also being pursued — even transplanting neuronal precursor cells that differentiate into inhibitory interneurons!

Learning Objective #6: *Outline the key differences between chemical and electrical synapses.*

The second major form of synaptic transmission is mediated by electrical synapses. At an electrical synapse, signals are transmitted from one cell to another by direct current flow through structures called gap junctions (Figure 9). Electrical synapses are less common than chemical synapses in our brain, but are often found in sensory systems (like the retina) and in non-neural tissues like epithelia, the liver, and heart. They play an especially important role in synchronizing groups of cells. In nonneuronal systems, only the phrase gap junction rather than the word synapse is used for such common cell-to-cell communication. In evolutionary terms, gap junction structures are ancient forms of cellular communication.

Gap junctions provide a non-selective conductance path between the two cells, permitting exchange of ions, second messengers, and small molecules between the cells. They are often identified based on their ability to pass small tracer molecules such as the yellow dye illustrated in Figure 10; here the dye was injected into one of the cells, and passed, via gap junctions, into the others. Because gap junctions provide a non-selective path for current to flow, they tend to equalize the voltage in the two cells. This is illustrated in Figure 11. If V1 > V2, current will flow from cell 1 to cell 2 and bring the voltages closer together. Vice-versa if V2 > V1. This property makes electrical synapses particularly good at synchronizing the electrical activity in populations of cells, a property essential for cell-to-cell propagation of action potentials in cardiac muscle.

Indeed, many cardiac diseases are marked by a disruption of normal patterns of gap junctions between the cells. Unfortunately, we have few good (i.e. specific) drugs that act on gap junctions. This hampers study of their function and the treatment of disorders associated with them.

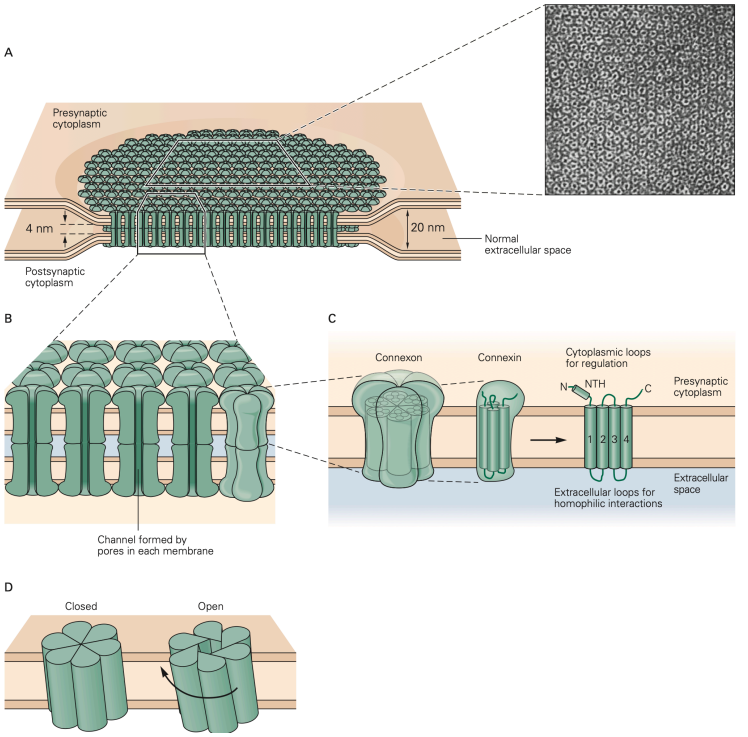

Figure 9: A three-dimensional model of the gap-junction channel, based on X-ray and electron diffraction studies. **A.** *The electrical synapse, or gap junction, is composed of numerous specialized channels that span the membranes of two neurons. These gap-junction channels allow current to pass directly from one cell to the other. The array of channels shown in the electron micrograph was isolated from the membrane of a rat liver. The tissue has been negatively stained, a technique that darkens the area around the channels and in the pores. Each channel appears hexagonal in outline. Magnification × 307,800.* **B.** *A gap-junction channel is actually a pair of hemichannels, one in each apposite cell that connects the cytoplasm of the two cells.* **C.** *Each hemichannel, or connexon, is made up of six identical subunits called connexins. Each connexin is approximately 7.5 nm long and spans the cell membrane. A single connexin has intracellular N- and C-termini, including a short intra- cellular N-terminal α-helix (NTH), and four membrane-spanning α-helixes (1–4). There are regions of similarity in the amino acid sequences of gap-junction proteins from many different kinds of tissue. These include the transmembrane helixes and the extracellular regions, which are involved in the homophilic matching of apposite hemichannels.* **D.** *The connexins are arranged in such a way that a pore is formed in the center of the structure. The resulting connexon, with a pore diameter of approximately 1.5 to 2 nm, has a characteristic hexagonal outline, as shown in part A. In some gap-junction channels the pore is opened when*

the subunits rotate approximately 0.9 nm at the cytoplasmic base in a clockwise direction.

The electrical synapse relies on current from one cell causing a voltage change in the connected cell. For this form of synaptic transmission to be effective, the two cells must have similar resistances. If the cells have very different resistances, current passed from the high resistance cell to the low resistance cell will not be very effective in changing its voltage. As an analogy, think of pressing a hot needle up against a cannon ball; the needle doesn't do much to heat the cannon ball because of its small size. A hot cannon ball, pressed up against the second cannon ball, would be much more effective. These reasons, for example, make gap junctions unsuitable at our neuromuscular junctions where motoneuron axons are small compared to the muscle cells that they innervate.

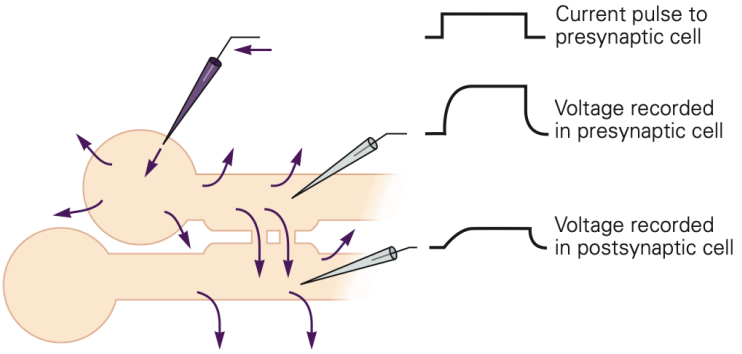

Figure 10: Electrical transmission is graded and occurs even when the current in the presynaptic cell is below the threshold for an action potential. This can be demonstrated by depolarizing the presynaptic cell with a small current pulse through one electrode while the membrane potential is recorded with a second electrode. A subthreshold depolarizing stimulus causes a passive depolarization in the presynaptic and postsynaptic cells. (Depolarizing or outward current is indicated by an upward deflection.)

Conclusion

To send and coordinate messages, the nervous system uses propagated action potentials in axons, chemical or electrical communication to the next cells at synapses, and both inhibitory and excitatory postsynaptic potentials to allow complex integration and decision making–computations that revolve around reaching firing threshold.

Terms used in ionic electricity

- *Charge*: Ions have a charge given in multiples of one elementary electron charge. Cations (Na^+, K^+, Ca^{2+}) are positively charged and anions (Cl^-) are negatively charged.
- *Two forces that move ions:*
    - Charges are moved by the force of electric fields: Opposites attract, so cations (positively charged) move towards a negative pole, and anions towards a positive pole.
    - In addition, ions are moved by diffusion (thermal agitation) down their concentration gradients–even in the absence of electric fields.
- *Current:* A net movement of charge is an electric current measured in amperes (A) and symbolized by I. Thus, a net flow of ions is an ion current The direction of current is defined as the direction of positive charge movement, so in neurophysiology, a positive current means positive charges moving out of a cell, an outward current.
- *Voltage (synonymous with potential)*: If cations are removed from a compartment (the cell), the compartment becomes more negative, a negative voltage will be set up inside the cell. A voltage is measured in units of volts (V) and symbolized by V. Positive charges want to move to regions of more negative

voltages in the absence of other forces.

- *Conductance*: Membrane conductance describes how easily ions can cross the membrane. Conductance, symbolized G, is proportional to the number of ion channels open. Each open channel contributes a small conductance to the total. (A closely related inverse concept, resistance, expresses how hard it is for ions to move. Mathematically, resistance is the reciprocal of conductance (1/G). An insulator has high resistance and low conductance.)
- *Capacitance*: Electrical capacitors store charge. Two conductors separated by a thin insulator (membrane) form an electrical capacitor. The magnitude of a capacitance (symbolized C) is defined as the number of charges that you have to move to achieve a certain potential change. Therefore, the capacitance of cell membranes tells us how many ions have to be moved across the membrane to change the membrane potential during signaling.
- *Equilibrium potential for an ion*: The membrane potential at which the electrical force and the diffusion force (due to the concentration gradient) exactly cancel for that particular ion. At the equilibrium potential there will be no net force on that ion across the membrane.
- *Reversal potential for an ion channel*: The membrane potential at which no net charge movement occurs in that ion channel. The channel may not be perfectly ion selective so that at the reversal potential one kind of ion may move one way and another may move the opposite way, but the net effect is no charge movement. When only one kind of channel is open, the ion flow in that channel tends to move the membrane potential to the reversal potential for that channel. Equilibrium potential is a thermodynamic property of an ion, given its gradient. Reversal potential is a property of an ion channel, given its ion selectivity and the gradients of the permeant ions.

Sensory Receptors

JOHN TUTHILL

Introduction

Sensory receptors are specialized, excitable cells that are designed to convert a physical stimulus into an electrical signal that can be transmitted to other nerve cells in the nervous system. The physical stimulus interacts with a specialized **receptor protein** embedded in the membrane of the sensory receptor leading to a change in membrane potential. Sensory receptors typically respond to only one type of stimulus and that specificity is conserved as the signal is propagated along neural pathways in the spinal cord and brain. The series of neurons that are connected by synapses from the sensory receptor to secondary sensory and third order sensory neurons in the nervous system is referred to as a '**labeled line'**. The labeled lines enable the brain to distinguish and process information about different types physical stimuli. In response to a constant stimulus, the response most sensory receptors declines over time, as process called **adaptation.** Some sensory receptors adapt slowly to a constant stimulus and others adapt more rapidly.

Learning Objectives

1. To know the general properties of sensory

receptors.

2. To understand the labeled-line principle of signal detection.
3. To compare the mechanisms of sensory transduction in different types of sensory receptors.
4. To appreciate how the intensity and duration of a stimulus are reflected in the receptor potential and action potential discharge rate of a sensory neuron.
5. To understand how sensory receptors adapt to a constant stimulus.

(Unless otherwise noted, all figures are from: Kandel ER, Schwartz JH, Jessell TM 2012, Siegelbaum SA, Hudspeth AJ. 'Principles of Neural Science, 5th ed. *McGraw-Hill, New York.*)

Learning Objective #1: *To know the general properties of sensory receptors.*

Sensory receptors are specialized, excitable cells that are designed to convert a physical stimulus in the external world (*e.g.*, light, sounds, odors, *etc.*) or one that occurs inside our bodies (*e.g.*, blood pressure, muscle force, osmolality, *etc.*) into an electrical signal that can be transmitted to other nerve cells in the nervous system. In all cases, the physical stimulus interacts with a specialized **receptor protein** embedded in the membrane of the sensory receptor leading to a change in membrane potential. The receptor proteins in sensory receptors typically respond to only one type of stimulus

that confers selectivity and specificity to the receptor. For example, the receptor protein in a cutaneous mechanoreceptor responds to the mechanical deformation of its membrane, whereas the receptor protein of a rod or cone photoreceptor in the retina responds to light energy. The form of energy that a receptor preferentially responds to is called the '**adequate stimulus**'.

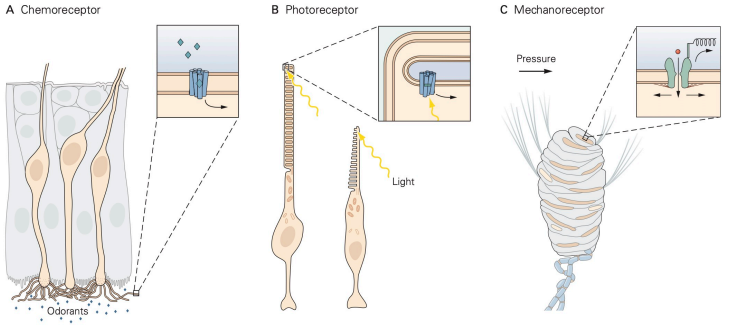

Figure 1: Sensory receptors are specialized to transduce a particular type of stimulus energy into electrical signals. Sensory receptors are classified as chemoreceptors, photoreceptors, or mechanoreceptors depending on the class of stimulus energy that excites them. They transform that energy into an electrical signal that is transmitted along pathways that serve one sensory modality. The insets in each panel illustrate the location of the ion channels that are activated by stimuli.

Sensory receptors can be grouped into one of three classes: **free nerve endings**, **nerve endings surrounded by accessory structures,** and **specialized sensory receptor cells .** For free nerve ending receptors, the stimulus interacts directly with the receptor proteins in the sensory receptor's membrane causing a localized depolarization called a **receptor potential.** This depolarization spreads electronically along the sensory receptor's axon where it encounters voltage-gated sodium and potassium channels that generate action potentials. The action potentials are propagated along the axon until they reach the central terminals of the sensory

neuron within the central nervous system (CNS) where they initiate the release of neurotransmitters at synapses that excite other nerve cells, called **secondary sensory neurons**. These secondary sensory neurons carry the signal from the sensory receptors to various regions of the CNS.

The sequence of events described above is the same for **sensory receptors with accessory structures** except that the stimulus interacts directly with the accessory structure rather than the receptor proteins directly. The accessory structure shapes or filters the original stimulus before in engages with the receptor proteins. For example, the cornea and lens of the eye are accessory structures that serve to focus incident light that enters the pupil onto the rod and cone sensory receptors in the retina located at the back of the eye. The third class of sensory receptors, **specialized receptor cells**, lacks an axon and thus, do not generate action potentials. When specialized receptor cells interact with their adequate stimulus, a change in membrane potential occurs which leads to either an increase or a decrease in the amount of neurotransmitter that they release onto post-synaptic sensory nerve cells.

Learning Objective #2. *To understand the labeled-line principle of signal detection.*

Sensory receptors typically respond to only one type of stimulus and that behavior confers selectivity and specificity to the receptor. The stimulus specificity of the receptor is conserved as the signal is propagated along neural pathways in the CNS. The series of neurons that are connected (by synapses) from the sensory receptor to secondary sensory and third order sensory neurons in the CNS is referred to as a '**labeled line**'. The figure below illustrates how the

labeled lines for several different sensory modalities are arranged. Because each neuron in the labeled line receives synaptic input from only one type of sensory neuron, each cell in the sequence or circuit retains the identity of the stimulus. The labeled lines enable the brain to distinguish and process information about different types physical stimuli.

Verification of the labeled line principle comes from a variety of experiments performed on human subjects both in laboratory settings and during neurosurgical procedures. One can record the action potentials generated by individual sensory neurons by inserting small wire electrodes into a peripheral nerve in the arm or leg. By probing the cutaneous surface, the experimenter can locate the area of skin that is innervated by the sensory neuron, referred to as the **receptive field**. Typically, pressing on that area increases the rate of action potentials produced by the sensory neuron. If the experimenter then delivers a series of minute electrical shocks through the same set of wire electrodes, the subject will experience the same sensation as that produced by the pressure. Moreover, the subject will be able to localize the sensation to the **receptive field** of the sensory neuron whose axon is being stimulated. Similarly, during a neurosurgical procedure to implant a brain stimulator, if the electrophysiologist on the surgical team delivers a small electrical shock through the brain probe near the optic nerve, the awake patient will report that they "saw" a flash of light. If the shock were delivered near the auditory nerve, the patient would report that they "heard" sound. The fact that the brain correctly associates the electrical impulses in a sensory neuron with the type of physical stimulus that normally activates the neuron forms the basis of 'neural prostheses' like cochlear implants to restore hearing.

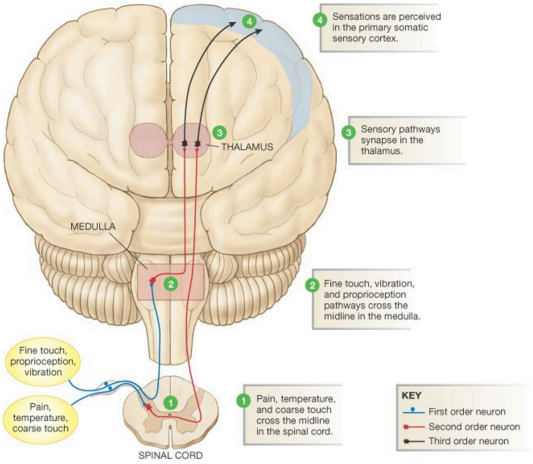

Figure 2: Labeled lines from different types of sensory receptors enable the CNS to distinguish different sensory modalities.

Learning Objective #3: *To compare the mechanisms of sensory transduction in different types of sensory receptors.*

All of the tissues in our body are endowed with sensory receptors with the ironic exception of the brain. In the following section, the mechanisms by which several different types of sensory receptors transform a physical stimulus into an electrical signal (i.e., **sensory transduction**) will be compared.

Light Receptors (Vision)

The principal light receptors (i.e., **photoreceptors**) in humans, rods and cones, are located in a thin layer of tissue lining the back of the

eye called the retina. The mechanisms by which the photoreceptors convert light energy to an electrical signal are shown in the figure below. In the absence of a light stimulus, a 'dark current' flows through the membrane of the receptors carried by an influx of Na^+ ions in the outer segment, and an efflux of K^+ ions through the membranes of the inner segments. This current depolarizes the receptor membrane in the dark to about -40 mV. At this membrane potential, Ca^{2+} ions flow into the synaptic terminals of the photoreceptors resulting in the release of neurotransmitter. When light enters the eye through the pupil and impinges on the retina, it activates as a complex G-protein coupled receptor protein (called a **photopigment)** embedded on membranous disks within the outer segment of the photoreceptor. The photopigments are composed of a transmembrane **opsin** moiety and retinal, a **chromophore**. Absorption of light by the chromophore activates the opsin and then **transducin**, a G-protein, resulting in a decrease in the concentration of cGMP through phosphodiesterase (PDE) in the cytosol. The decrease in cGMP concentration in turns allows some of the Na^+ channels to close, reducing the dark current, hyperpolarizing the receptor, reducing the influx Ca^{2+} in the synaptic terminals and consequently deceasing the release of neurotransmitter. This sequence of events is referred to as the **phototransduction cascade**. The process is terminated by the phosphorylation of the photopigment by a kinase and the binding of another protein called arrestin.

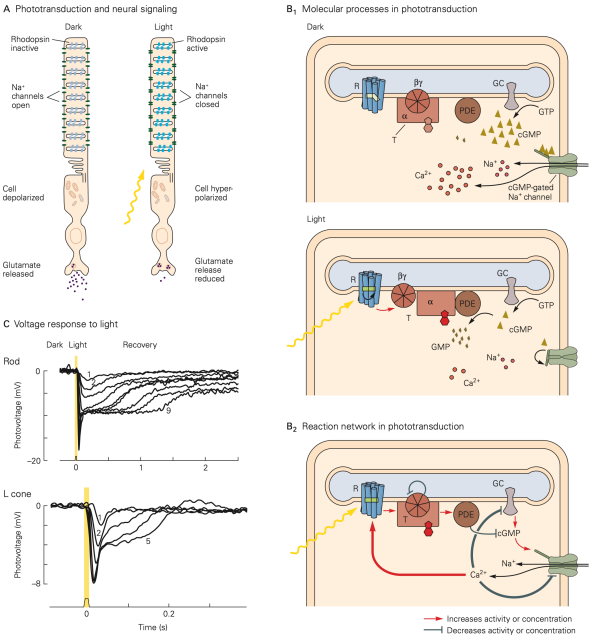

Figure 3: Phototransduction. **A.** *The rod cell responds to light. Rhodopsin molecules in the outer-segment discs absorb photons, which leads to the closure of cGMP-gated channels in the plasma membrane. This channel closure hyperpolarizes the membrane and reduces the rate of release of the neurotransmitter glutamate.* **B.** *1. Cyclic GMP (cyclic guanosine 3'-5' monophosphate) is produced by a guanylate cyclase (GC) and hydrolyzed by a phosphodiesterase (PDE). In the dark the phosphodiesterase activity is low, the*

cGMP concentration is high, and the cGMP-gated channels are open, allowing the influx of Na^+ and Ca^{2+}. In the light rhodopsin (R) is excited by absorption of a photon, then activates transducin (T), which in turn activates the phosphodiesterase; the cGMP level drops, the membrane channels close, and less Na^+ and Ca^{2+} enter the cell. The transduction enzymes are all located in the internal membrane discs, and the soluble ligand cGMP serves as a messenger to the plasma membrane. 2. Calcium ions have a negative feedback role in the reaction cascade in phototransd

Chemoreceptors (Smell and Taste)

Sensory receptors that are designed to detect the presence of chemicals in the air we breathe, the substances we ingest and the fluids that circulate through our tissues are called **chemoreceptors.** The most familiar of these are **olfactory sensory neurons** (OSNs) embedded in the olfactory mucosa and the **taste receptor cells** found in the taste buds on the tongue and elsewhere in the oral cavity. The OSNs are endowed with **olfactory cilia** that extend into the mucosa of the oral cavity and interact with airborne odorant molecules. The olfactory cilia of each OSN express only one of the approximately 400 different olfactory receptor G-proteins such that each OSN is activated by a restricted set of odorant molecules. The **olfactory sensory transduction cascade** is quite similar to the **phototransduction cascade** except that the **G-protein** interacts with cyclase to increase cAMP rather than cGMP. The resultant depolarizing receptor potential leads to the generation of action potentials along the axon of the OSN. As is the case in

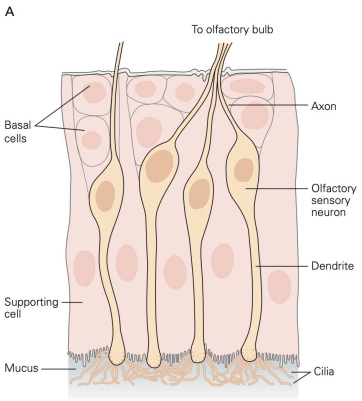

Figure 4: The olfactory epithelium contains sensory neurons interspersed with supporting cells as well as a basal layer of stem cells. Cilia extend from the dendrite of each neuron into the mucus lining the nasal cavity. An axon extends from the basal end of each neuron to the olfactory bulb.

phototransduction, the OSN receptor potential lasts until the olfactory receptor protein is phosphorylated by a kinase and bound to an arrestin-like protein so that the cAMP that was produced is hydrolyzed by PDE.

uction. Stimulation of the network by light leads to the closure of the cGMP-gated channels. This causes a drop in the intracellular concentration of Ca^{2+}. Because Ca^{2+} modulates the function of at least three components of the cascade—rhodopsin, guanylyl cyclase, and the cGMP-gated channel—the drop in Ca^{2+} counteracts the excitation caused by light. **C.** *Voltage response of a primate rod and cone to brief flashes of light of increasing intensity. Higher numbers on the traces indicate greater intensities of illumination (not all traces are*

labeled). For dim flashes the response amplitude increases linearly with intensity. At high intensities the receptor saturates and remains hyperpolariz ed steadily for some time after the flash; this leads to the afterimages that we perceive after a bright flash. Note that the response peaks earlier for brighter flashes and that cones respond faster than rods.

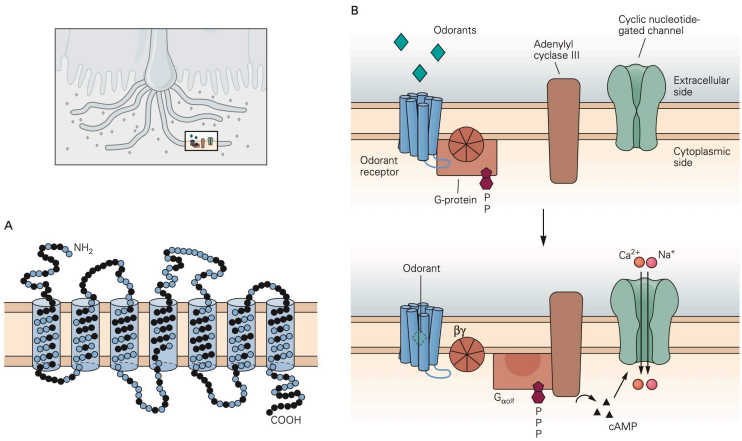

Figure 5: **A.** *Odorant receptors have the seven transmembrane domains characteristic of G protein-coupled receptors. They are related to one another but vary in amino acid sequence (positions of highest variability are shown here as black balls). Humans have approximately 350 different odorant receptors, and mice have approximately 1,000.* **B.** *Binding of an odorant causes the odorant receptor to interact with $G\alpha_{olf}$, the α-subunit of a heterotrimeric G protein. This causes the release of a GTP-coupled $G\alpha_{olf}$, which stimulates adenylyl cyclase III, leading to an increase in cAMP. The elevated cAMP in turn induces the opening of cyclic nucleotide-gated cation channels, causing cation influx and a change in membrane potential in the ciliary membrane. (cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate.*

There are several different types of **taste cells** found in individual taste buds and three different types of sensory transduction mechanisms. Tastant molecules contained in ingested materials enter the taste buds through a **taste pore** on the surface of the tongue and other oral cavity structures and interact with **taste receptors** located on the **microvilli** of the taste cells. Full elucidation of the different transduction mechanisms underlying taste remains elusive, but the following descriptions are reasonable secure. Sweet, bitter and savory (umami) molecules bind to G-protein coupled taste receptors called, **gustducins,** leading to a depolarizing receptor potential in the taste that increases the

release of neurotransmitter from the distal synaptic terminals. Sour, acidic substances and CO2 produce H+ ions in solutions that bind to sour taste receptor proteins and also flow through non-specific cation channels in the microvilli of sour taste cells. Finally, edible salts dissociate in liquid and the Na+ ions enter salty taste cells through ion channels in their microvilli leading to depolarization.

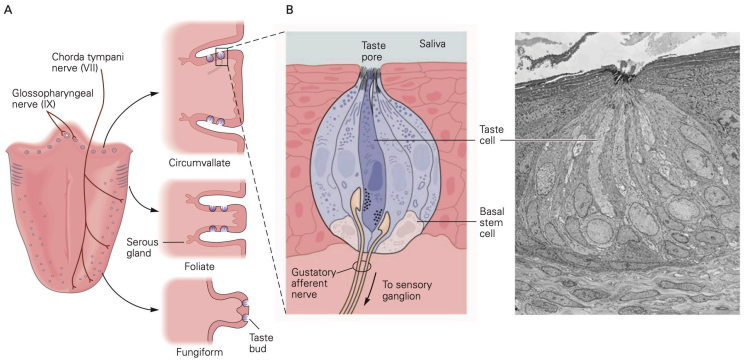

Figure 6: Taste buds are clustered in papillae on the tongue. **A.** *The three types of papillae—circumvallate, foliate, and fungiform—differ in morphology and location on the tongue and are differentially innervated by the chorda tympani and glossopharyngeal nerves.* **B.** *Each taste bud contains 50 to 150 elongated taste receptor cells, as well as supporting cells and a small population of basal stem cells. The taste cell extends microvilli into the taste pore, allowing it to detect tastants dissolved in saliva. At its basal end the taste cell contacts gustatory sensory neurons that transmit stimulus signals to the brain. The scanning electron micrograph shows a taste bud in a foliate papilla in a rabbit.*

Mechanoreceptors (Touch and Proprioception)

Virtually of the tissues in the body are endowed with some type of mechanoreceptor designed to detect physical displacement, i.e., movement. For example, blood vessels are innervated by **baroreceptors** that respond to stretch of the vessel wall and thereby signal blood pressure to the CNS. Skeletal muscles contain two

types of **proprioceptors**, called **muscle spindles** and **Golgi tendon organs**, which transduce muscle length and muscle force, respectively, into electrical signals that permit the CNS to precisely control movement. Common to all mechanoreceptors are membrane receptor proteins that form ion channels whose gating depends mechanical forces exerted on the channel protein. For example, applying stretch to a muscle also stretches the muscle spindles that reside within the muscle. This causes the stretch-sensitive channels in the membrane of the muscle spindle sensory receptors to open allowing Na^+ ions to flow into the sensory neuron which in turn results in a depolarizing receptor potential. Relaxing the muscle stretch, removes the force on the mechanosensitive channels on the muscle spindle sensory receptors and the membrane of the sensory neuron returns to its resting level.

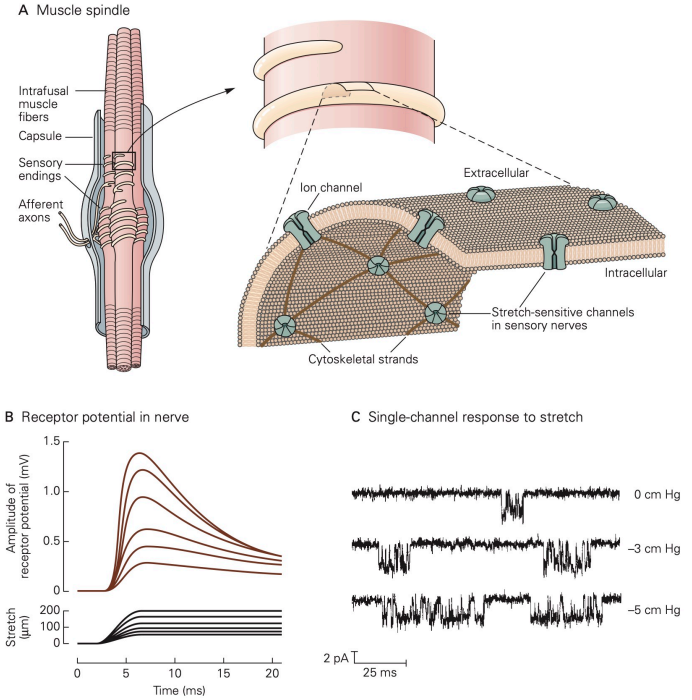

Figure 7: The muscle spindle is the principal receptor mediating proprioception. **A.** *The muscle spindle is located within skeletal muscle and is excited by stretch of the muscle. It consists of a bundle of thin (intrafusal) muscle fibers entwined by a pair of sensory axons, and is also innervated by several motor axons (not shown) that produce contraction of the intrafusal muscle fibers. Stretch- sensitive ion channels in the sensory nerve terminals are linked to the cytoskeleton by the protein spectrin.* **B.** *The depolarizing receptor potential recorded in a group* I_a *fiber innervating the muscle spindle (upper record) is proportional to both the velocity and amplitude of muscle stretch parallel to the myofilaments (lower record). When stretch is maintained at a fixed length, the receptor potential decays to a lower value.* **C.** *Patch clamp recordings of a single stretch-sensitive channel in myocytes. Pressure is applied to the receptor cell membrane by suction. At rest (top record) the channel opens sporadically for short time intervals. As the pressure applied to the mem- brane increases (lower records) the channel opens more often and remains in the open state longer. This allows more current to flow into the receptor cell, resulting in higher levels of depolarization.*

Skin is richly endowed with a host of different types of

mechanoreceptors; each specialized to detect a particular form of mechanical stimulation. Also found in skin and many other tissues are **nociceptors** that are designed to detect noxious stimuli like extreme pressure, extreme temperature and dangerously high levels of chemical substances.

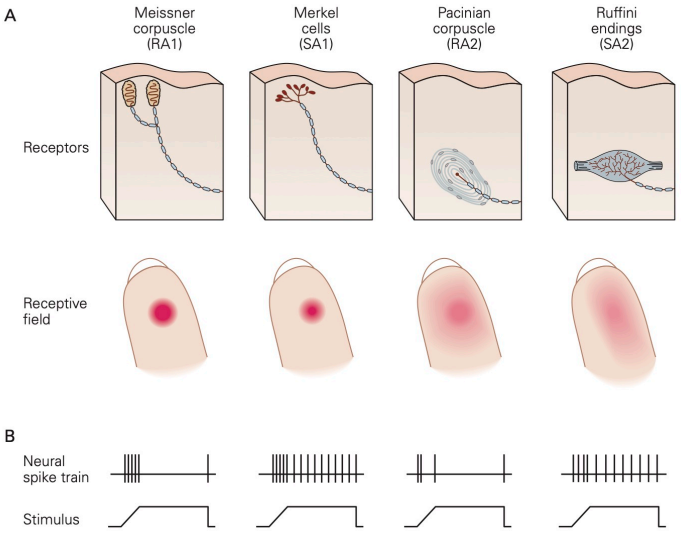

Figure 8: Touch is mediated by four types of mechanoreceptors in the human hand. The terminals of myelinated sensory nerves innervating the hand are surrounded by specialized structures that detect contact on the skin. The receptors differ in morphology, innervation patterns, location in the skin, receptive field size, and physiological responses to touch. **A.** *The superficial and deep layers of the glabrous (hairless) skin of the hand each contain distinct types of mechanoreceptors. The superficial layers contain small receptor cells: Meissner corpuscles and Merkel cells. The sensory nerve fibers innervating these receptors have branching terminals such that each fiber innervates multiple receptors of one type. The deep layers of the skin and subcutaneous tissue contain large receptors: Pacinian corpuscles and Ruffini endings. Each of these receptors. is innervated by a single nerve fiber, and each fiber innervates only one receptor. The receptive field of a mechanoreceptor reflects the location and distribution of its terminals in the skin. Touch receptors in the superficial layers of the skin have smaller receptive fields than those in the deep layers. (RA1, rapidly adapting type 1; RA2, rapidly adapting type 2; SA1, slowly adapting type 1; SA2, slowly adapting type 2.)* **B.** *The nerve fibers innervating each type of mechanoreceptor respond differently when activated. The spike trains show responses of each type of nerve when its receptor is activated by constant pressure against the skin. The RA type fibers that innervate Meissner and Pacinian corpuscles adapt rapidly to constant stimulation while the SA type nerves that innervate Merkel cells and Ruffini endings adapt slowly.*

Hair Cell Receptors (Hearing and Balance)

A highly specialized form of mechanoreceptor called the **hair cell** is found in the four different sense organs that reside within the bony labyrinth (i.e., cochlea, semicircular canals, utricle and saccule). The hair cells in the cochlea transduce sound waves that impinge on the tympanic membrane into changes in membrane potential and the hair cells in the semicircular canals, utricle and saccule are activated by movements of the head and body to help maintain object fixation in the visual field and balance.

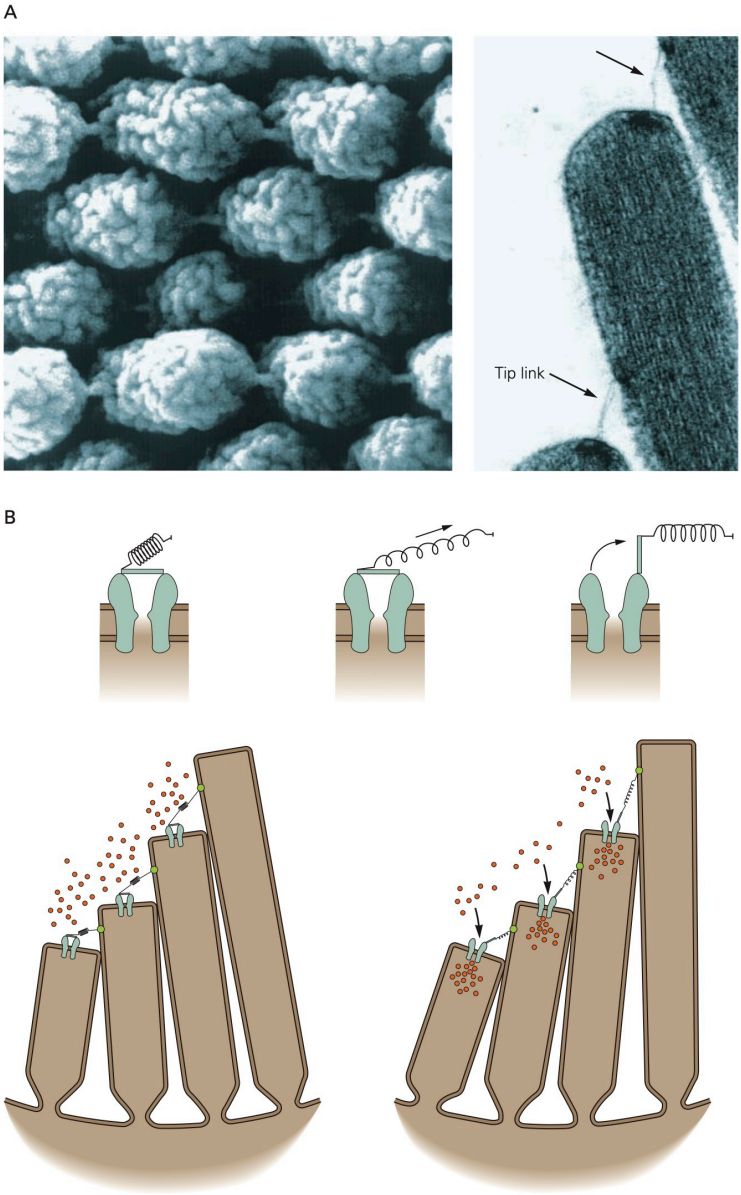

Figure 9: Mechanoelectrical transduction by hair cells. **A.** *A tip link connects each stereocilium to the side of the longest adjacent stereocilium, as seen in a scanning electron micrograph (left) and a transmission electron micrograph*

(right) of a hair bundle's top surface. Each tip link is only 3 nm in diameter. The links appear stouter in the illustration on the left because of metallic coating during specimen preparation. ***B. Top:*** *Ion flux through the channel that underlies mechanoelectrical transduction in hair cells is regulated by a molecular gate. The opening and closing of the gate are controlled by the tension in an elastic element, the gating spring, that senses hair-bundle displacement.* ***Bottom:*** *When the hair bundle is at rest each transduction channel clatters between closed and open states, spending most of its time shut. Displacement of the bundle in the positive direction increases the tension in the gating spring, here assumed to be in part a tip link, attached to each channel's molecular gate. The enhanced tension promotes channel opening and the influx of cations, thereby producing a depolarizing receptor potential.*

Hair cells reside in an unusual extracellular milieu: the apical end that include the stereocilia that give the hair cells their name projects into endolymph which has an ionic composition similar cytosol (i.e., high K^+, low Na^+), while the remainder of the cell is bathed in perilymph with an ionic concentration similar to other extracellular fluids (high Na^+, low K^+). Pump molecules in the adjacent epithelia cells maintain the disparate ionic concentrations in these two compartments of the hair cells. K^+ ions flow through non-selective cation channels located in the tips of the stereocilia, some of which are open at rest. Displacement of the stereocilia either stretches or relaxes molecular linkages between adjacent stereocilia that opens or closes the K^+ channels, respectively. Opening more K^+ channels leads to a depolarizing receptor potential whereas closing the K^+ channels leads to a hyperpolarizing receptor potential. Depolarizing receptor potentials activate voltage-gated $Ca2^+$ channels in the hair cell that results in more neurotransmitter release. With hyperpolarizing receptor potentials, the $Ca2^+$ channels remain closed and the amount of neurotransmitter released is decreased.

Learning Objective #4: *To appreciate how the intensity and duration of a stimulus are reflected in the receptor potential and action potential discharge rate of a sensory afferent neuron.*

Unlike the all-or-none action potential, **receptor potentials are graded and are proportional to the strength of the stimulus that evokes them**. Further, receptor potentials normally do not display refractoriness: They persist as long as the stimulus is present. The magnitude of the receptor potential is then reflected in either the rate or action potential generation (i.e., discharge or firing rate) in the sensory neuron or in the amount of neurotransmitter released in a specialized sensory receptor cell that lack axons. The relationship between the magnitude of the receptor potential and the firing rate of the sensory neuron (or the amount of neurotransmitter released for a specialized receptor cell that does not produce action potentials) forms the basis of the **neural code**, the firing rate or amount of transmitter released 'reports' the strength of a stimulus.

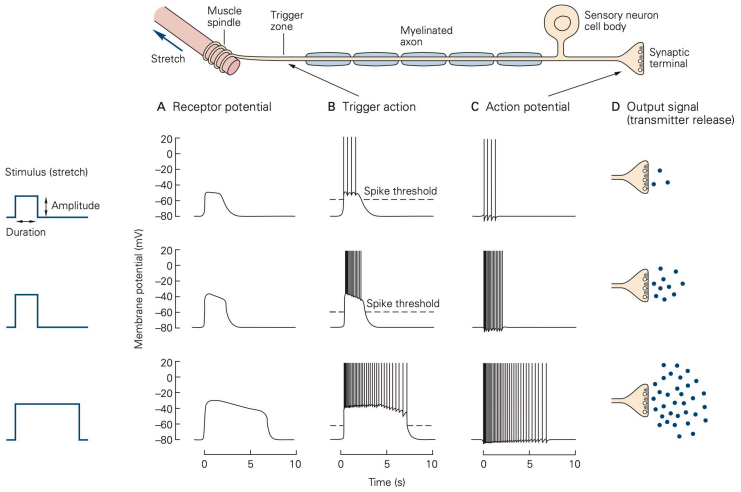

Figure 10: Each of the neuron's four signaling components produces a characteristic signal. The figure shows a sensory neuron activated by stretching of a muscle, which the neuron senses through a specialized receptor, the muscle spindle. **A.** *The input signal, called a receptor potential, is graded in amplitude and duration, proportional to the amplitude and duration of the stimulus.* **B.** *The trigger zone sums the depolarization generated by the receptor potential. An action potential is generated only if the receptor potential exceeds a certain voltage threshold. Once this threshold is surpassed, any further increase in amplitude of the receptor potential can only increase the frequency with which the action potentials are generated, because action potentials have a constant amplitude. The duration of the receptor potential determines the duration of the train of action potentials. Thus the graded amplitude and duration of the receptor potential is translated into a frequency code in the action potentials generated at the trigger zone. All action potentials produced are propagated faithfully along the axon.* **C.** *Action potentials are all-or-none. Because all action potentials have a similar amplitude and duration, the frequency and duration of firing represents the information carried by the signal. D. When the action potential reaches the synaptic terminal, it initiates the release of a neurotransmitter, the chemical substance that serves as the output signal. The frequency of action potentials determines how much neurotransmitter is released by the cell.*

Learning Objective #5. *To understand how sensory receptors adapt to a constant stimulus.*

In response to a constant stimulus, most sensory receptors do not maintain a constant receptor potential, but rather one that declines over time. This process is called **adaptation** and is mediated by several different mechanisms in different receptors including changes in the behavior of the accessory structures, intracellular signal cascades and even changes in the threshold for generating action potentials at the axon hillock of the sensory neuron. Some sensory receptors adapt slowly to a constant stimulus and others adapt more rapidly. **Slowly-adapting receptors** are sometimes called **tonic receptors** because their sensory neurons maintain a tonic or sustained level of discharge as long as the stimulus is present. Likewise, rapidly-adapting sensory receptors are referred to as **phasic receptors** because they only discharge during specific phases of the stimulus, normally at the onset, but sometimes also at the offset. Tonic receptors are designed to signal the continued presence of a stimulus, whereas phasic receptors primarily signal the start time and end time of the stimulus. Many tissues, like skin and sense organs, like muscle spindles are innervated by both tonic and phasic receptors.

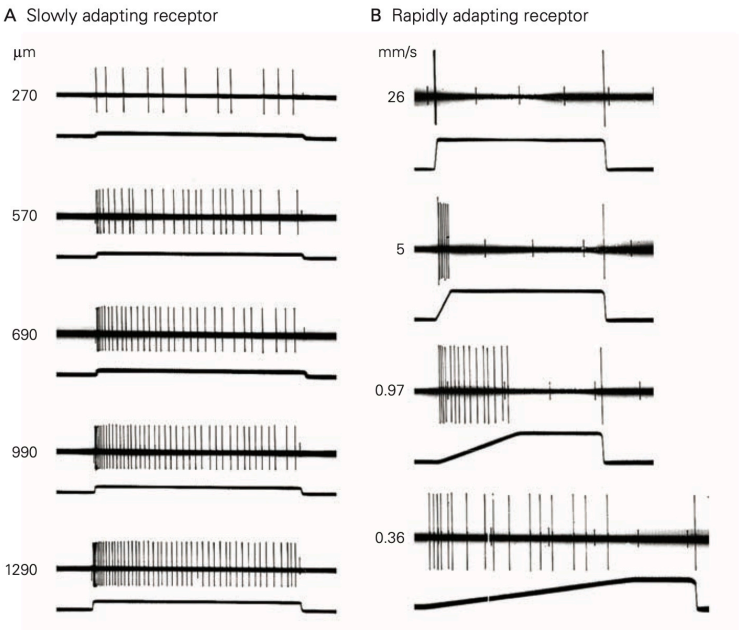

Figure 11: Firing rates of sensory neurons convey information about the stimulus intensity and time course. These records illustrate responses of two different classes of touch receptors to a probe pressed into the skin. The stimulus amplitude and time course are shown in the lower trace of each pair; the upper trace shows the action potentials recorded from the sensory nerve fiber in response to the stimulus. **A.** *A slowly adapting mechanoreceptor responds as long as pressure is applied to the skin. The total number of action potentials discharged during the stimulus is proportional to the amount of pressure applied to the skin. The firing rate is higher at the beginning of skin contact than during steady pressure, as this receptor also detects how rapidly pressure is applied to the skin. When the probe is removed from the skin, the spike activity ceases.* **B.** *A rapidly adapting mechanoreceptor responds at the beginning and end of the stimulus, signaling the rate at which the probe is applied and removed; it is silent when pressure is maintained at a fixed amplitude. Rapid motion evokes a brief burst of high-frequency spikes, whereas slow motion evokes a longer-lasting, low-frequency spike train.*

Autonomic Nervous System Physiology

JOHN TUTHILL

Learning Objectives and Quick Synopses

1. Compare and contrast the neurotransmitters and receptor types in the somatic motor division, parasympathetic autonomic efferent division, and sympathetic autonomic efferent division of the nervous system. Include the neurotransmitter / receptor pairs in the ANS two-neuron pathways.

Preganglionic nerve fibers exit the central nervous system (CNS) and release acetylcholine (ACh) on postganglionic neurons in autonomic ganglion. This second postganglionic neuron releases norepinephrine on targets if it is sympathetic, or ACh if it is parasympathetic. The ACh receptors on the ganglion are nicotinic type, whereas the distal receptors are muscarinic type on parasympathetic target cells and adrenergic on sympathetic target cells.

2. Identify epinephrine / norepinephrine receptor types and their effects on various target organs.

There are two categories of alpha adrenergic receptors and

three types of beta adrenergic receptors. They are G-protein coupled receptors (GPCRs). You will learn the G proteins they each couple to and some principal cell types they are expressed in (see Tables).

3. Compare nicotinic and muscarinic acetylcholine receptor (AChR) activation and identify acetylcholine receptor types and their effects on various target organs

Nicotinic nAChRs are fast, ligand-gated nonselective cation channels. Muscarinic mAChRs are GPCRs and not ion channels. You will learn the G proteins that M2 and M3 muscarinic receptors couple to and some principal cell types they are expressed in (see Tables).

4. Identify the role of ATP and nitric oxide in smooth muscle relaxation and blood vessel-dilation.

ATP is released by exocytosis from synaptic vesicles as a cotransmitter from autonomic nerve fibers. It can initiate contraction of smooth muscles in arterioles and in the vas deferens. The diffusible gas nitric oxide (NO) is synthesized enzymatically in neurons of the genitalia and in endothelium of the cardiac coronary circulation. It relaxes smooth muscle, stimulates blood flow, and signals erection.

5. Describe the baroceptor reflex in response to high or low blood pressure.

The baroceptor reflex is an autonomic reflex that excites the parasympathetic NS and inhibits the sympathetic NS when blood pressure rises. The reflex path is: Pressure receptor -> CNS -> ANS.

6. Define orthostatic hypotension and discuss how the baroreflex counters it in healthy patients.

Orthostatic hypotension is a loss of blood pressure in the

upper body that happens when you stand up. Pressure receptors mount a corrective response called the baroreflex.

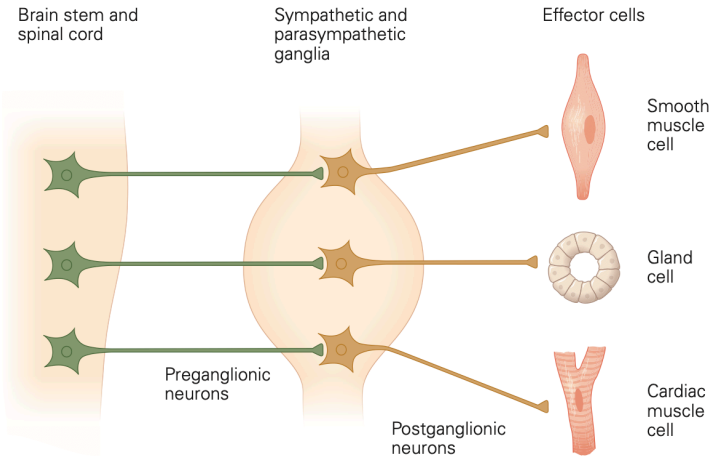

Figure 1: Autonomic pathways have three basic cell types. Autonomic motor neurons lie outside the central nervous system in clusters or ganglia and are controlled by preganglionic neurons in the spinal cord and brain stem. Specialized neurons in ganglia regulate specific types of effector cells, such as smooth muscle, gland cells, and cardiac muscle.

The word *efferent* means "carrying messages away from the central nervous system (CNS)." The CNS communicates to the rest of the body through three major classes of efferent nerve fibers: (i) somatic, (ii) sympathetic preganglionic, and (iii) parasympathetic preganglionic. The first controls familiar voluntary muscle motor activity. The latter two belong to the autonomic nervous system (or simply, ANS), the subject of this chapter. The word autonomic implies involuntary, happening by itself. The agenda of these three

efferent systems is different, crudely: (i) voluntary movement, (ii) fight & flight, and (iii) rest & These three classes of efferent nerve fibers have their cell bodies in the spinal cord or brain stem and send axons out as nerve bundles to the periphery. Such efferent nerve fibers all act by releasing the neurotransmitter acetylcholine (ACh) on their initial targets, so by definition they are called cholinergic (colored blue here). Here we focus on the neurotransmitters, receptors, and specialized cellular effector actions of ANS signaling.

Aside: *Another branch of the peripheral nervous system related to the ANS is the Enteric Nervous System regulating the gut, which we will not deal with now. It lies in the peritoneum.*

(Unless otherwise noted, all figures are from: Kandel ER, Schwartz JH, Jessell TM 2012, Siegelbaum SA, Hudspeth AJ. 'Principles of Neural Science, 5th ed. *McGraw-Hill, New York.*)

Learning Objective #1: *Compare and contrast the neurotransmitters and receptor types in the somatic motor, parasympathetic autonomic motor, and sympathetic autonomic motor divisions of the nervous system. Include the neurotransmitter- receptor pairs in the ANS two-neuron pathways.*

Somatic nervous system efferents

Efferent somatic motoneurons innervate skeletal muscle forming a very large synapse: the neuromuscular junction (NMJ) or motor endplate. They excite action potentials in the muscle by opening depolariz- ing nicotinic ACh receptor ion channels (nAChRs) at the NMJ. Each skeletal muscle fiber is an obligatory follower of the motor axon that innervates it. Each presynaptic action potential propagating in a somatic motor axon elicits one postsynaptic action potential in the skeletal muscle fibers it innervates and one muscle twitch.

Autonomic nervous system efferents

Almost every organ of the body is innervated by the two *autonomic* peripheral systems called the sympathetic and parasympathetic nervous systems. In both, the innervation is via a two-neuron relay: first efferent preganglionic neurons and then peripheral postganglionic neurons. The efferent ANS nerve fibers that exit the CNS are called preganglionic because they release their ACh in the peripheral autonomic ganglia to excite the second postganglionic neurons that in turn innervate the peripheral targets of the ANS. The ANS efferent preganglionic nerve fibers form standard, focal, and fast nicotinic synapses on ANS postganglionic neurons. As it is for skeletal muscle, the ACh effect within the ganglion is depolarizing and excitatory, and the postganglionic neuron fires action potentials, acting mostly as a follower: Each preganglionic action potential elicits one or a few postganglionic action potentials in the second neuron. Thus, the somatic motor efferents and the ANS efferents share a common neurotransmitter (ACh) and act on quite similar (but genetically distinct) nicotinic receptors.

Note about nomenclature: The CNS neurons giving rise to autonomic efferents are sometimes called autonomic motor neurons, but to avoid any confusion with somatic motoneurons, we stick to calling them just autonomic efferents here.

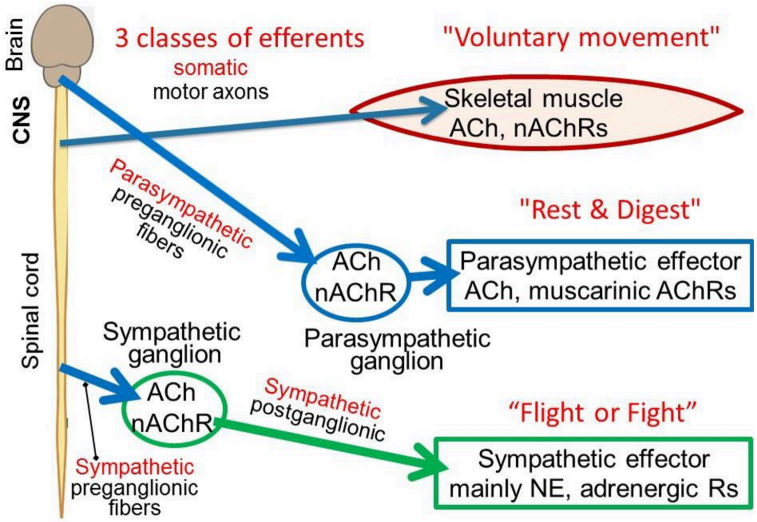

Figure 2: Comparison of somatic and ANS efferent pathways.

Autonomic postganglionic nerve fibers

We have emphasized that autonomic efferent signals are relayed in a second step by axons of post-ganglionic neurons to their final targets in a two-neuron efferent pathway (Figure 2)–we call this the "two-neuron rule." The targets include glands, all muscles, the major

organs, epithelia, and blood vessels. Instead of forming terminal focal synapses with point-to-point connections, the postganglionic neurons (the second neurons) of the ANS usually deliver neurotransmitters more diffusely throughout a target tissue by "sprinkled" release from numerous varicosities along the length of their axons. The varicosities are swellings–looking like beads on a string–that you could think of as sprinkler heads arranged along a water pipe. As is true of fast, focal synapses, the varicosities release their neurotransmitters in response to passing action potentials by calcium-dependent exocytosis from intracellular neurotransmitter vesicles concentrated in the varicosity. You should think of the actions of autonomic nervous system axons as broadly regulating groups of cells and tissues rather than as targeting a specific single cell.

An exception to the two-neuron rule

There is an exception to the two-neuron rule in the adrenal gland. The epinephrine-secreting chromaffin cells of the adrenal medulla are directly innervated by the "preganglionic" cholinergic efferent nerve rather than by a two-neuron pathway. However, loosely, one might regard the neuron-related, specialized secretory chromaffin cells as being like postganglionic neurons that deliver their epinephrine hormone/neurotransmitter by vesicular exocytosis, but now in endocrine fashion. They are round cells with no axons but release the hormone from the cell body to blood that circulates everywhere.

Sympathetic versus parasympathetic postganglionic neurotransmitter

We have emphasized the common features of postganglionic

neurons. We now concentrate on the specialized properties that differentiate the postganglionic sympathetic from the postganglionic parasympathetic nerve fibers–with reference again to Figure 2.

- Sympathetic postganglionic nerve fibers are adrenergic. They release norepinephrine (NE) by exocytosis from synaptic vesicles in their varicosities. (Colored green in Figure 2)
- Parasympathetic postganglionic fibers are cholinergic. They release ACh by exocytosis from synaptic vesicles in their varicosities. (Colored blue in Figure 2.)
- Both classes of postganglionic nerve fibers release additional mediators, sometimes called cotransmitters, in addition to NE or ACh. This may include neuropeptides and other small molecules such as ATP or nitric oxide. We do consider them later, but here we emphasize the principal neurotransmitters NE and ACh and their actions.
- *Exception:* At eccrine sweat glands, sympathetic postganglionic fibers release ACh instead of NE.

> **Aside:** *Eccrine glands are the common sweat glands of your skin. They are distinguished from the often more smelly apocrine sweat glands found in certain hair follicles.*

Receptors at each synapse

Nicotinic ACh receptors (nAChRs) are fast ligand-gated ion channels used at the cholinergic synapses on skeletal muscle endplates and at the synapses on all postganglionic neurons in autonomic ganglia. They are depolarizing (excitatory), nonselective cation channels that

initiate action potentials. These nAChRs relay electrical excitation (action potentials) from the presynaptic neuron to a postsynaptic cell.

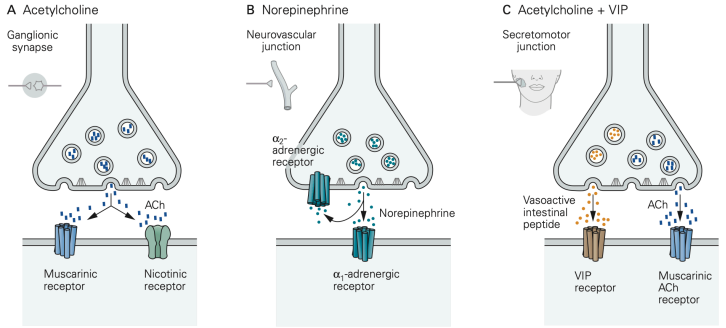

Figure 3: Synaptic transmission in the peripheral autonomic system. **A.** *In sympathetic ganglia ACh can activate both nicotinic and muscarinic receptors to produce fast and slow postsynaptic potentials, respectively.* **B.** *At neurovascular junctions norepinephrine can simultaneously activate postsynaptic α1-adrenergic receptors to produce vasoconstriction and presynaptic α2-adrenergic receptors to inhibit further transmitter release.* **C.** *Co-transmission involves the co-activation of more than one type of receptor by more than one transmitter. Parasympathetic postganglionic nerve terminals in the salivary glands release both ACh and vasoactive intestinal peptide (VIP) to control secretion. Autonomic synapses with end-organs sometimes employ more elaborate combinations, activating three or more receptor types.*

Thus, in the ganglion, they help to relay preganglionic action potential messages as postganglonic action potential messages on their way to target organs.

By contrast, various G-protein coupled receptors (GPCRs) are the principal receptors for neurotransmitter in the target organs of the postganglionic sympathetic and parasympathetic nerve fibers. Recall that GPCRs are not ion channels. Rather they initiate cascades of G-protein, calcium, and protein kinase signaling. Thus, action potentials and transmitter release from the postganglionic nerve fibers initiate biochemical signaling cascades in the target

tissues, rather than action potentials. Like the postganglionic varicosities that stimulate them, these GPCRs on the target are not discretely organized in specialized postsynaptic structures but rather are diffusely spread over the surface of target cells and tissues. For ANS responses the key GPCRs are:

- Muscarinic ACh receptors (mAChRs, several kinds) mediating parasympathetic effects.
- Adrenergic receptors (α1, α2, and β) mediating sympathetic effects.
- Additional receptors responding to other cotransmitters like ATP, peptides, and nitric oxide (NO).

Table 1: Neurotransmitters and receptors of the ANS.

Transmitter	Receptor	Responses
Norepinephrine	α_1	Stimulates smooth muscle contraction in arteries, urethra, gastrointestinal tract, iris (pupillary dilation), uterine contractions during pregnancy, ejaculation; glycogenolysis in liver; glandular secretion (salivary glands, lacrimal glands)
	α_2	Presynaptic inhibition of transmitter release from sympathetic and parasympathetic nerve terminals; stimulates contraction in some arterial smooth muscle
	β_1	Increases heart rate and strength of contraction
	β_2	Relaxes smooth muscle in airways and gastrointestinal tract; stimulates glycogenolysis in liver
	β_3	Stimulates lipolysis in fat cells; inhibits bladder contraction
Acetylcholine	Nicotinic	Fast EPSP in autonomic ganglion cells
	Muscarinic: M_1, M_2, M_3	Glandular secretion; ocular circular muscle (pupillary constriction); ciliary muscles (focus of lens); stimulates endothelial production of NO and vasodilation; slow EPSP in sympathetic neurons; slows heart rate; presynaptic inhibition at cholinergic nerve terminals; bladder contraction; salivary gland secretion
Neuropeptide Y	Y_1, Y_2	Stimulates arterial contraction and potentiates responses mediated by α_1-adrenergic receptors; presynaptic inhibition of transmitter release from some postganglionic sympathetic nerve terminals
Nitric oxide	Diffuses through membranes; often acts to stimulate intracellular soluble guanylate cyclase	Vasodilation, penile erection, urethral relaxation
Vasoactive intestinal peptide	VIPAC1, VIPAC2	Glandular secretion and dilation of blood vessels supplying glands
ATP	P_{2X}, P_{2Y}	Fast and slow excitation of smooth muscle in bladder, vas deferens, and arteries

ATP, adenosine triphosphate; EPSP, excitatory postsynaptic potential; NO, nitric oxide.

Learning Objective #2: *Identify epinephrine / norepinephrine receptor types and their effects on various target organs.*

Norepinephrine, Epinephrine, and their Adrenergic receptors

Norpinephrine (NE) and epinephrine (E) are the principal sympathetic neurotransmitters.

Note about nomenclature: The word "Adrenaline" was registered in a US patent in 1901 and blocked from generic use in the US. Therefore, in the US we are technically supposed to say epinephrine for the compound that all the rest of the world, as well as our popular culture, calls adrenaline. The words "epi nephros" mean over the kidney in Greek, and the words "ad renal" mean next to the kidney in Latin.

- NE is released locally on tissue targets by most sympathetic postganglionic nerve fibers at their varicosities. The released NE is taken up again into the sympathetic nerve fibers by a transporter for reuse.
- Both E and NE are released by the adrenal medulla (4:1 ratio) by exocytosis from the secretory chromaffin granules of chromaffin cells. Like other typical endocrine hormones, these hormones are secreted into the circulatory system. This is the source of circulating E.

- Release of NE from sympathetic nerves along visceral blood vessels is the principal source of circulating NE.

E and NE bind with different affinities to adrenergic receptors, which are GPCRs coupled to different signaling G proteins and stimulating different intracellular pathways. The table below shows that each of the major families of heterotrimeric G proteins is activated by one of the adrenergic receptors. This material will be essential in preparation for the ANS Pharmacology sessions that will follow soon. More complete tables are at the end of the chapter.

Table 2: Location and coupling of **adrenergic** receptors.

Name of Receptor	**One typical location**	**Couples to G-protein α-subunit type**
α1	smooth muscle	**Gαq** (smooth muscle contraction)
α2	on presynaptic side of sympathetic nerve varicosities	**Gαi** (inhibits excessive NE release from nerve varicosities, a function called autoinhibition)
β1	heart, juxtaglomerular cells	**Gαs** (promotes contractility
β2	smooth muscle	**Gαs** (smooth muscle relaxation)
β3	fat cells bladder cells	**Gαs** (lipid breakdown) **Gαs (bladder relaxation)**

Learning Objective #3: *Compare nicotinic and*

muscarinic acetylcholine receptor activation and identify acetylcholine receptor types and their effects on various target organs.

Distinguishing nicotinic and muscarinic ACh receptors

There are two major categories of AChRs: nicotinic (nAChR) and muscarinic (mAChR). Except that they both live in the plasma membrane and bind ACh, nicotinic and muscarinic receptors are quite different in structure, function, and pharmacology. Both have many isoforms (genes).

Nicotinic nAChRs are fast, **ligand-gated cation channels** opened directly by binding of ACh. Opening these channels can depolarize a cell to 0 mV. (Technically, being permeable to Na^+ and K^+, their reversal potential is around 0 mV.) Outside the central nervous system, nACHRs are located both at the skeletal neuromuscular junction (NMJ) and at synapses onto postganglionic neurons in all ANS ganglia and onto adrenal chromaffin cells. The nAChR isoforms (genes) expressed in nerve versus muscle are different, so it is possible to use drugs to block the neuronal and muscle receptors selectively. However, many nAChR pharmacological agents will affect both, including ACh, which is the natural agonist for both. The ACh binding sites of nAChRs are blocked by the paralytic drug curare and related compounds. The nicotinic name comes from the early discovery that nicotine and smoke from tobacco can activate these receptors as well. There are no nAChRs on cardiac muscle, smooth muscle, or on non-nerve/muscle tissue cells such as liver, kidney, epithelia, etc.

Muscarinic mAChRs are **G-protein coupled receptors** (GPCRs),

not ion channels. They are located on the target tissues of parasympathetic innervation and at the sympathetic sweat gland—indeed on most cells of the body. There are five types, but the two primary receptor types to consider here are M2 and M3. All are inhibited by the drug atropine (an antagonist competing with ACh at the binding site) from deadly nightshade. Atropine does not affect nicotinic receptors.

M2 couples to Gαi. It slows heart rate through both:

1) activation of a K^+ channel via Gβγ subunits, leading to hyperpolarization of pacemaker, and

2) turning off the formation of the stimulatory cAMP second messenger through inhibiting adenylyl cyclase via Gαi.

M3 couples to Gαq. The following three actions all result from the ability to elevate intracellular Ca^{2+} through Gq and the PLC/IP3 pathway.

- Contracts smooth muscle.
- Stimulates glands to secrete (including eccrine sweat glands).
- Elevates nitric oxide (NO) production in endothelial cells leading to smooth muscle relaxation (vasodilation).

Synthesis and cycling of ANS neurotransmitters

We now review synthesis and degradation of the neurotransmitters E/NE and ACh (see also ANS Pharmacology sessions):

NE and E are catecholamines, members of the biogenic amine family. They are released from postganglionic varicosities, taken up again by a Na^+/Cl^--coupled NE uptake transporter, and degraded by cytoplasmic monoamine oxidase (MOA).

You do NOT need to memorize their chemical structures.

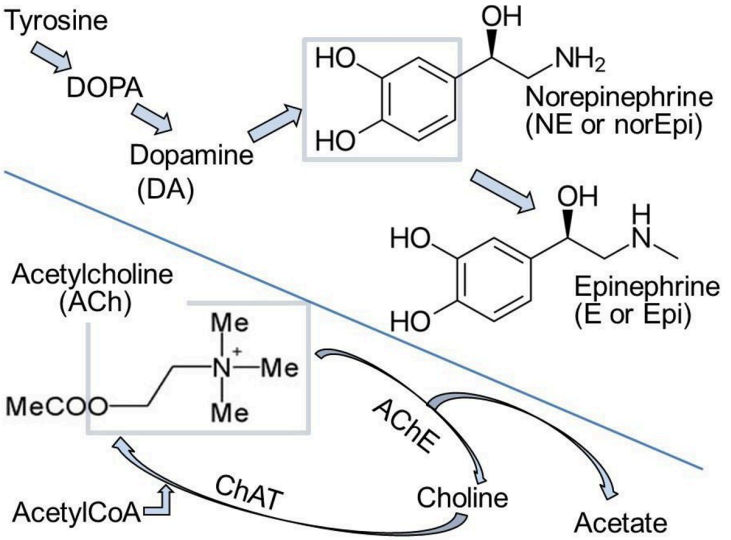

Figure 4: Formation of principle ANS neurotransmitters. ***Top right:*** *Synthesis of NE and E. The gray box on NE outlines the catechol moiety that gives the catecholamine its name.* ***Bottom left:*** *Synthesis of ACh by ChAT is intracellular and hydrolysis by AChE is extracellular. The gray box on ACh outlines the choline moiety of ACh. The quaternary nitrogen has a permanent positive charge. Me stands for methyl -CH_3. You will NOT need to be able to draw these structures.*

- *Synthesis* (Figure 4): The precursor tyrosine is taken up into varicosities by a Na^+/tyrosine cotransporter. Cytosolic enzymes convert tyrosine to DOPA and then to dopamine.
- Dopamine is transported into secretory vesicles.
- Inside vesicles, dopamine is converted to NE.
- *In the adrenal gland*, NE leaks out again to the cytosol where NE is methylated to form E (Figure 4), and E is re-transported into the giant secretory vesicles called "chromaffin granules" in the adrenal gland.

NE is normally released from vesicles by exocytosis, but NE also leaks from nerve into serum. This leak is increased if cytoplasmic

monoamine oxidases (MAOs) are inhibited by MAO inhibitors (MAOIs).

ACh is an ester formed from choline and acetate (Figure 4). Choline and Acetyl-CoA are combined by choline acetyl transferase (ChAT) in the presynaptic terminal. ACh is then transported into secretory vesicles and released at varicosities and synaptic terminals. ACh is inactivated in the extracellular space by hydrolysis back to choline and acetate by the highly active extracellular enzyme acetylcholinesterase (AChE) present in all cholinergic tissues. The enzyme AChE is the target of nerve gases, such as CX, and organophosphate insecticides. Death is by accumulation of excess circulating ACh and, for humans, by exaggerated parasympathetic action. Choline is recycled by uptake into the nerve by a Na^+-coupled cotransporter–conservation. There, choline acetyl transferase resynthesizes ACh. Of all the steps mentioned here, the AChE enzyme will be the most important one for you to remember now.

You do NOT need to remember the structures.

> **Learning Objective #4:** *Identify the role of ATP and nitric oxide in smooth-muscle relaxation and blood-vessel dilation.*

In addition to NE, E, and ACh, the neurotransmitters ATP and NO (nitric oxide) can play important roles in the ANS.

> **Aside:** *ANS terminals also release many active neuropeptides, but those ANS actions are not yet targets*

of pharmaceutical interventions, and we do not amplify on them here.

NO, nitric oxide, is a hydrophobic gas (like CO, carbon monoxide) that diffuses readily from one cell to the next. It relaxes vascular smooth muscle. NO is synthesized inside cells by nitric-oxide synthase (NOS) in response to Ca^{2+}/calmodulin following cytosolic Ca^{2+} rises. The diffusible NO gas stimulates guanylyl cyclase inside cells. This enzyme related to adenylyl cyclase generates cyclic-GMP (cGMP), which activates protein kinases that promote smooth muscle relaxation increasing blood flow. Figure 5 illustrates two possible sources of NO. In the male and female genitalia, NOS seems to be in the varicosities of the postganglionic nerve fibers, and so NO synthesis is increased by the same presynaptic Ca^{2+} rise that also mediates exocytosis of vesicles of ACh. The NO potentiates blood flow during sexual arousal. Viagra and other erectile dysfunction drugs act by prolonging the lifetime of cGMP (stopping its breakdown by a PDE) and hence promoting vascular smooth muscle relaxation. In the cardiac coronary circulation, NOS is in the endothelial cells that line the blood vessels. Calcium rises through ACh acting on endothelial M3 receptors. NO then diffuses to the blood vessel smooth muscle to allow vasodilation and enhance coronary blood flow. Some nitro drugs that release NO, such as nitroglycerine, are used clinically to improve coronary circulation.

ATP is obligatorily co-packaged and co-released with NE, E, and ACh as the polyvalent anion that counters the positive charge of these neurotransmitters inside the synaptic vesicles and chromaffin granules. It is co-released during exocytosis as a cotransmitter. The released extracellular ATP can activate purinergic receptors that are ligand-gated non-selective cation channels. Activation of such purinergic receptors initiates contraction of smooth muscle in arterioles and in the vas deferens.

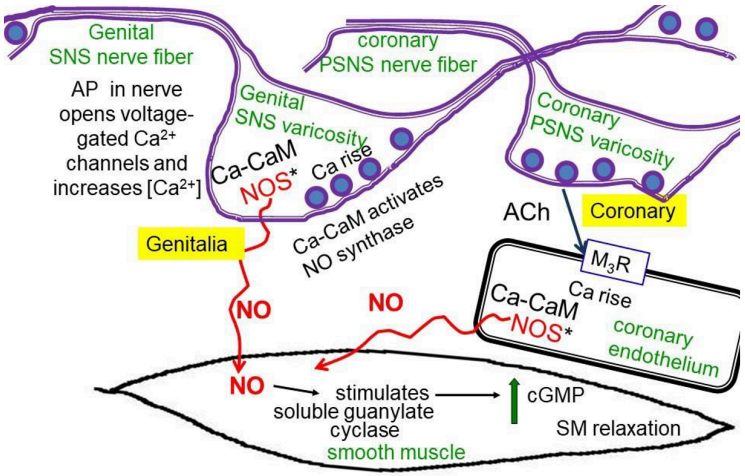

Figure 5: Two sources of nitric oxide synthesis. Calcium-stimulated NOS can make NO inside ANS postganglionic nerve varicosities and in endothelial cells of blood vessels.

Learning Objective #5: *Describe the Baroreceptor reflex in response to high or low blood pressure.*

An autonomic reflex regulating blood pressure

The peripheral somatic system has reflexes such as the familiar tendon-jerk reflex involving a short and involuntary arc through the spinal cord leading to motor output. Similarly the ANS has short and involuntary reflex arcs from peripheral receptors through the CNS and out again through ANS ganglia to ANS effectors (Fig. 4).

We now describe the **Baroreceptor Reflex,** an autonomic reflex that regulates blood pressure. Like all reflexes it has three parts:

- (i) afferent input from sensory receptors,
- (ii) a processing center in the spinal cord or brain, and
- (iii) efferent output, which in the case of the baroreceptor reflex is mediated by efferent activity in sympathetic and parasympathetic nerve fibers and adjusts blood pressure.

> **Note about nomenclature:** *Baro-* and *bary-* mean pressure or weight — as in *barometer* and *bariatric* surgery.

Here's how the baroreceptor reflex works:

1. *Sensory receptor afferent input.* Baroreceptors are stretch-sensitive mechanoreceptors in the walls of the aortic arch and carotid sinus that monitor blood pressure by responding to changes in the tension of the arterial wall. Stretch activates them.
2. *Central processing center.* Afferent input carried by spike activity (action potentials) in baroreceptor axons increases with elevated blood pressure and is sent to the medulla of the brainstem for integration.
3. *Efferent outflow.* Increased input from receptor afferents excites the parasympathetic NS and inhibits the sympathetic NS, which lowers blood pressure.

The baroreceptor reflex stabilizes blood pressure by adjusting the activity of the sympathetic NS and the parasympathetic NS. For example, a drop in blood pressure reduces tension in the walls of the aortic arch and carotid sinus, decreasing excitation of the

stretch sensitive baroreceptors that results in a corrective **increase** in segmental sympathetic outflow and a **decrease** in parasympathetic outflow (in the Vagus nerve) as illustrated in Figure 6.

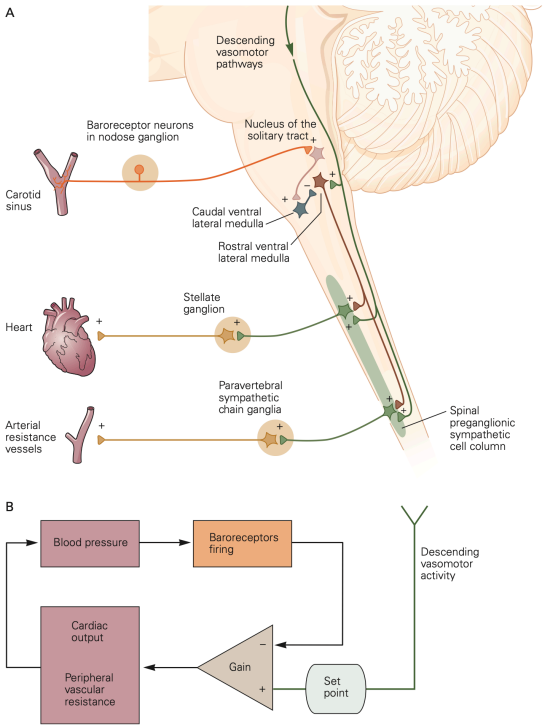

Figure 6: The baroreceptor reflex behaves as a negative feedback loop with gain. **A.** *Arterial blood pressure is sensed by baroreceptor s, a type of stretch receptor neuron, in the carotid sinus near the base of the brain. After integration in the medulla this information provides negative feedback control of the cardiovascul ar system. The sympathetic component of the circuit includes outputs that stimulate the heart's pumping capacity (cardiac output) by increasing heart rate and the strength of contractions. In addition, sympathetic*

stimulation causes arteries to contract, which raises the hydraulic resistance to blood flow. Together the effects of increased cardiac output and increased vascular resistance raise mean arterial blood pressure.

Learning Objective #6: *Define orthostatic hypotension and discuss how the baroreflex counters it.*

Orthostatic hypotension (postural hypotension) is a sudden decrease in blood pressure that occurs when a person quickly assumes a standing position and blood pools down in the lower extremity under the influence of gravity.

Importantly, inhibitory projections from the caudal to the rostral ventral lateral medulla create negative feedback so that an increase. in blood pressure inhibits sympathetic activity, whereas a decrease raises sympathetic activity. Although omitted for simplicity, parasympathetic neurons in the cardiac

Note about nomenclature: *Orthostatic* means "upright posture," and *hypotension* means low blood pressure.

The gravitational redistribution of blood volume reduces blood flow to the heart causing the amount of blood pumped by the heart to decrease, a drop in arterial blood pressure, less flow to the brain, and potential dizziness. The baroreceptor firing rate falls and triggers the sequence of events in the baroreceptor reflex . The increased sympathetic outflow and reduced parasympathetic outflow speed the heart rate and constrict the vessels. This "autonomic reflex" boosts the blood pressure and baroreceptor spike activity in a manner that ultimately restores blood pressure to its normal resting set point. It is involuntary and quick. You are not aware that it is happening except that you may realize that it is good

advice to the elderly to support themselves for a few seconds after getting out of bed or after standing up from a crouching position–like after retrieving objects off a bottom shelf.

ganglion also contribute to the reflex by creating an inhibitory cardiac input that is functionally antagonistic to the sympathetic pathway. During baroreceptor reflexes parasympathetic activity within the heart is therefore increased by hypertension and reduced by hypotension. **B.** *The neurons mediating the baroreceptor reflex behave as a negative feedback loop with gain. By amplifying the activity that provides the signals for cardiovascular control, neurons in this circuit can accurately control blood pressure. In a healthy individual with reflex gain of 8,*

systemic blood pressure can be maintained within 10% of its set point.

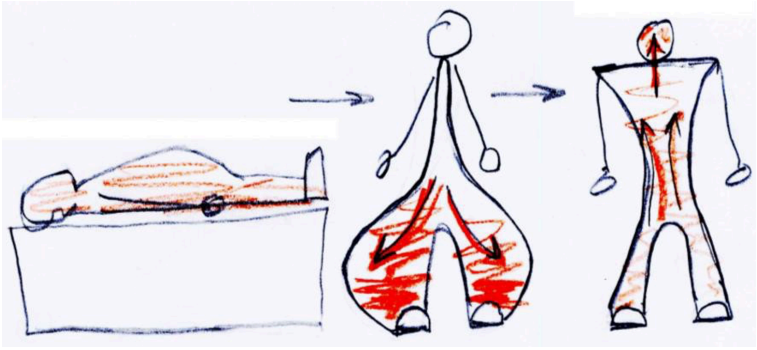

Figure 7: Orthostatic (postural) hypotension. Blood pools in the legs initially upon getting out of bed. The baroreflex helps to restore homeostasis by vasoconstriction in the legs and speeding heart beat.

Conclusion

The autonomic nervous system controls many involuntary actions including regulatory, homeostasis, and housekeeping functions of the body. The sympathetic and parasympathetic branches using NE and ACh, respectively, usually exert opposite physiological effects: flight or fight versus rest and digestion. They can be understood from the different G proteins that are engaged. Many patients arriving in the emergency room need immediate pharmacological intervention of their autonomic function.

Note: *The following two tables can help you begin to learn the types of ANS receptors and their locations in anticipation of ANS pharmacology. To help you work out some of these ideas by logical reasoning, remember that the sympathetic adrenergic effects aid "flight or fright" and the parasympathetic muscarinic effects aid "rest and digestion."*

Table 3: Clinically relevant ANS receptors and their signal-transduction mechanisms (*begin to memorize them*).

Adrenergic:				
Alpha1*	Gq	PKC, Ca^{2+}	Contr./secrete	Smooth muscle/ glands
Alpha2*	Gi	Gβγ- sub-units and -cAMP	varies	On presynaptic side of adrenergic nerve terminals, acts to inhibit subsequent NE release from the terminal–called autoinhibition.
Beta1	Gs	+cAMP	Increase heart rate & contractility	Heart muscle, kidney juxtaglomerular cells (renin release), fat cells
Beta2	Gs	+cAMP	Relax	Smooth muscle, glands & enteric neurons
Beta3	Gs	+cAMP	Relax; prevent urination	Bladder; also fat cells (lipolysis and thermogenesis)
Muscarinic:				
M2	Gi	-cAMP	Slow heart rate	Primarily heart; a few in smooth muscle that cause contraction
M3	Gq	PKC, Ca^{2+}	Contract or relax**	Smooth muscle, salivary glands, eyes, vascular endothelium

*There are 3 distinct alpha1 receptor isoforms (genes) and 3 distinct alpha2 isoforms; the distinctions are beginning to be exploited clinically and you will learn more in later blocks.

** Because the M3 receptor is Gq-coupled and mediates an increase in intracellular calcium, it typically causes contraction of smooth muscle, such as bronchoconstriction. However, in the vasculature, activation of M3 receptors on vascular endothelial cells accelerates synthesis of NO, via Ca^{2+} which diffuses to

adjacent vascular smooth muscle cells and relaxes them (Figure 5), explaining the contrasting parasympathetic effects on vascular tone versus bronchiolar tone. Direct stimulation of vascular smooth muscle M3 receptors mediates vasoconstriction in pathologies that disrupt the vascular endothelium. M3 receptors stimulate secretion in many glands, including the salivary glands and lacrimal glands.

Table 4: Effector organs and ANS innervation. (*Use this table to interpret actions of different pharmaceutical agents.*)

Effector organ	Parasympathetic action	Target muscarinic receptor	Sympathetic action
Eye			
1) Lacrimal glands	Secretion	M2/M3	–
2) Pupil sphincter (para) or dilator/radial (symp)	Constriction	M3	Dilation
Ciliary muscle (para) Ciliary process / Aqueous humor	Contraction –	M3 –	– Increase flow
Heart rate and output	Decrease in rate	M2, M4	Increase in rate & contractility
Arterioles GI tract, genitals Almost all others In Skeletal muscle	Dilate/ relax – –	*M3 / via nitric oxide (NO)	Constrict Constrict Relax
Lungs Bronchial muscle	Constrict	M3	Dilate
GI Tract Mobility and secretion Sphincters	Increase/excite Relax	M3, M1 M3	Decrease/inhibit Constrict
Sweat glands	–		Secretion
Adrenal Medulla	–		Secretion
Bladder Bladder wall	Contract	M3	Relax
Reproductive organs	Erection, vaginal lubrication	*M3 / via NO	Ejaculation
Exocrine glands	Salivation	M1, M3, M4	

*__Nitric oxide__'s (__NO__) role in arteriole dilation is discussed in footnote to Table 3 and in Figure 5.

**Note two non-adrenergic exceptions for sympathetic receptors: nAChR is the type of receptor found on all postganglionic neurons of the ANS (different isotype from nAChRs found on skeletal muscle). Sweat glands have either mAChRs or adrenergic receptors, not both.

Nociception and Spinal Reflexes

JOHN TUTHILL

Summary: Nociception and Spinal Reflexes

Nociceptors are sensory neurons that respond to physical stimuli strong enough to cause tissue damage. They innervate every tissue of the body except, ironically, the brain. Nociceptors are classified by the type of physical stimuli (i.e., modality) that they respond to, such as pressure, heat, or cold. Their activity gives rise to the perception of pain.

A **spinal reflex** is a rapid activation of muscle produced by the stimulation of sensory receptors. Spinal reflexes are mediated by nerve cells in the peripheral nervous system (**sensory neurons**) and the spinal cord (**spinal interneurons**, cells confined to the spinal cord and **motor neurons**, the cells that innervate skeletal muscle). Unlike planned, intentional movements, reflexes are involuntary and can be evoked without the involvement of the brain.

The simplest spinal reflex is called the **tendon jerk or knee jerk** and it is widely used in neurological examinations to examine the integrity of sensory and motor functions of the spinal cord. This spinal reflex is mediated by actions of just two populations of neurons: the primary sensory receptors of a sense organ called the **muscle spindle** and **motor neurons,** the cells that activate skeletal muscle fibers.

Application of a noxious stimulus to the skin results in widespread and powerful activation of ipsilateral flexor muscles that generally produces a withdrawal of the limb away from the stimulus, called the **flexion reflex**. The opposite reaction, called the **crossed-extension reflex,** is often observed in the contralateral limb.

Lesions in the brain or spinal cord and several disease processes can be manifested in excessive spinal reflexes and spasticity.

Learning Objectives

1. To know how nociceptors are activated and sensitized.
2. To understand the difference between reflexes and other types of movements.
3. To differentiate the neural circuits underlying the flexion and stretch reflexes.
4. To understand how reflex testing can be used clinically to diagnose neuro-pathologies that affect motor and sensory function.

(Unless otherwise noted, all figures are from: Kandel ER, Schwartz JH, Jessell TM 2012, Siegelbaum SA, Hudspeth AJ. 'Principles of Neural Science, 5th ed. *McGraw-Hill, New York.*)

Learning Objective #1: *To know how nociceptors are activated and sensitized.*

Nociceptors are classified by the type of physical stimuli (i.e., modality) that they respond to. Some nociceptors respond to more

than one modality and are referred to as **polymodal nociceptors**. Others, called sleeping or silent nociceptors, normally do not respond to any type of stimulus, but become activated under conditions of inflammation or tissue damage.

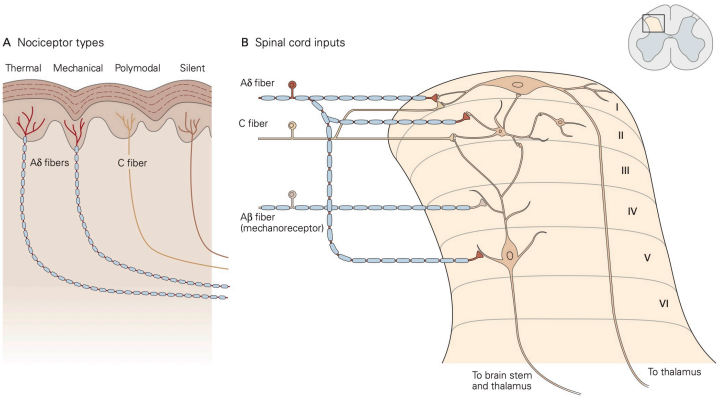

Figure 1: Nociceptive fibers terminate in the dorsal horn of the spinal cord. **A.** *Peripheral nociceptor classes.* **B.** *Neurons in lamina I of the dorsal horn receive direct input from myelinated (Aδ) nociceptive fibers and both direct and indirect input from unmyelinated (C) nociceptive fibers via interneurons in lamina II. Lamina V neurons receive low- threshold input from large-diameter myelinated fibers (Aβ) of mechanoreceptors as well as inputs from nociceptive afferent fibers (Aδ and C fibers). Lamina V neurons send dendrites to lamina IV, where they are contacted by the terminals of Aβ primary afferents. Dendrites in lamina III arising from cells in lamina V are contacted by the axon terminals of lamina II interneurons. Aα fibers innervate motor neurons and interneurons in the ventral spinal cord (not shown).*

Thermal nociceptors are activated by noxious (i.e., tissue damaging) hot or cold temperatures within the receptive field acting on **TRP channels**. **TRPV1 channels** are expressed on thermal nociceptors that respond to heat/pain temperatures above 42 °C. Other temperatures in the warm–hot range are transduced by different thermal nociceptors that express a different TRP channel. Cold stimuli are sensed by thermal nociceptors that express **TRPM8** channels. An interesting finding related to cold stimuli is that tactile

sensibility and motor function deteriorate while pain perception persists.

Mechanical nociceptors respond to excessive mechanical deformation of the tissue they innervate. They also respond to wounds that break the epidermal surface. These mechanical nociceptors that also express TRP channels frequently have polymodal characteristics, responding to thermal stimuli and chemical stimuli.

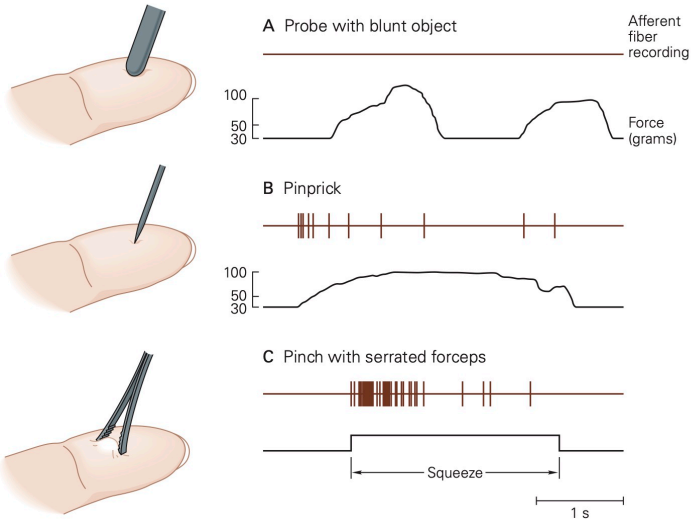

Figure 2: Mechanical nociceptors respond to stimuli that puncture, squeeze, or pinch the skin. Sensations of sharp, pricking pain result from stimulation of Aδ fibers with free nerve endings in the skin. These receptors respond to sharp objects that puncture the skin (B) but not to strong pressure from a blunt probe (A). The strongest responses are produced by pinching the skin with serrated forceps that damage the tissue in the region of contact (C).

Chemical nociceptors express TRP channels that respond to a wide variety of molecules including spices like capsaicin (8-methyl-N-vanillyl-6-nonenamide), the active, hot sensation

producing substance in chili peppers and environmental irritants like acrolein, a component of cigarette smoke. Chemical nociceptors also respond to endogenous ligands, and certain fatty acid amines that arise from damage to internal tissues.

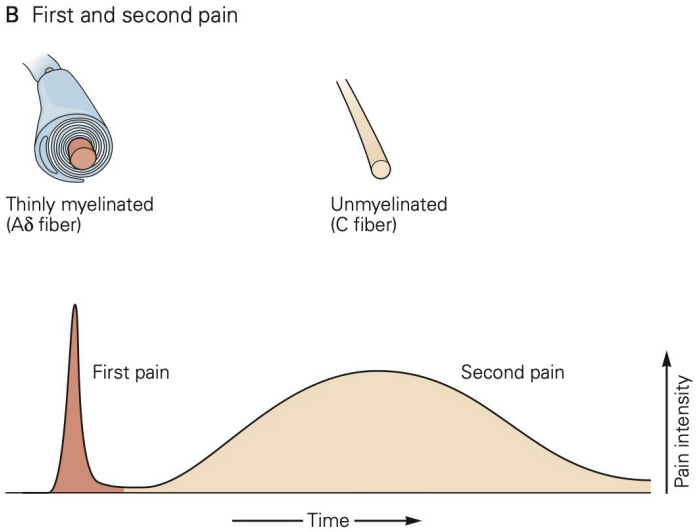

Figure 3: First and second pain are carried by two different primary afferent fibers.

Individual nociceptors are grouped into two different classes, which have distinct types of axons: **A-∂ fibers** are myelinated and conduct action potentials quickly, within 10-30 m/s, while unmyelinated **C-fibers** conduct action potentials more slowly, from 0.5 – 2 m/s. Due to these differences in axonal conduction speed, nociception and the **affective perception of pain** often come in two phases: The first phase, initial sharp and localized pain, is mediated by the fast-conducting Aδ fibers and the second phase, continuous, long-lasting and more diffuse pain, is generated by activation of the slower-conducting C-fibers.

Response of nociceptors to tissue damage

Exposure to noxious stimuli often results in tissue damage. When this occurs, the plasma membrane of individual cells is breached and proteases are released. Proteases cleave proteins and produce peptides, including one termed **bradykinin**, a very potent pain-inducing substance. Cleaved bradykinin acts by binding to its receptors: B2 and B1. B2 receptors are constitutively expressed and are important in mediating pain detection and vasodilation. B1 receptors are upregulated in damaged tissue and play an important role in chronic pain. Bradykinin receptors on nerve terminals, especially free nerve endings, will depolarize in response to bradykinin binding.

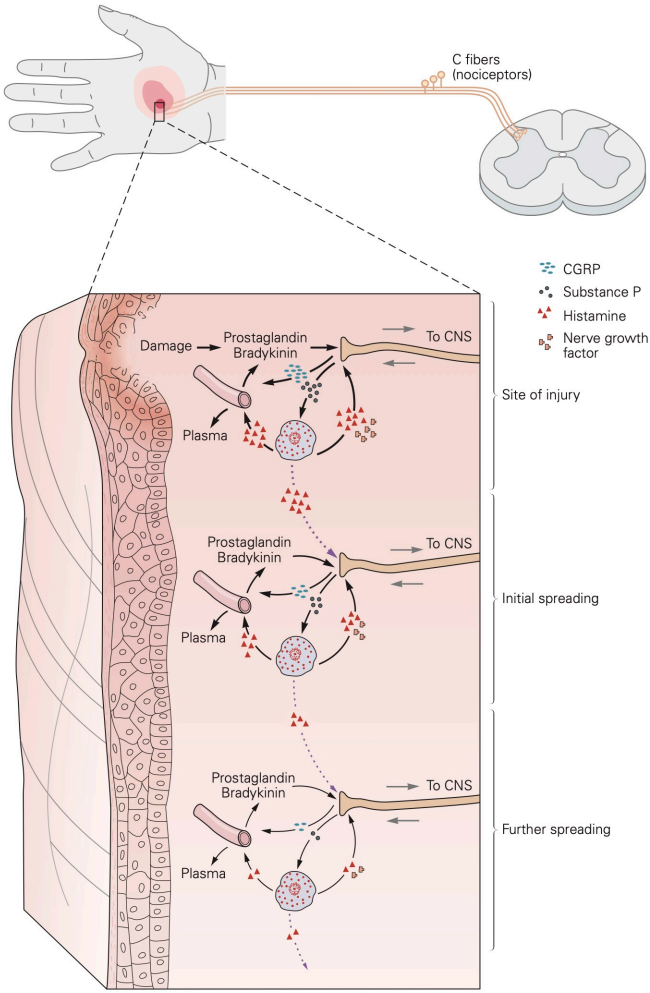

Figure 4: Injury or tissue damage releases bradykinin and prostaglandins, which activate or sensitize nociceptors. Activation of nociceptors leads to the release of substance P and CGRP (calcitonin gene–related peptide). Substance P acts on mast cells in the vicinity of sensory endings to evoke degranulation and the release of histamine, which directly excites nociceptors. Substance P produces plasma extravasation, and CGRP produces dilation of peripheral blood vessels; the resultant edema causes additional liberation of bradykinin. These mechanisms also occur in healthy tissue, where they cause secondary or spreading hyperalgesia.

Sensitization of nociceptors

The responsiveness of nociceptors to stimulation can be modified, a process called **sensitization**, by a number of substances that interact with the receptive membrane. In the extreme, a nociceptor can be transformed from a noxious stimulus detector into one that responds to innocuous stimuli as well. When sensitized in this way, low intensity, innocuous stimuli associated with regular activity, can activate nociceptors and thus, generate a painful sensation. This is commonly known as **hyperalgesia**. Inflammation is one common cause of nociceptor sensitization. Normally hyperalgesia desists when the inflammation is resolved. Occasionally, however, repeated injury or chronic use of opioid analgesics can result in **allodynia,** a condition in which a completely non-noxious stimulus like light touch causes extreme pain. Allodynia can also be caused when nociceptor afferent fibers are damaged in the peripheral nerves.

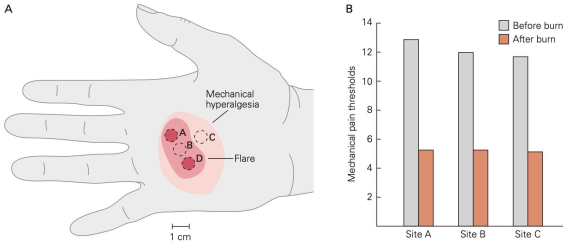

Figure 5: Hyperalgesia results from sensitization of nociceptors. **A.** *Mechanical thresholds for pain were recorded at sites A, B, and C before and after burns at sites A and D. The areas of reddening (flare) and mechanical hyperalgesia resulting from the burns are shown on the hand of one subject. In all subjects the area of mechanical hyperalgesia was larger than the area of flare. Mechanical hyperalgesia was present even after the flare disappeared.* **B.** *Mean mechanical pain thresholds before and after burns. The mechanical threshold for pain is*

significantly decreased after the burn.

Learning Objective #2: *To understand the difference between reflexes and other types of movements.*

Learning Objective #3: *To differentiate the neural circuits underlying the flexion and stretch reflexes.*

Stimulation of nociceptors triggers a reflexive withdrawal of the affected body part away from the stimulus. A **reflex** is a rapid activation of muscle produced by the stimulation of sensory receptors. **Spinal reflexes** are mediated by nerve cells in the peripheral nervous system (**sensory neurons**) and the spinal cord (**spinal interneurons**, cells confined to the spinal cord and **motor neurons**, the cells that innervate skeletal muscle). Unlike planned, intentional movements, reflexes are involuntary and can be evoked without the involvement of the brain. Importantly, many reflexes are flexible and can be altered depending on body posture and behavioral context.

Flexion reflex

Application of a noxious stimulus to the cutaneous surface results in widespread and powerful activation of ipsilateral flexor muscles that generally produces a withdrawal of the limb away from the stimulus. One can also evoke a weak contraction of the same flexor muscles located in a given area by probing the skin with a sharp-pointed probe. Both of these responses are referred to as the **flexion reflex**.

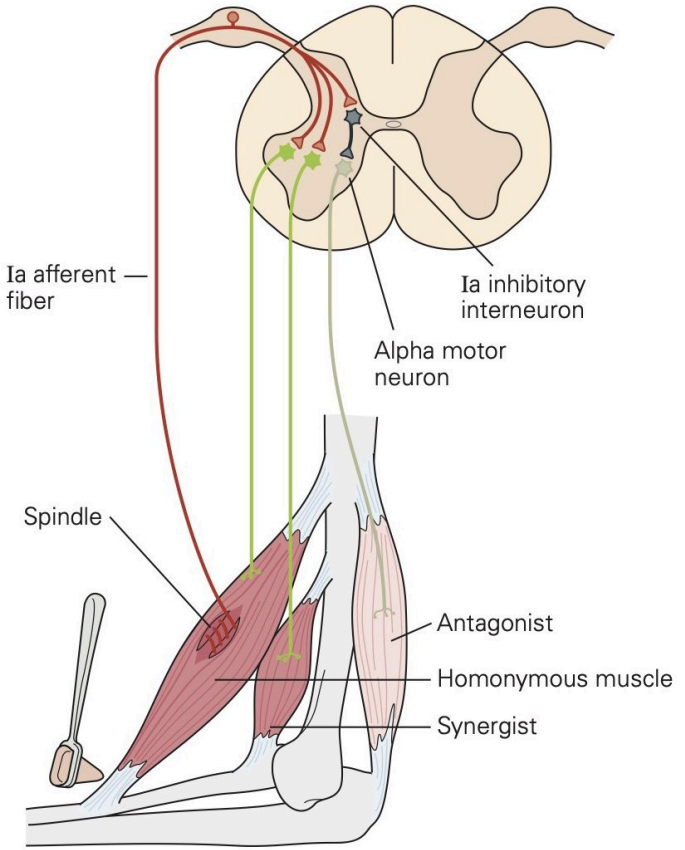

Figure 6: Monosynaptic pathways mediate stretch reflexes. Afferent axons from muscle spindles make excitatory connections on two sets of motor neurons: alpha motor neurons that innervate the same (homonymous) muscle from which they arise and motor neurons that innervate synergist muscles. They also act through interneurons to inhibit the motor neurons that innervate antagonist muscles. When a muscle is stretched by a tap with a reflex hammer, the firing rate in the afferent fiber from the spindle increases. This leads to contraction of the same muscle and its synergists and relaxation of the antagonist. The reflex therefore tends to counteract the stretch, enhancing the spring-like properties of the muscles.

The reason that both noxious and innocuous stimuli can evoke flexion reflexes is that the sensory neurons associated with different modalities converge onto common interneurons in the spinal cord. The heterogeneous groups of sensory afferent fibers that evoke flexion reflexes are called the **flexor reflex afferents** (FRA). The FRA include A-b, A-∂ and C-fibers that innervate mechanoreceptors, chemoreceptors, temperature receptors and nociceptors found in virtually all of our tissues. The FRA generate excitatory postsynaptic potentials (**EPSPs**) that lead to action potentials in the spinal interneurons they contact synaptically. Some of these spinal interneurons generate EPSPs in **flexor motor neurons** and other spinal interneurons generate inhibitory post-synaptic potentials (**IPSPs**) in **extensor motor neurons.**

Crossed-extension reflex

Some of the interneurons that receive excitation (EPSPs) from the FRA send collaterals of their axons to the contralateral segment of the cord via the anterior commissure. These collaterals excite other interneurons which in turn influence the excitability of both flexor and extensor motor neurons. In general, the net effects are excitation (EPSPs) of contralateral extensor motor neurons and inhibition (IPSPs) of contralateral flexor motor neurons, often resulting in what is called the **crossed-extension reflex.** One might reasonably speculate that this reflex pattern evolved to facilitate the contralateral limb's assuming additional weight bearing in the event of a limb withdrawal from a harmful stimulus.

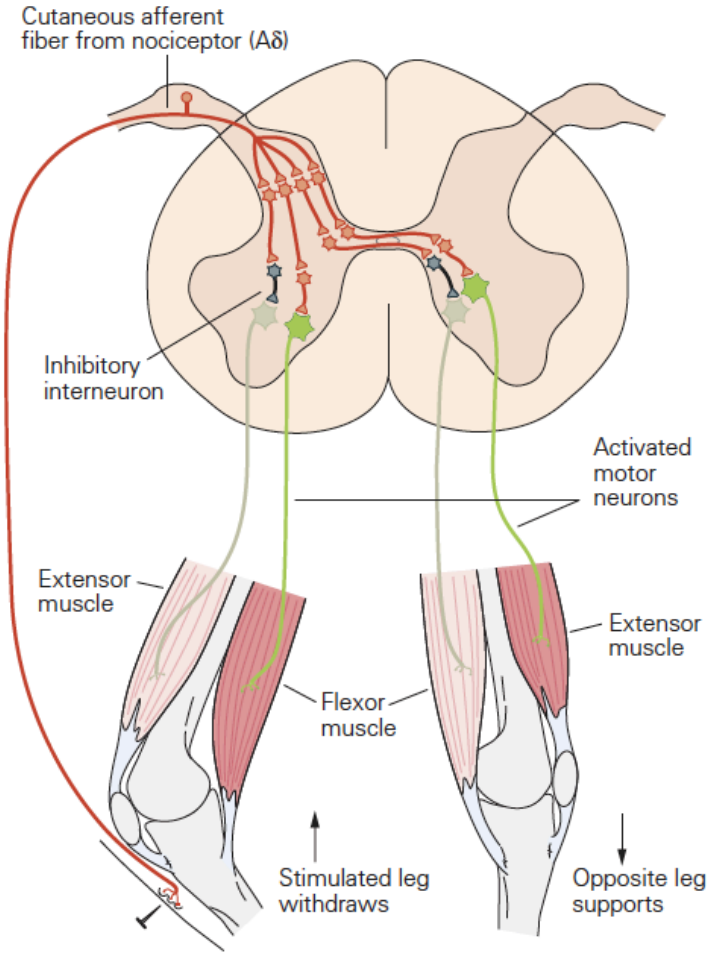

Figure 7: Polysynaptic pathways in the spinal cord mediate flexion and crossed-extension reflexes. *One excitatory pathway activates motor neurons that innervate ipsilateral flexor muscles, which withdraw the limb from noxious stimuli. Another pathway simultaneously excites motor neurons that innervate contralateral extensor muscles, providing support during withdrawal of the limb. Inhibitory interneurons ensure that the motor neurons supplying antagonist muscles are inactive during the reflex response.*

Learning Objective #4: *To understand how reflex testing can be used clinically to diagnose neuropathologies that affect motor and sensory function.*

Tendon-jerk/knee-jerk reflex

The simplest spinal reflex is called the **tendon jerk or knee jerk** and it is widely used in neurological examinations to examine the integrity of sensory and motor functions of the spinal cord. This spinal reflex is mediated by actions of just two populations of neurons: the primary sensory receptors of a sense organ called the **muscle spindle** and the a-**motor neurons,** the cells that activate skeletal muscle fibers. The *neural circuit* of the tendon jerk is outlined in the figure below.

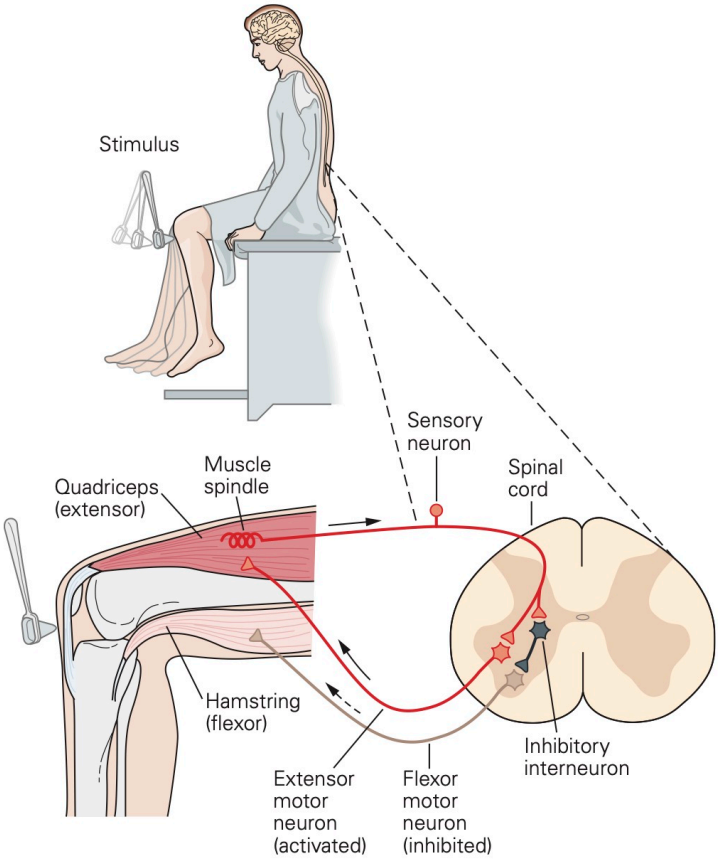

Figure 8: The knee-jerk reflex is controlled by a simple circuit of sensory and motor neurons. Tapping the kneecap with a reflex hammer pulls on the tendon of the quadriceps femoris, a muscle that extends the lower leg. When the muscle stretches in response to the pull of the tendon, information regarding this change in the muscle is conveyed to the central nervous system by sensory neurons. In the spinal cord the sensory neurons form excitatory synapses with extensor motor neurons that contract the quadriceps, the muscle that was stretched. The sensory neurons act indirectly, through interneurons, to inhibit flexor motor neurons that would otherwise contract the opposing muscle, the hamstring. These actions combine to produce the reflex behavior. In the drawing each extensor and flexor motor neuron represents a population of many cells.

The tendon jerk reflex can be easily assessed in patients. Applying a brisk tap to the patellar tendon with a reflex hammer just below the knee normally results in a small contraction of the quadriceps muscles that extends or jerks the knee slightly. The mechanism underlying this reflex is as follows: The tendon tap produces a small, rapid stretch of the quadriceps muscles. Muscle stretch is the adequate stimulus for the **primary or annulospiral sensory receptors** of the muscle spindles. There are many muscle spindles scattered throughout the muscles, and each spindle has one annulospiral receptor. The muscle stretch evokes a depolarizing receptor potential from annulospiral receptors of the muscle spindles within the quadriceps muscles. These depolarizing receptor potentials result in a few action potentials that are propagated to the spinal cord by the **group Ia sensory neurons** that terminate as synaptic contacts, called 'boutons' on neurons in the spinal cord.

Each of the group Ia afferent fibers gives rise to many synaptic boutons in the spinal cord that contact all of the alpha motor neurons innervating the quadriceps muscles. The group Ia fiber synaptic boutons produce EPSPs in these alpha-motor neurons. The EPSPs depolarize some of these alpha motor neurons to threshold causing them to generate action potentials. The action potentials are then propagated to the muscle resulting in a twitch contraction that extends the knee.

The neural circuit mediating the tendon jerk is referred to as a **monosynaptic pathway** because there is only one synapse between the afferent (group Ia afferent fiber) and efferent (alpha motor neuron) elements of the circuit. Most neural circuits are multi- or polysynaptic, like the flexion and crossed- extensor reflex spinal circuits that were outlined earlier.

The same population of group Ia afferent fibers that produces the tendon jerk reflex also makes direct (monosynaptic) connections with alpha motor neurons that innervate **synergist muscles**, i.e., muscles that exert a similar mechanical action on the same joint. However, the group Ia afferent fibers make fewer contacts with

these synergist motor neurons, such that the EPSPs generated in them are smaller than those generated in their own (called *homonymous*) motor neurons. The resulting depolarization in the synergist motor neurons fails to bring them to threshold and, thus, normally no reflex contraction occurs in the synergist muscles.

One can also measure tendon jerk reflexes by recording **electromyographic (EMG)** signals from muscles in response to a tendon tap. The EMG is a consequence of the action potentials generated by the active muscle fibers.

H-reflex

Group Ia afferent fibers can also be stimulated electrically in human subjects when the peripheral nerve in which they are contained lies just below the skin surface. In general, the group Ia afferent fibers are slightly larger in diameter than the axons of the a motor neurons in the same nerve and, thus, they have a lower threshold to an externally applied electrical shock. Applying a single shock through electrodes placed over the popliteal fossa, for example, produces a brisk reflex contraction of the soleus muscle, which closely resembles the response elicited by a tendon tap stimulus. This technique evokes what is called the **H- or Hoffman Reflex**. H-reflex testing is widely used both in the laboratory and the clinic. The H-reflex can be measured by recording an EMG signal from either surface electrodes or intramuscular electrodes. (As the strength of the electrical stimulus is increased, the H-reflex is preceded by an **M-wave** that results from direct activation of the a-motor neuron axons in the same peripheral nerve.)

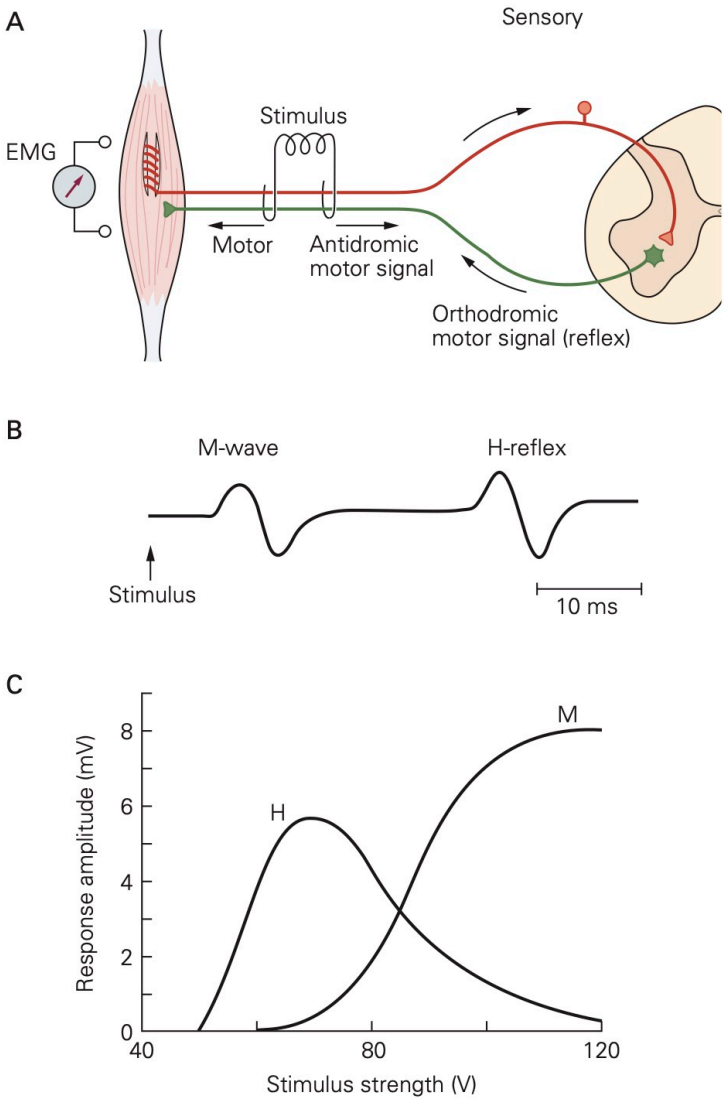

Figure 9: The Hoffmann reflex. **A.** *The Hoffmann reflex (H-reflex) is evoked by electrically stimulating Ia sensory fibers from muscle spindles in mixed nerves. The sensory fibers excite alpha motor neurons, which in turn activate the muscle. Muscle activation is detected by the electromyogram (EMG).* **B.** *At intermediate stimulus strengths motor axons in the mixed nerve are excited in addition to the spindle afferents. Excitation of the motor neurons produces an M-wave that precedes the H-wave (H-reflex) in the EMG.* **C.** *At low*

stimulus strengths only an H-wave is produced because only the spindle afferents are excited. As the stimulus strength increases, the magnitude of the H-reflex also increases, then declines, because the orthodromic motor signals generated reflexively by the spindle affer- ents are cancelled by antidromic signals initiated by the electrical stimulus in the same motor axons. At very high stimulus strengths only an M-wave is evoked.

Reciprocal innervation

As a general rule, afferent fibers that excite one group of alpha motor neurons inhibit alpha motor neurons that innervate antagonist muscles. Antagonist muscles are those with opposing actions across a joint (i.e., flexion vs. extension; adduction vs. abduction). This common pattern has been referred to as reciprocal innervation.

The simplest inhibitory pathway is **di-synaptic**, that is, there are two synapses interposed between the afferent fibers and the a-motor neurons. For example, a Ia afferent fiber originating from a muscle spindle in a biceps muscle makes synaptic contacts with the biceps motor neurons and with a group of spinal interneurons in the spinal cord. (A spinal interneuron is a cell whose soma, axon and dendrites are contained completely within the spinal cord.) The synaptic boutons of the group Ia afferent fiber generate EPSPs in both the alpa motor neurons and these spinal interneurons. This excitation leads to the generation of action potentials in some of the interneurons. The interneurons in turn make synaptic contacts with alpha motor neurons innervating the antagonist triceps muscles. The interneurons produce IPSPs in the triceps motor neurons and, thus, inhibit the activation of these muscles. These interneurons are called **Ia inhibitory interneurons** because they are activated by group Ia afferent fibers and inhibit a-motor neurons.

Spasticity and the clasp-knife reflex

Spasticity is a condition characterized by **hypertonicity** (increased muscle tone), **clonus** (a series of rapid muscle contractions), exaggerated tendon jerk reflexes, involuntary muscle spasms, scissoring (involuntary crossing of the legs), and stiff joints caused by co-contraction of antagonist muscles. The degree of spasticity varies from mild muscle stiffness to severe, painful, and uncontrollable muscle spasms. Spasticity can interfere with rehabilitation in patients with certain disorders, and often interferes with daily activities. Spasticity is usually caused by damage to brain or spinal neurons involved in the control of movement. It may occur in association with spinal cord injury, multiple sclerosis, cerebral palsy, anoxia, brain trauma, severe head injury, and diseases such as adrenoleukodystrophy, amyotrophic lateral sclerosis (ALS), and phenylketonuria. Inferences drawn from an animal model suggest that one of the principal mechanisms of spasticity is an increase in the excitability of motor neurons consequent to the lesion in the brain or spinal cord. The increased excitability results in sustained repetitive discharge of a-motor neurons in response to brief stimuli.

The **clasp-knife reflex** (also called the **inverse myotatic reflex**) is a pathological reflex that is only observed following lesions of the central nervous system that produce spasticity. In response to a rapid muscle stretch, a patient with spasticity first exhibits a strong stretch reflex, followed by a relaxation of the muscle. It is the sudden release of muscle tension when a contracting muscle is forcibly stretched that gives the reflex its name: The spastic limb initially resists flexion (because of the stretch reflex) and then collapses on itself like the blade of a jack- or clasp-knife. The reflex is thought to be mediated by A-b and A-d sensory neurons with free nerve ending receptors located in muscle fascia and aponeuroses. The spinal circuits responsible for the clasp-knife reflex are

normally suppressed and only become operational after lesions to the spinal cord or brain.

Muscle Physiology

CHARLES ASBURY

Introduction: Why is muscle physiology important?

You may see patients with muscle-related diseases and you may prescribe treatments that act directly on muscles. Muscle physiology can also have a major impact on surgery and interventions that are intended to treat other organs. In addition, a wide variety of medical problems, including ionic disturbances, exposure to toxins or drugs, and congenital disorders can lead to detectable changes in muscle function, which therefore serve as diagnostic clues. Understanding how electrical excitation of muscle leads to its contraction will also help you learn about neurophysiology, signal transduction pathways, and motor proteins.

Session Learning Objectives

1. Explain the mechanism by which muscle contracts, outlining how the sliding of actin filaments in sarcomeres is driven by ATP-dependent chemo-mechanical cycling of myosin motor proteins.
2. Explain excitation-contraction coupling and

relaxation in skeletal muscle by identifying the roles of the t-tubules, calcium channels ($Cav_{1.1}$ and the ryanodine receptor), thin filament regulators (troponin and tropomyosin), and ATP-dependent calcium pumps.

3. Compare twitch contractions for slow/type 1 and fast/type 2 skeletal muscle fibers and explain the molecular bases for the differences in twitch behavior. Define isometric and isotonic contractions.
4. Explain how smooth, graded contractions of a skeletal muscle are produced by changes in stimulus intensity and by the size principle of motor unit recruitment.
5. Understand the differences in excitation-contraction coupling between skeletal, cardiac, and smooth muscle. Describe the two-stage phospho-regulatory cascade that initiates smooth muscle contraction.
6. Compare and contrast how skeletal, cardiac and smooth muscle are controlled by the nervous system. Define single-unit vs. multi-unit smooth muscle types.

Muscle generates force by contracting against external load. There are three main types of muscle, **skeletal**, **cardiac**, and **smooth**. All three use the same fuel source, ATP, which supplies chemical energy that muscles convert into mechanical work and heat. All three share the same fundamental mechanism for generating force. However, they vary in their morphology, physiology, biochemical properties, and mechanics. Here you will begin to distinguish similarities and differences in how they function (in their physiology). We will start by reviewing skeletal muscle.

Note about muscle nomenclature: Some familiar cell structures are given muscle-cell-specific names. It will be useful to know these terms:

- *sarcolemma* = muscle outer (plasma) membrane (*sarco = Greek for "flesh"*; *lemma = "husk"*)
- *sarcoplasm* = muscle cytoplasm
- *sarcoplasmic reticulum* = specialized endoplasmic reticulum in muscle; stores Ca^{2+}

Learning objective #1. *Explain the mechanism by which muscle contracts, outlining how the sliding of actin filaments in sarcomeres is driven by ATP-dependent chemo-mechanical cycling of myosin motor proteins.*

The contraction machinery in skeletal muscle is organized hierarchically

Recall from your prior histology sessions that skeletal muscles are organized in a hierarchical manner, from the whole organ down to the individual molecules that drive its contraction (see Figure 1 and notes from Muscle Histology). Their organization gives them a striated appearance in the light and electron microscopes. It is also important for their function.

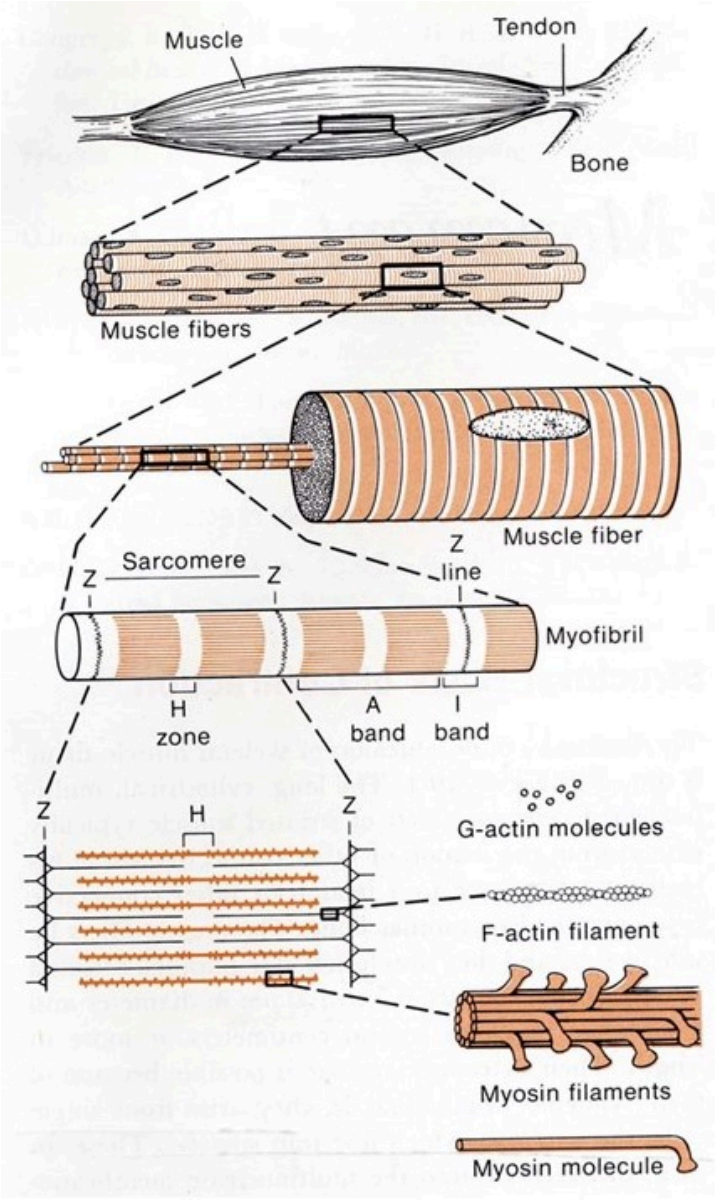

Figure 1. Hierarchical structure of skeletal muscle. *(From Eckert, Animal Physiology)*

Each whole muscle is composed of hundreds of muscle **fibers**, roughly ten to twenty micrometers in diameter. Each fiber is a single multi-nucleated cell, surrounded by a plasma membrane (for muscle cells this is often given a special name, the **sarcolemma**) and containing nuclei, mitochondria, and other organelles common to all living cells. Every muscle fiber contains hundreds of **myofibrils**, each about a micrometer in diameter. Every myofibril is a series of **sarcomeres**, linked end-to-end down the entire muscle fiber. Inside each sarcomere is a set of hundreds of parallel **thick** and **thin filaments** (Figure 2). The thin filaments extend into the sarcomere from the left and right sides. The thick filaments extend outward from the centerline, and these contain the primary force-generating molecules, called **myosin**.

Muscle shortening is driven by relative sliding of thick and thin filaments

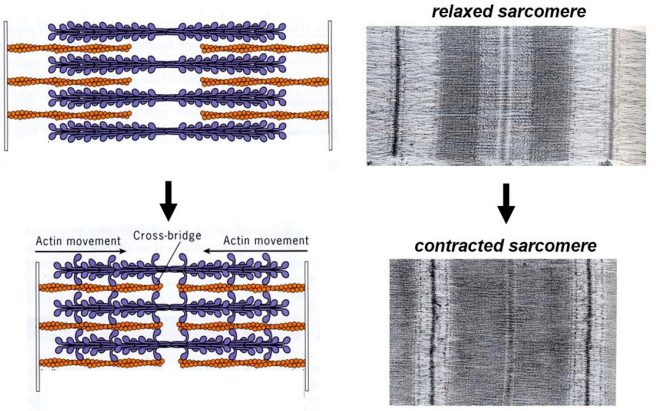

Figure 2. Sarcomeres, fundamental contractile units. Interactions between thick and thin filaments drive filament sliding. Thick and thin filaments are drawn in blue and orange, respectively, in the drawings at left. The images at right show real sarcomeres viewed by electron microscopy. (From Karp, Cell & Molecular Biology)

Normally the thin and thick filaments overlap. When a muscle cell contracts, the amount of overlap between thick and thin filaments increases and the thick filaments in one sarcomere move closer to those in the next sarcomere of the series (i.e., the dense striations move closer together, as can be seen in the electron microscope – see Figure 2). Muscle contraction is driven by interactions between the thick and thin filaments that drive sliding of the two sets of filaments relative to one another. The thick filaments literally *crawl* along the thin filaments, and that crawling generates active force.

Biophysics note: Sarcomeres contain *active* contractile elements and *passive* elastic elements. The active elements convert stored chemical energy (in the form of ATP) into mechanical work, defined as force acting through distance. The passive elements only become tense when stretched, behaving like rubber bands. A consequence of the sliding filament mechanism is that the amount of *active* force generation is proportional to the amount of overlap between thick and thin filaments, which decreases as the sarcomeres are stretched. (Caution: this proportionality applies *only* to the *active* force. The force of *passive* stretching will increase, rather than decrease, as the muscle is stretched. The *total* force generated by a muscle will be the *sum* of active and passive components. Later, in the Circulatory Systems block, you will learn that passive stretching makes a major contribution to the force generation of cardiac muscle, which is greatest when the heart tissue is most stretched.)

High-resolution electron micrographs show tiny projections from the thick filaments that often bridge across and attach to an

adjacent thin filament. In some images, these **cross-bridges** appear to tilt all in the same direction (particularly when the muscle is in "**rigor**", i.e. lacking ATP and therefore tightly locked up). Tilted cross-bridges led Hugh Huxley to propose (in 1969) that they drive filament sliding by cyclically attaching to a thin filament, tilting, detaching, and then un-tilting. The tilting of cross-bridges around a hinge point within the myosin head is now well-proven.

Key molecules of the sarcomere

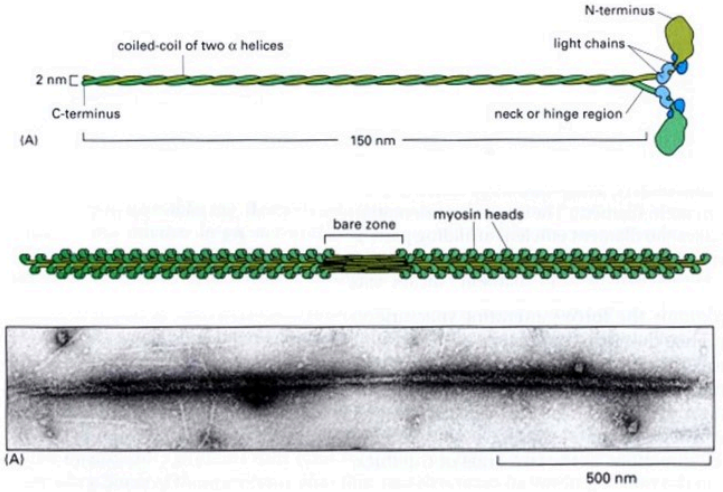

Figure 3. Thick filaments are primarily made of myosin. Upper drawing shows a single myosin rod, composed of heavy and light chains. Below is drawn a thick filament assembled from about 300 myosin rods and also shown is an electron micrograph of an actual, isolated thick filament (note the much different scale). (From Alberts, Molecular Biology of the Cell)

The thick filaments are composed primarily of the protein **myosin** (Figure 3). Myosin is made of three protein chains that form a six-member, rod-like complex (two copies of each chain). The largest

protein chain (sometimes called the 'heavy chain') forms a long coiled-coil with twin globular 'head' domains at one end. Smaller protein chains ('light chains') bind at the junction between the coiled-coil rod and the heads (these form the 'lever arm', that rotates, as described below). To form one thick filament, several hundred myosin molecules assemble in a bipolar fashion, with their coiled-coil rods packed together in a bundle, and with their globular heads sticking out from the surface of the bundle at regular intervals. These heads sticking out from the bundle are the cross-bridges that drive sarcomere shortening by cyclic interactions with the thin filaments. Another element of the thick filament is **titin** (Figure 4). Titin is a very long and elastic protein that binds along each thick filament and extends beyond the tip of each thick filament to the side/end of the sarcomere (to the **Z-discs**). Even when a muscle is relaxed, titin provides it with passive elasticity, and titin also keeps the thick and thin filaments in register with one another, especially when their degree of overlap is low during a strong muscle stretch.

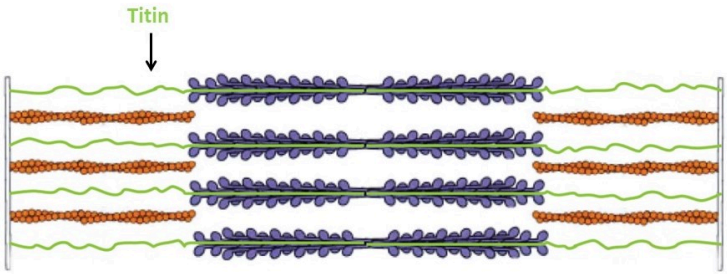

Figure 4. Muscles contain active and passive elements. Depiction of where the elastic molecule, titin, is found in a sarcomere.

The thin filaments are composed primarily of actin. Actin is a "globular" (i.e., roughly spherical) protein that self-assembles into a helical polymer. The globular form is sometimes called G-actin, and the polymeric form, F-actin (F for "filamentous"). To form F-

actin, hundreds of G-actin subunits pack together into two rows that gently wrap around one another, in a rope-like arrangement. Thin filaments also contain some additional elements besides actin. Of these, **tropomyosin** and **troponin** are the most important. Tropomyosin is a rod-like protein that binds along the groove of F-actin. Troponin is a globular protein that binds periodically on the thin filament, interacting with both the actin and the tropomyosin. Tropomyosin and troponin regulate skeletal muscle contraction, and we will discuss them in some detail in the section on excitation contraction coupling, below.

Atomic-level structures of myosin fragments suggest a detailed picture of myosin's cyclical action (Figure 5). Each myosin head contains a lever-arm between the actin-binding portion and the coiled-coil rod. The lever arm is found in a very different orientation in ATP- versus ADP-bound structures, suggesting that it undergoes a large rotation during the myosin cycle. Biochemical experiments show that myosin binds more tightly to actin in the absence of nucleotide (or in the presence of ADP) than with ATP. These observations together with the atomic structures provide strong evidence supporting the tilting cross-bridge model for muscle contraction.

Chemo-mechanical cycle of myosin

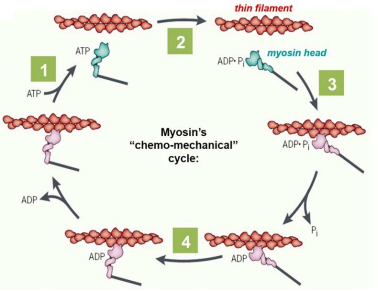

Figure 5. Rotation of myosin's lever arm is coupled to ATP hydrolysis.

The lever-arm motion of a myosin head (cross-bridge) is tightly coupled to the hydrolysis of ATP, through its "chemo-mechanical cycle" (Figure 5). The nucleotide (i.e., ATP or ADP) participates in several key transitions in the cycle: First, (1) ATP binding to a myosin head triggers detachment of the myosin from the actin filament. Next, (2) hydrolysis of the bound ATP converts the head into a pre-stroke state, in which its lever arm adopts a mechanically strained configuration. It is literally 'cocked' into a high energy state, similar to the firing pin of a gun. A myosin head in the pre-stroke state has a higher affinity for actin. (3) Attachment of the head to actin triggers release of ADP and phosphate, which in turn (4) triggers the 'power stroke', when the lever arm rotates back to its low energy configuration. This power stroke drives movement of the actin filament and generates force. The chemo-mechanical cycle of myosin is diagrammed in Figure 5 and animated in the 2-minute video below (*includes narration*).

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Biophysics side note: *How much force does an individual myosin molecule need to generate to produce the strength of a typical muscle?* To estimate this unitary force, the number of myosins acting together in parallel must be estimated. Cross-sections through skeletal muscle viewed by electron microscopy show ~400 thick filaments per square μm. A muscle 5 cm in diameter would therefore have 800 *billion* (800×10^9) thick filaments acting in parallel (i.e., 2.5×10^4 μm squared times π, times 400 thick filaments per square μm). Electron micrographs of isolated thick filaments suggest ~300 myosin heads emanating from each thick filament (e.g., see Figure 3). Multiplying these two numbers gives 2×10^{14} myosin heads acting in parallel over the cross section of the entire muscle (i.e., 800×10^9 thick filaments times 300 myosins per thick filament). ***This is an amazingly large number!!!*** Skeletal muscles have evolved such an orderly structure that 10^{14} molecules can all be tugging simultaneously on the same load. That's 10,000 *times more molecules cooperating within a single muscle than there are people on earth!* Dividing a 50 lbs (225 Newtons) force by this huge number of myosins gives about 1×10^{-12} N, or 1 pN per myosin. (There is no need to memorize these numbers. Just appreciate their magnitude).

Using laser traps (a.k.a. 'optical tweezers'), it is possible to directly measure the motion and force produced when a single myosin molecule binds an actin filament and undergoes one lever arm rotation (one 'power stroke'). A single myosin from skeletal muscle

can generate ~10 nm of movement against ~3 pN of force, more than enough to explain muscle contraction given the massive numbers of molecules involved.

Simple exercises to check what you recall

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Learning objective #2: *Explain excitation-contraction coupling and relaxation in skeletal muscle by identifying the roles of the t-tubules, calcium channels ($Cav_{1.1}$ and the ryanodine receptor), thin filament regulators (troponin and tropomyosin), and ATP-dependent calcium pumps.*

The job of skeletal muscle is to contract when commanded by the nervous system. The command comes in the form of synaptic input from a motor neuron, which impinges on the muscle cell at the neuromuscular junction. Here we will cover how this input triggers muscle contraction, a process called **excitation-contraction (EC) coupling**. We will begin by focusing on EC coupling in skeletal muscle. This will serve as a foundation for our discussion below about EC coupling in cardiac and smooth muscle.

The mechanism of excitation-contraction coupling in skeletal muscle can be summarized in six steps, listed here and shown schematically in Figure 6.

1. Somatic motor neuron releases acetylcholine (ACh) at neuromuscular junction.
2. Entry of Na^+ through nicotinic ACh receptor (nAChR) channels initiates a muscle action potential.
3. Action potential travels deep into muscle fiber along t-tubules.
4. Voltage change in t-tubules triggers conformational change in Ca^{2+} channels ($Cav_{1.1}$ and ryanodine receptor [RyR]) to release Ca^{2+} from internal stores.
5. Ca^{2+} binding to regulatory proteins (troponin and tropomyosin) exposes myosin binding sites on actin, allowing contraction to begin
6. Ca^{2+} is pumped into internal stores or extracellularly to stop contraction.

Each step is explained in more detail in the descriptions that follow.

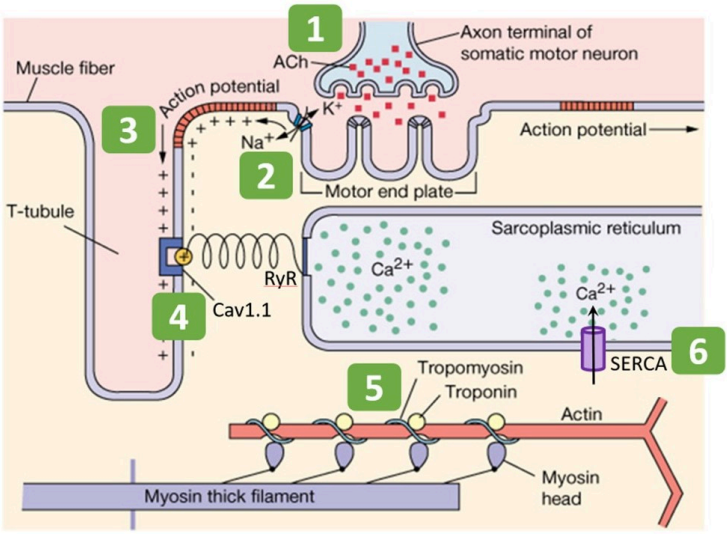

Figure 6. Basic steps of excitation-contraction coupling in skeletal muscle.

Step 1: *Somatic motor neuron releases acetylcholine at neuromuscular junction*

You have already learned in previous sessions on action potentials and synapses the basic steps for a neuron to release neurotransmitter. Skeletal muscle receives synaptic input from a somatic alpha motor neuron at the neuromuscular junction. This is the initial step in excitation. In this case the neurotransmitter is **acetylcholine** (ACh). Muscle contraction relies on the binding of acetylcholine to postsynaptic **nicotinic acetylcholine receptors** (nAChRs) at the motor end plate (Figure 6). Acetylcholine that does not get immediately degraded by acetylcholinesterase in the synaptic cleft binds to nAChRs. (Acetylcholinesterase is omitted from Figure 6.)

Pharmacology note: nAChRs are so named because nicotine is an agonist for these receptors. Do not confuse these with muscarinic acetylcholine receptors (mAChRs), which can be bound by muscarine, and are involved in smooth and cardiac muscle synaptic transmission (as described in sessions on autonomic nervous system). Note also the two different signal transduction schemes: nAChRs are fast ligand-gated ion channels, whereas mAChRs are G-protein coupled receptors (*not* ion channels).

Step 2: *Net entry of Na^+ through receptor channel initiates a muscle action potential*

nAChRs are direct ligand-gated cation channels. Their activation leads to depolarization of a portion of the sarcolemma. The depolarization is called an **end-plate potential**, or EPP (Fig. 9). nAChRs are permeable to both Na^+ and K^+ (Figure 6), which move through the channels according to their electrochemical gradients (as explained in the prior session on membrane electricity). Since Na^+ and K^+ have roughly equal concentration gradients in opposite directions, the membrane potential of the muscle goes to about halfway between E_{Na} and E_K, around 0 mV, assuming equal permeability to both ions. (To review your understanding of membrane electricity, *consider what would happen to the EPP if the permeability of the nAChR for Na^+ were greater than for K^+?*) The combined influx of Na^+ and efflux of K^+ is the **end-plate current**. (*How would a change in extracellular ion concentrations alter the end-plate current?*)

The end-plate potential is a graded depolarization – its amplitude will vary with the number of receptors bound by ACh. Typically, however, the end plate potential in a muscle cell is always large enough to reach the threshold for activating the voltage-gated Na^+ channels along the sarcolemma that will elicit a muscle action potential, the beginning of the excitation / contraction coupling of the muscle fiber.

Pharmacology note: The toxin *curare*, derived from Central and South American plants, reversibly inhibits nAChRs. Historically, curare was used in scientific studies and also clinically, to paralyze joint muscles during surgery. It has since been replaced by other inhibitors. (See Figure 7.)

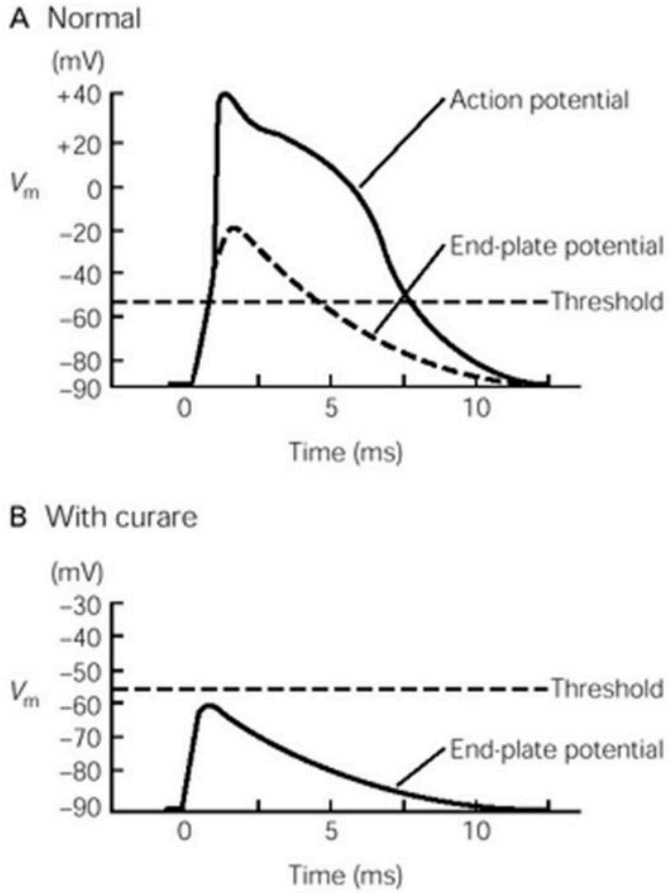

Figure 7. End-plate potentials (EPPs). (A) The normal EPP exceeds threshold and causes an action potential. (B) Curare blocks the receptor so the muscle will not contract.

Step 3 : *Action potential travels deep into the fiber along t-tubules*

The action potential propagates deep into the muscle cell along **t-tubules**, membranous tubes that are continuous with the

sarcolemma. The t-tubule network is akin to a system of telegraph wires that deliver the message, "contract!", very quickly and nearly simultaneously to all the contractile machinery inside the muscle cell. To do so, the t-tubules pass very close to another membranous compartment inside the cell, the **sarcoplasmic reticulum** (SR), which stores lots of calcium. The junction where t-tubules and SR pass very close together, when viewed by electron microscopy, contains regularly spaced, dense particles bridging between the two membranes (Figure 8). These particles, sometimes called "end feet", contain two key membrane-embedded proteins, **$Cav_{1.1}$** and the **ryanodine receptor** (RyR). (Note that $Cav_{1.1}$ is also known as the dihydropyridine or DHP receptor, and also as the L-type calcium channel.)

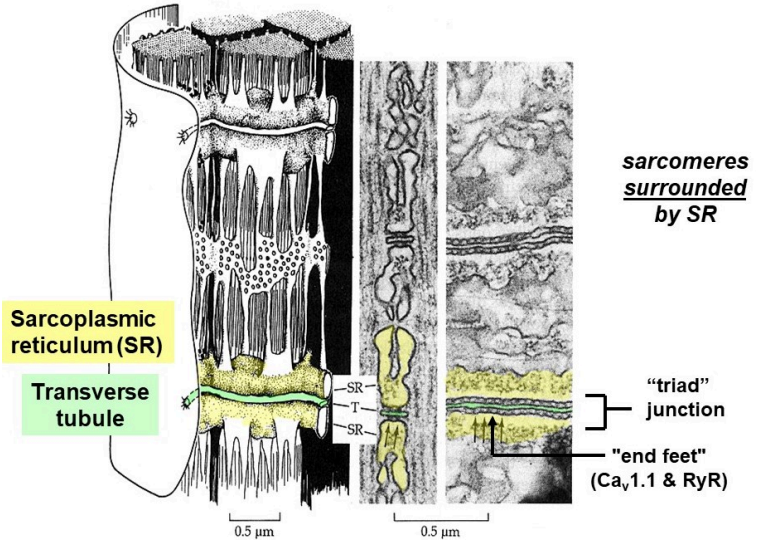

Figure 8. The triad contains $Cav_{1.1}$ channels embedded in the t-tubule membrane that associate directly with ryanodine receptors embedded in the SR membrane. (*From Hille 1992 Ionic channels of excitable membranes*)

Step 4: *Voltage change in t-tubules causes conformational*

change in channels to release calcium from internal stores

The $Cav_{1.1}$ proteins are voltage-sensitive calcium channels embedded in the t-tubule membranes. When an action potential arrives at the t-tubule, $Cav_{1.1}$ channels open and allow calcium to flow from the lumen of the t-tubule (which is continuous with the exterior of the cell) into the muscle cell sarcoplasm (Figure 8). However, the amount of calcium entering the muscle cell from the exterior via $Cav_{1.1}$ channels is low compared to what is needed for contraction. A more substantial, internal source of calcium is required, called the sarcoplasmic reticulum (SR). The SR is a system of internal membrane-bound calcium storage compartments surrounding each sarcomere and located very nearby each t-tubule. In skeletal muscle, the t-tubule $Cav_{1.1}$ channels are bound directly to another class of calcium release channels embedded in the SR membrane, called ryanodine receptors (RyRs). This direct, mechanical coupling of t-tubule $Cav_{1.1}$ channels to RyRs in the SR allows the two channels to cooperate and quickly flood the muscle cell with calcium. Voltage-dependent conformational changes in $Cav_{1.1}$ directly push the RyRs open, releasing a flood of calcium from within the SR into the muscle cell sarcoplasm.

Step 5: *Calcium binding to regulatory proteins exposes myosin binding sites on thin filaments, allowing contraction to begin*

In resting muscle, actin and myosin do not interact because tropomyosin on the thin filaments obstructs their binding. Calcium released into the muscle sarcoplasm binds to the regulatory protein, **troponin**, located on the thin filaments. Calcium binding to troponin causes a conformational change that moves **tropomyosin** out of the myosin-binding groove on actin (Figure 9). Exposure of the myosin-binding groove on actin enables chemo-mechanical

cycling of myosin to generate contractile force (see Figure 5 and Learning Objective #1 above for details).

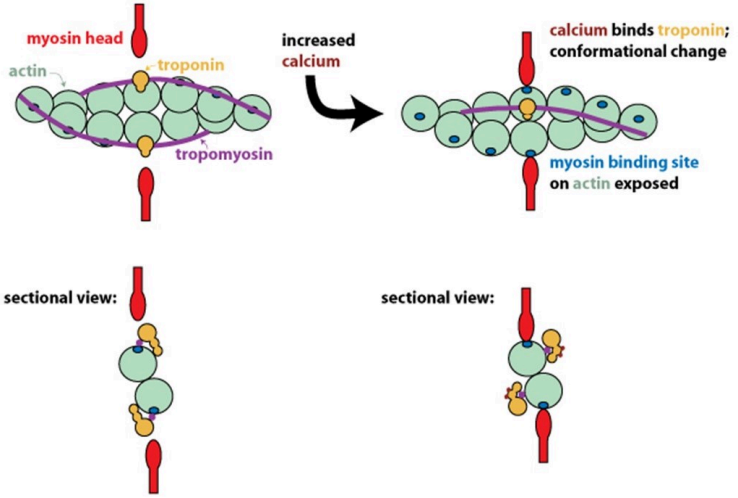

Figure 9. When calcium is absent from the sarcoplasm, tropomyosin covers the myosin-binding sites on actin, preventing contractile activity. When calcium floods the sarcoplasm, it binds troponin. A calcium-dependent conformational change causes tropomyosin to move out of the way of the myosin binding sites and allow contraction to begin.

Step 6: To stop contraction, calcium is pumped back into internal stores and extracellularly

Because calcium is the trigger for muscle contraction, its presence in the sarcoplasm is highly regulated. To stop contraction, calcium ions are sequestered into the sarcoplasmic reticulum by membrane transport proteins called **sarco/endoplasmic reticulum calcium ATPases** (SERCAs). Sodium-calcium exchangers also pump calcium out of the sarcoplasm into the extracellular space through secondary active transport (not shown in Figure 6).

Skeletal muscle relaxation also requires that the alpha motor neuron stops firing action potentials. ACh is continuously removed

from the synaptic cleft by **acetylcholinesterase** (AChE), thereby terminating the input signal to the muscle. Cross-bridge cycling will cease when $Cav_{1.1}$ and RyR channels close, and when SERCAs on the SR pump the calcium out of the sarcoplasm. As calcium levels in the sarcoplasm fall, troponin releases calcium and allows tropomyosin to slide back into place over the myosin-binding sites. Actin and myosin are then prevented from interacting. Relaxation can occur quickly but is not instantaneous, because it takes some time to sequester the calcium. Chloride ions (Cl^-) are also involved. Chloride enters the skeletal muscle cell during relaxation, when voltage-gated CLC-1 channels open. The influx of chloride ions speeds the repolarization of the muscle cell's membrane potential, thereby accelerating the relaxation process.

Pharmacology note: Acetylcholinesterase (AChE) inhibitors are chemicals that inhibit the AChE enzyme from breaking down ACh, thereby increasing the level and prolonging the action of the neurotransmitter. These pharmacological agents are used to assist people with certain neuromuscular junction disorders, but they can lead to side effects in the autonomic nervous system.

Simple exercises to check what you recall

Drag the six events (*gray boxes at left*) to the corresponding steps (*green numbers*) in the EC coupling diagram:

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Learning objective #3: *Compare twitch contractions for slow/type 1 and fast/type 2 skeletal muscle fibers and explain the molecular bases for the differences in twitch behavior. Define isometric and isotonic contractions.*

As mentioned above, a single action potential in a *skeletal* muscle cell nearly always releases a maximal amount of calcium from the SR, producing a twitch contraction of nearly uniform strength in every instance. The action potential itself is fast, lasting only a couple of milliseconds, and calcium floods the sarcomeres after only a short delay. Peak (maximum) tension may take a few milliseconds to develop, or a few tens of milliseconds, depending on the fiber type. The rate at which force develops mainly reflects the rate at which calcium binds troponin, moves tropomyosin, and gradually allows engagement of myosin motors with thin filaments. If the electrical stimulation then ceases, the tension falls back to zero in about 50 to 500 ms, again depending on the fiber type. The rate of

relaxation reflects the rates at which myosin releases F-actin, and the rate of re-sequestration of calcium.

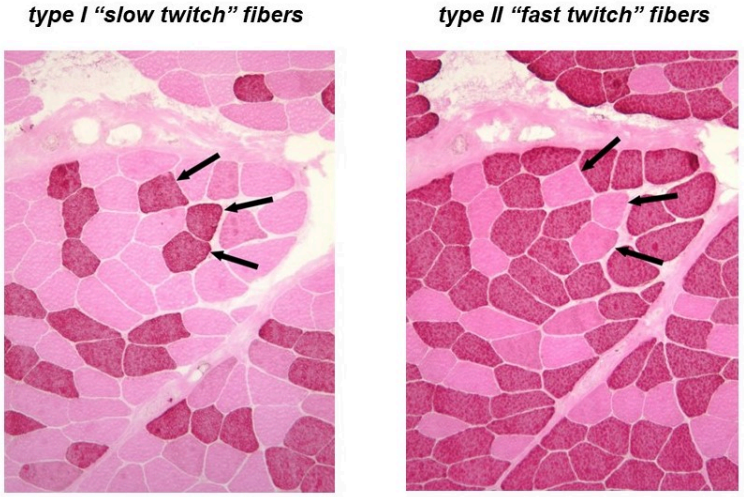

Figure 10. Skeletal muscles are mosaics consisting of mixtures of slow- and fast-twitch fibers (each of which is a single, multi-nucleate cell). Here, the same piece of tissue is stained for both the MHC-1 isoform of myosin (left image) and the MHC-2 isoform (right image). Three slow-twitch fibers are indicated by arrows. (RJ Seidman 2010, http://emedicine.medscape.com)

Recall that a whole skeletal muscle consists of many individual muscle fibers, each of which is a single, multinucleated cell (Figure 1). Whole skeletal muscles are mosaics, consisting of mixtures of 'slow twitch' (type I) and 'fast twitch' (type II) fibers (Figure 10). In response to a single action potential, type I slow twitch fibers develop force more slowly and they also relax more slowly (Figure 11). Their slower development of force is partly explained by a lower density of RyR and $Cav_{1.1}$ channels, which implies that calcium will be released more slowly from their SR. Their slow relaxation is partly explained by a lower density of SERCA pumps, which implies that calcium will be sequestered into their SR more slowly. The different fiber types also have different isoforms of myosin, with

different ATPase rates, and therefore different intrinsic speeds. Intrinsic speeds for the various myosins, measured in gliding filament assays correlate well with shortening speeds of the fiber types in which they are found (Figure 12). (*Note: Gliding filament assays were explained in the prior session on Cytoskeleton & Motor Proteins.*) This correspondence suggests that 'slow twitch' fibers are slow in part because the myosin isoform they contain has an intrinsically slower chemo-mechanical cycling rate.

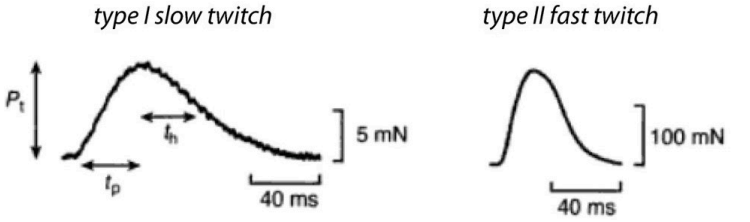

Figure 11. Different fiber types exhibit different twitch behaviors. The response of each fiber type to a single action potential is shown. Note the very different vertical scale bars. (RM Enoka 2008 "Neuromechanics of human movement")

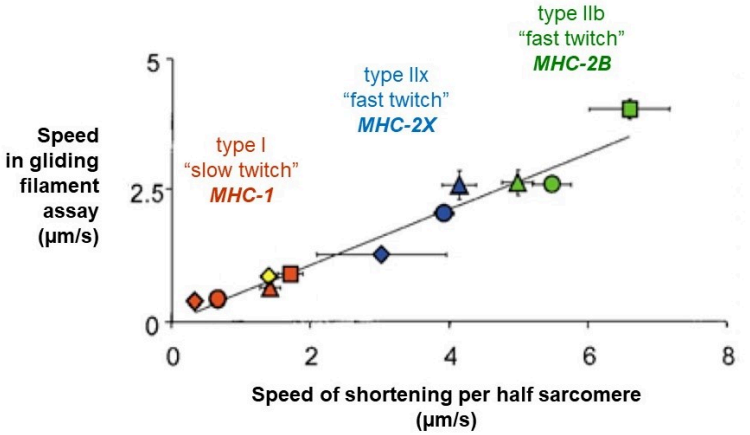

Figure 12. Different myosin isoforms have intrinsically different speeds. (*MA Pellegrina 2003 J Physiol 546(3):677-89.*)

Muscle fibers are often classified into more than two types. A common, three-type scheme designates three classifications, slow oxidative (Type I), fast oxidative (Type IIa), and fast glycolytic (Type IIb or IIx). These are based on twitch contraction speeds, on the primary isoform of myosin expressed in the fiber, and on the fiber's capacity for oxidative phosphorylation (see metabolism sessions). In addition to variations in speed and myosin ATPase activity, the fibers can also differ considerably in their myoglobin content, glycogen stores, and rate of fatigue.

A note about exercise: Skeletal muscle responds to resistance training by becoming larger and stronger. Muscle enlargement and strengthening after exercise occurs by *hypertrophy*, an increase in the diameter of the muscle cells (fibers) – not by hyperplasia, an increase in the number of cells. Exercise can also cause minor changes in the myosin isoforms expressed within the fibers, causing minor changes in the fiber-type distribution of the muscle. For example, the fraction of fast oxidative type-IIa fibers might increase slightly, with a corresponding decrease in the fraction of fast glycolytic type-IIx fibers. However, these changes in myosin isoform expression are modest compared to the changes in cell diameter. Some smooth muscles do undergo hyperplasia, such as those in the uterus, which continue to grow in response to hormonal signals like estrogen, during puberty.

Simple exercises to check what you recall

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Learning objective #4: *Explain how smooth, graded contractions of a skeletal muscle are produced by changes in stimulus intensity and by the size principle of motor unit recruitment.*

The degree of control we have over the amount of force our skeletal muscles exert is exquisite. Using the very same muscles we can gently manipulate a delicate object or exert a crushing grip of 100 lbs or more. As you have learned, the neuro-muscular junction acts as a simple, binary relay – essentially every action potential that reaches the motor end plate elicits an action potential on the muscle fiber and therefore a twitch contraction. How then do we regulate muscle force? Muscle contraction can be adjusted in two ways: by changing the frequency of stimulation, and by **recruitment** of more and larger motor units.

Motor units are quantal elements of muscle action. A skeletal muscle motor unit consists of a motor neuron and the several muscle fibers that it innervates (Figure 13). Upper motor neurons, from the primary motor cortex in the frontal lobe, synapse onto lower motor neurons in the brainstem or spinal cord to control voluntary movements. The details of these central nervous system pathways will be covered later, in your Mind Brain Behavior block. Here we focus on how motor units allow fine adjustments of muscle force.

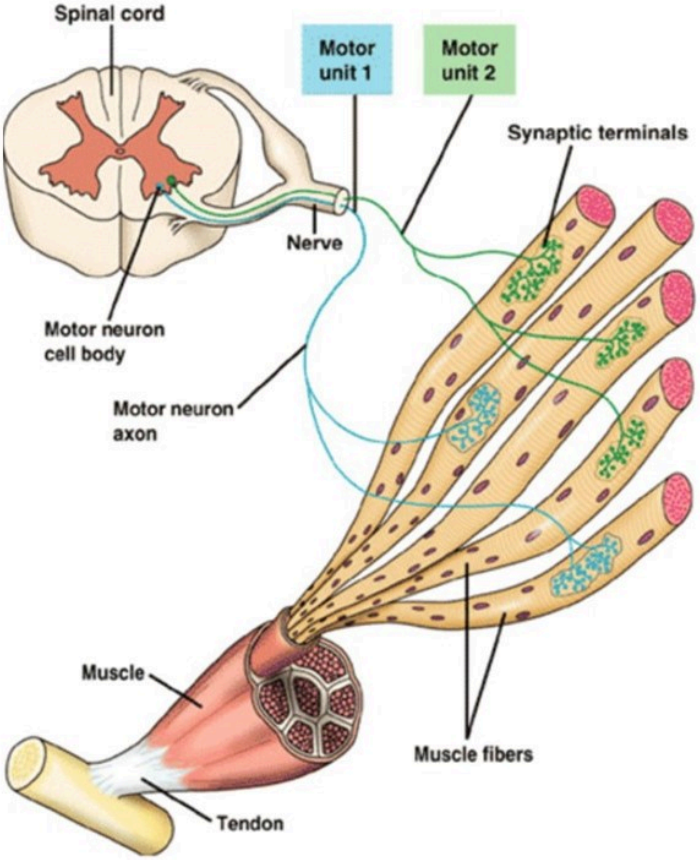

Figure 13. Basic structure of the skeletal muscle motor unit. Two motor units are shown, one unit (blue) with a neuron innervating two muscle fibers and another unit (green) with a neuron innervating three fibers.

Increasing the stimulus frequency can increase skeletal muscle force, but only modestly

An action potential is all-or-none, but the force a muscle fiber develops as a result is not all-or-none. The force can be increased to some degree by increasing the frequency of action potentials, up until a **fused tetanus** is reached, where the fiber produces its maximal force (Figure 14). With faster stimulation, calcium release outpaces calcium sequestration, so more calcium builds up in the cytoplasm. Higher calcium allows a greater fraction of the myosins to bind F-actin, thereby allowing more force to be generated. Notably, however, this 'temporal summation' mechanism allows variation of muscle fiber force over only a limited range. Other mechanisms, which regulate the number of muscle fibers that are recruited during a muscle contraction, are required for fine control across a broad range of forces.

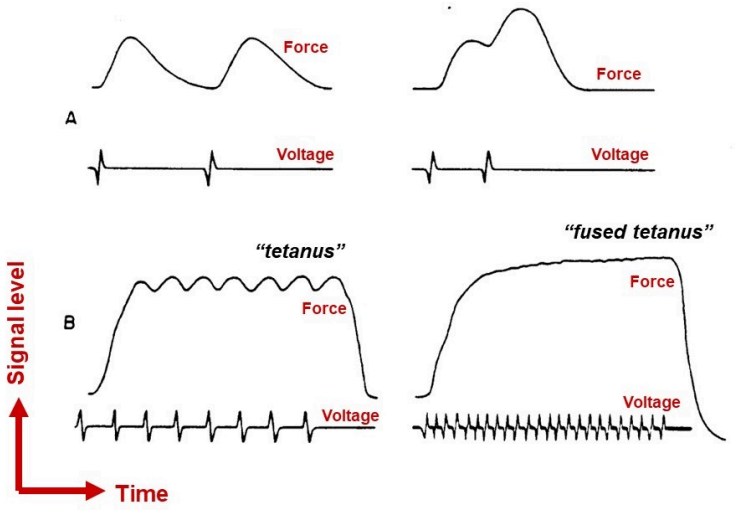

Figure 14. As stimulation frequency increases, the amount of force a muscle fiber exerts increases. Tetanus occurs when stimulation is frequent enough to produce maximal fiber force. Note how changing stimulation frequency can only adjust the force by a modest amount. At very low stimulation frequencies, the force is pulsatile, not steady. Thus, fine motor control requires additional mechanisms.

Regulation of skeletal muscle force occurs primarily by changes in motor unit recruitment

When the central nervous system sends stimulatory input to a particular muscle, the motor units innervating that muscle are recruited in an orderly manner, following the **size principle**: The first motor units to be activated are those with the smallest axons. These small units have relatively few, weak fibers and thus they generate the weakest contractile forces, allowing the total muscle force to be finely graded. As more units are recruited, motor neurons with progressively larger axons are activated, generating progressively larger amounts of force. This size-ordered recruitment enables fine motor control when little force is needed, and also enables very large forces to be generated when necessary.

Biophysics side note: A simple mechanism underlies size-ordered recruitment of skeletal muscle motor units: Motor neurons come in a range of sizes, some smaller and some larger in diameter. The smaller motor neurons have fewer parallel ion channels and thus higher electrical resistance (R). Synaptic input current (I) will thus generate bigger excitatory post-synaptic potentials in the smaller motor neurons (by Ohm's Law, $V = I \cdot R$), bringing them to threshold for action potentials sooner than larger motor neurons. The smaller motor neurons also happen to innervate fewer muscle fibers, so they elicit less force. Thus the neurons that are excited first are the ones that elicit the smallest (finest) increases in total muscle force.

Simple exercise to check what you remember

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Learning objective #5: *Understand the differences in excitation-contraction coupling between skeletal, cardiac, and smooth muscle. Describe the two-stage phospho-regulatory cascade that initiates smooth muscle contraction.*

CARDIAC MUSCLE

Cardiac muscle cells (cardiac myocytes, or cardiomyocytes) are striated, just like skeletal muscle cells, because their contractile filaments are very similarly organized, into sarcomeres connected end-to-end to form myofibrils. Unlike skeletal muscle, however, cardiac muscle contracts involuntarily, in response to intrinsic

pacemaker signals that are regulated by the autonomic nervous system. The molecular mechanisms underlying excitation-contraction coupling are very similar in cardiac and skeletal muscle, but there are a few key differences. To understand these differences, consider again the steps of excitation-contraction coupling, but focusing now on cardiac rather than skeletal muscle.

Voltage changes in the t-tubules of cardiomyocytes release calcium indirectly, via calcium-induced calcium release (CICR)

Like skeletal muscle, the t-tubules in a cardiac myocyte have voltage-sensitive channels, called **$Cav_{1.2}$**, embedded in them. When an action potential arrives at a cardiac t-tubule, the voltage change likewise causes the $Cav_{1.2}$ channels to open, allowing external calcium from the lumen of the t-tubule to flow into the cell. However, unlike in skeletal muscle, the $Cav_{1.2}$ channels in cardiac myocytes are not directly linked to RyRs embedded in the SR. Cardiac and skeletal muscle cells contain different isoforms of RyRs. The cardiac RyRs are less abundant than in skeletal muscle, and they do not associate directly with $Cav_{1.2}$ channels. Instead, external calcium entering a cardiac cell through $Cav_{1.2}$ must bind to the RyRs to induce them to open – a process called calcium-induced calcium release (CICR). This indirect form of coupling has an important advantage over the more direct coupling found in skeletal muscle, because it allows graded regulation of the strength of heart muscle contractility by the autonomic nervous system. An action potential in a *skeletal* muscle cell nearly always releases a maximal amount of calcium from the SR, producing a twitch contraction of nearly uniform strength in every instance. By contrast, in a *cardiac* muscle cell, the amount of force is adjustable, via autonomic regulation of the amount of calcium released. Thus, calcium levels in cardiomyocytes serve not only as an "on-off switch", but also as an adjustable "rheostat" that controls how forcibly the cell contracts.

Autonomic innervation regulates heart rate

The autonomic nervous system (ANS) regulates both the rate and forcefulness of cardiac muscle contraction. Heart rate is controlled by both parasympathetic and sympathetic innervation of the SA node, via modification of the rhythm of pacemaker cells in the node. The membrane potential of these cells oscillates in a regular, rhythmic pattern due to special properties of their ion channels, the details of which will be covered later, in your Circulatory Systems block. For now, understand that the parasympathetic **vagus nerve** innervates the pacemaker cells and acts to decrease their firing rate, while sympathetic nerves act to increase their firing rate. (Some details are summarized in the box below.)

Note about ANS regulation of the SA node: The parasympathetic vagus nerve decreases heart rate by releasing acetylcholine (ACh), which binds muscarinic ACh receptors (mAChRs) on pacemaker cells in the SA node and in the atrio-ventricular (AV) node. This in turn activates a $G\alpha_i$-based signaling cascade which ultimately opens a potassium channel to hyperpolarize the pacemaker cells. Conversely, sympathetic nerves increase heart rate by releasing onto the SA and AV nodes norepinephrine, which binds β1 receptors on the pacemaker cells, ultimately accelerating inactivation of the potassium channels and increasing the opening probability of sodium and calcium channels, thereby depolarizing the cells more quickly.

Autonomic innervation also regulates the contractility of the heart

In addition to regulating heart rate, the ANS also controls the forcefulness (**contractility**) of cardiac muscle contraction. Cardiac contractility is regulated by the sympathetic nervous system, which innervates the entire heart. Sympathetic postganglionic fibers synapse onto the cardiomyocytes and release norepinephrine, which binds β_1-adrenergic receptors to activate a signaling cascade (via $G\alpha_s$). This G-protein-based cascade increases the amount of calcium released per heartbeat, and thus the contractility of the cardiomyocytes, in at least two ways (*depicted in the interactive diagram below*, and explained in detail in the following two paragraphs). Circulating epinephrine secreted by the adrenal medulla has the same effects.

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β_1-adrenergic stimulation increases contractility by at least two mechanisms, regulating the voltage-gated $Cav_{1.2}$ channels that allow influx of external calcium, and also regulating the SERCA pumps that sequester calcium into the SR. Both mechanisms occur via activation of a $G\alpha_s$ pathway that you should recall from your earlier sessions on G-protein coupled receptors: $G\alpha_s$ activates the membrane enzyme adenylyl cyclase, which converts ATP into cytoplasmic cAMP (3',5'-cyclic adenosine monophosphate), which in turn activates PKA (cAMP-dependent protein kinase). PKA directly phosphorylates $Cav_{1.2}$ channels, thereby increasing their open-probability and allowing more external calcium to enter the cardiomyocytes during each heartbeat. Higher calcium results in

more activation of thin filaments (see above, *Step* 5 of EC coupling in skeletal muscle), and thus a more forceful contraction.

PKA affects SERCA pumps too: Just as in skeletal muscle, the relaxation of cardiac muscle cells requires calcium to be returned to the SR by SERCA pumps. (Some calcium is also extruded to the outside of cardiac cells by sodium-calcium exchangers, but the SERCA pumps are responsible for removal of most of the calcium that enters during each contraction.) In cardiomyocytes, these SERCA pumps are regulated by another protein on the SR membrane called **phospholamban**. Phospholamban binds SERCAs and acts like a brake, partially inhibiting (slowing) their pumping action. The inhibition is relieved by PKA-dependent phosphorylation of phospholamban, thereby allowing SERCAs to pump more calcium into SR more quickly. When more calcium is pumped back into SR, more will be released from SR during the subsequent beat. Thus, the same sympathetic nerve input that increases heart rate by accelerating pacemaker activity in the SA node (as described in the previous section above), also increases the influx of extracellular calcium (via phosphorylation of $Cav_{1.2}$ channels) and increases the speed and amount of calcium sequestration into SR (via phosphorylation of phospholamban). The overall result is a heartbeat that is both faster and more forceful.

SMOOTH MUSCLE

Smooth muscle surrounds hollow cavities and tubes in our bodies. At the most fundamental level, the contraction of smooth muscle is driven by essentially the same chemo-mechanical cycle of myosin as in skeletal and cardiac muscle (see Figure 5). However, smooth muscle is not striated like skeletal or cardiac muscle, because its contractile proteins are arranged less regularly. Smooth muscle drives constriction of blood vessels to control blood flow, contraction of the uterus during childbirth, contraction of the

bladder (for *micturation*, a.k.a. peeing), peristaltic pumping (*peristalsis*) in the esophagus for swallowing and in the intestines during digestion and closing of various sphincters of the digestive and urinary tracts (e.g., *pyloric sphincter* at outlet of stomach, *anal sphincters*, *internal urethral sphincter*). Generally, these occur in response to involuntary signals from the autonomic nervous system. (The anus and urethra have both internal and external sphincters. Internal sphincters are subconscious and controlled by smooth muscle. External sphincters are conscious and controlled by skeletal muscle.)

Smooth muscle cells lack t-tubules and have less SR

Excitation-contraction coupling in smooth muscle differs substantially from skeletal or cardiac muscle. There are no t-tubules in smooth muscle cells, so action potentials can only propagate along their surface. They contain less SR and thus may not store enough calcium for full contraction. Instead, much of the required calcium enters from outside the cell, through voltage or ligand-gated channels in the plasma membrane. This external calcium must diffuse across the width of the cell to reach all the contractile machinery, limiting the speed at which full contraction can develop. Note how different this situation is from skeletal and cardiac muscle, where every sarcomere is surrounded by SR and can be quickly flooded with calcium that needs only to diffuse a very short distance. The slow response of smooth muscle cells to stimulation is not a disadvantage, since they generally drive processes that are slow.

Calcium controls smooth muscle myosin via a two-stage phospho-regulatory cascade

The contractile proteins in smooth muscle are activated by a very different, slower calcium-dependent mechanism than in skeletal and cardiac muscle. There is no troponin in smooth muscle, and thus the F-actin filaments are not regulated. Instead, it is the myosin that is regulated. (Note that different myosin isoforms are found in smooth, cardiac, and skeletal muscle.) *Skeletal* muscle myosin is always ready to cycle; but in resting *smooth* muscle, the myosin is in a quiescent state (unphosphorylated). Calcium entry into a smooth muscle cell initiates a cascade (*depicted in the interactive diagram below*) where calcium first binds **calmodulin**, and then calcium and calmodulin together bind a kinase enzyme, **myosin light chain kinase** (MLCK), activating it. Active MLCK phosphorylates myosin (on one of its two light chains) to initiate chemo-mechanical cycling of the myosin. Smooth muscle myosin is turned off by an enzyme, **myosin phosphatase**, that removes the activating phosphates. Notably, removal of phosphates from myosins that are already attached to F-actin slows (rather than accelerates) their detachment from the actin. This so-called latch (or latch-bridge) behavior is thought to help smooth muscles maintain luminal pressures (e.g., in the vasculature) more economically than they would otherwise, by allowing them sustain tension over long times while using less ATP.

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A simple exercise to check what you recall

Can you identify similarities and differences in EC coupling across the three muscle types? **Drag the features of EC coupling (*gray boxes at bottom*) onto the correct muscle types.** Hints: Some features apply to more than one muscle type, and each muscle type should get four features (*one from each column*).

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Learning objective #6: *Compare and contrast how skeletal, cardiac and smooth muscle are controlled by the nervous system. Define single-unit vs. multi-unit smooth muscle types.*

CARDIAC MUSCLE

Depolarization of cardiomyocytes occurs via gap junctions or pacemaker cells

Cardiac myocytes are linked end-to-end by intercalated discs, which allow mechanical forces to propagate from one cell to the

next, and which also contain gap junctions (Figure 15). The gap junctions allow electrical currents and chemical signals to spread between neighboring cells, creating one functional unit composed of many interconnected cells. Note how this total interconnectedness is unlike the situation in skeletal muscle, where only individual cells (fibers) can be activated by a particular motor neuron. Cardiac muscle contraction is triggered by a set of pacemaker cells located on the right atrium of the heart, called the **sinoatrial (SA) node**, which generates action potentials rhythmically. (The basis for pacemaker activity in SA node cells will be discussed in more detail later, in your Circulatory Systems block.) When the command to contract is issued by the pacemaker, it spreads throughout the heart by the extensive branching of the connected myocytes.

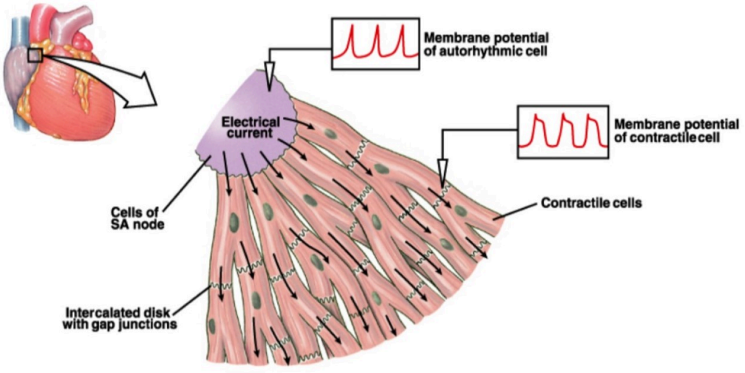

Figure 15. Intercalated disks allow for the spread of electrical excitation in cardiac muscle.
(From Silverthorn 2007 Human Physiology)

Just as in skeletal muscle, each cardiac muscle cell contains t-tubules continuous with the sarcolemma, although not as many, and an extensive network of internal sarcoplasmic reticulum (SR) calcium stores surrounding each sarcomere. Just as in skeletal muscle, the t-tubules in cardiac myocytes are located nearby the

SR, allowing action potentials to penetrate the cell and deliver the "contract" signal nearly simultaneously to all the contractile machinery in the cell.

Cardiac muscle contraction always involves all fibers

The gradual recruitment of motor units in a skeletal muscle (described above under Learning objective #4) contrasts with what occurs in the heart. With each beat, all the cardiac muscle fibers in the heart are involved in generating force as a single unit. There is no option for recruiting additional motor units. Instead, to increase the forcefulness of the heartbeat, the calcium permeability of cardiac muscle fibers is regulated by the autonomic nervous system (as discussed above, under Learning objective #5).

In addition to their regulation by the autonomic nervous system, cardiac muscle fibers are intrinsically stretch-activated: Cardiac sarcomeres normally operate over a length range where the amount of active force they generate increases with their length. This stretch-activation of heart muscle was first discovered in 1914 and is known as the Frank-Starling Law or as Starling's Law of the heart. It causes the heart to beat more forcefully when it becomes more filled, enabling it to automatically pump all the blood that returns to it, even in the absence of neuronal or hormonal input. (You will learn more about cardiac output and Starling's Law later, in your Circulatory Systems block.)

SMOOTH MUSCLE

Different smooth muscles can be innervated in two distinct patterns, multi-unit or single-unit (Figure 16). Their innervation patterns influence how they behave.

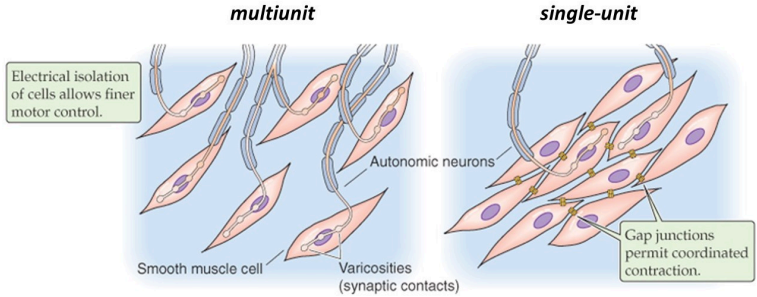

Figure 16. Patterns of smooth muscle innervation.
(*Boron & Boulpaep 2009 Medical Physiology*)

In a **multi-unit smooth muscle**, each cell is directly innervated by a post-ganglionic autonomic motor neuron, similar to the situation in skeletal muscle. Like in skeletal muscle, the innervation of individual cells in a multi-unit smooth muscle allows graded recruitment, and thus fine control of force generation. Because multi-unit smooth muscles are capable of maintaining sustained contractions, they are sometimes referred to as "tonic" smooth muscles. They are found for example in large airways, in the ciliary and iris muscles of the eye (which control focus and aperture, respectively), in sphincters, vasculature, and arrector pili (the tiny muscles that raise hairs and goose-bumps on your skin).

By contrast, the cells in a **single-unit smooth muscle** (also called unitary smooth muscle) are connected together by gap junctions, which allow electrical signals to spread from one cell to its neighbors. The interconnected cells act together as a single unit, with waves of electrical activity passing through them. Smooth muscles can contain pacemaker cells, similar to the SA node of the heart, and autonomic neurons may also synapse on a some of the cells to help regulate the contractility of the muscle. Because unitary smooth muscles contract transiently when stimulated, they are also known as "phasic" smooth muscles. They are found for

example in the gastrointestinal tract and urogenital walls, where peristalsis is coordinated down the length of a tube.

Many smooth muscles are blends of both phasic and tonic, which allows them to respond to a range of stimuli. Smooth muscles in different body organs vary substantially in fiber organization, innervation and responsiveness to various stimuli.

A simple exercise to check what you recall

Drag each muscle type (*gray boxes at right*) to the category (1 *or* 2) where it belongs:

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/physiology/?p=74#h5p-15

Extra Q&A from past students that might be helpful

Professor Asbury,

I was hoping to ask you one question in regard to the cardiac and smooth muscle. I understand that in skeletal muscle, we have a clear neuromuscular junction. However,

for cardiac and smooth muscle, it seems it can be done via an electrical action potential (SA node pacemaker and nerve attachment respectively) and by G protein pathways (Gs and Gq respectively). Am I thinking of that the right way?

You're absolutely correct about skeletal muscle, but you might be misunderstanding something about cardiac muscle: In cardiac muscle, contraction is *always* triggered by an electrical action potential. The action potential (AP) is delivered to cardiac cells in a different manner than delivery to skeletal muscle cells. For cardiac muscle cells, the AP is delivered by direct transmission from neighboring cells via gap junctions (and, ultimately, if you trace it back, the AP is initially created by the pacemaker cells in the SA node). But in both skeletal and cardiac, contraction is *always* triggered by an electrical AP.

What might be confusing is that cardiac muscle cells receive additional input from the autonomic nervous system (ANS). This ANS input does *not* trigger cardiac muscle contraction. Instead, it regulates how *forcefully* the cardiac muscle cells contract whenever they receive an AP from the pacemaker. The ANS input is received by GPCRs on the cardiac muscle cell surface (beta-1 adrenergic), then it gets relayed through G-alpha-s, and ultimately increases the forcefulness of the contractions elicited from each SA-generated AP (via increasing calcium influx through Cav1.2, and also by promoting faster SERCA pumping).

Smooth muscles are quite variable. In some cases, smooth muscle cells are directly electrically coupled to their neighbors via gap junctions, so APs propagate from

cell-to-cell just like in heart muscle. These so-called "single-unit" smooth muscles can sometimes have pacemaker cells too, so that the whole muscle undergoes waves of contractions, again similar to the heart (for example, smooth muscles that drive peristalsis). In other cases, each individual smooth muscle is innervated by a single neuron. This arrangement is similar to skeletal muscle, except that the neurons will be ANS neurons, and the receptors will usually be GPCRs. And just to make smooth muscle even a little bit more complicated, contraction of some smooth muscles can be triggered by non-neural inputs. For example, stretch-activated channels in vascular smooth muscle cells can open to allow calcium in – this input is called "myogenic" because it's generated intrinsically by the myocytes themselves. Another example is histamine, which can be released from immune cells and bind GPCRs on airway smooth muscles, promoting their contraction – this is a cause of asthma attacks.

Hi Dr. Asbury,

I'm confused about this except from the syllabus: "Phospholamban binds SERCAs and acts like a brake, partially inhibiting (slowing) their pumping action. The inhibition is relieved by PKA-dependent phosphorylation of phospholamban, thereby allowing SERCAs to pump more calcium into SR more quickly. When more calcium is pumped back into SR, more will be released from SR during the subsequent beat." Why would sequestering calcium increase contractility? Wouldn't you want calcium to stick around so that more actin binding sites are exposed, and more are involved in contraction?

This is a very good question. It illustrates that you're

thinking carefully about the physiology of the heart. That last sentence in the passage quoted from the syllabus is important: "When more calcium is pumped back into SR, more will be released". It is also helpful to understand that the effects of ANS on cardiac contractility happen over minutes, a time scale that is much longer than the duration of a single heartbeat. Two things happen simultaneously in response to PKA activation, (1) more opening of the Cav1.2 channels is promoted, and (2) SERCA activity is also increased. This means that over time, the heart cells are bringing in more calcium from outside, via number 1 above, and — during the relaxation phases of the heartbeat cycle — they are also stuffing the extra calcium into stores (into sarcoplasmic reticulum), via number 2 above. Due to this combination, and after some minutes of ANS input, the amount of calcium released from SR during the contraction phase of each beat will be greatly increased. The higher peak calcium levels in the sarcomeres will uncover more myosin binding sites on thin filaments, allowing more force to be generated.