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SUMMER COLLEGE IN BIOPHYSICS

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THE NERVOUS SYSTEM

Assorted Notes - Part 3

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NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Structure of Sensory Receptors and Organization of the Peripheral Autonomic Nervous System

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1. PRIMARY SENSORY NEURONS OF THE SOMATOSENSORY SYSTEM

Much of the information we receive about the conditon of our environment is derived from stimuli impinging upon our skin. We are all keenly aware of the sensations of touch, pressure, pain, warmth or coldness, i.e., modality or quality of sensation, produced by a particular object in contact with the skin. Our central nervous system also receives important information from tissues deep within the body; for example, signals from muscles, tendons and joints is critical for determining the position of our limbs, and producing smooth coordinated muscle movements. Sensory information is also derived from the viscera; for example, visceral information is responsible for the sensation of hunger, and distention of the bladder. Signals form the blood vessels regulate blood pressure.

Sensory information from the skin, muscles, tendons, joints and viscera is transmitted to the central nervous system by neurons whose cell bodies in nearly every case are situated in ganglia. Each of the 31 paired dorsal roots has a sensory ganglion responsible for innervating specific areas of the trunk and limbs. Cranial nerves V, VII, IX and X also have conspicuous sensory ganglia for conveying information from the head. The nerve cells are the primary sensory neurons in pathways of increasingly higher order neurons that terminate in areas of the CNS which in turn influence behavioral patterns. As pointed out in previous lectures these primary sensory neurons are unipolar; in fact, they are the only unipolar cells in the vertebrate nervous system. Their single process undergoes a T-shaped bifurcation with the ganglion: one limb of the T extends to the CNS where it makes synapses with second order neurons; the other limb of the T extends peripherally to terminate on or near the skin, muscles, joints, tendons or a viscus. The two limbs together form a single process which looks and performs like axons of the common multipolar neurons. For descriptive purposes it is often convenient to order the sensory neurons according to their source of stimulation. Sensory neurons that respond to stimuli from the external environment are called exteroceptors; receptors from the eye and ear as well as cutaneous receptors would fall into this class. Sensory neurons responding to activity in muscles, ligaments and joints are proprioceptors, and those responding to activity in viscera are interceptors.

Today I am going to discuss the cutaneous receptors and proprioceptors. These sensory neurons comprise the initial steps in the somatosensory system (soma=body wall) which will be considered in great detail in other lectures. Specifically I am going to describe the structure of cutaneous receptors and proprioceptors and the role that structural relationships play in determining the function of particular receptor types within these two classes.

Adequate stimulus

Discrimination between the various types of stimuli we encounter might in theory occur if primary sensory neurons were to respond equally well to all sorts of stimuli and code the quality of a particular stimulus by the pattern of impulses they conducted. Thus a receptor might be equally sensitive to mechanical deformation of the skin and heat but depending upon whether a stimulus was a caress or a warm breath of air the axon would generate a series of impulses with intervals characteristic for each. In fact, this sort of notion was proposed over 50 years ago and was suggested again as late as the mid-1960's. However, it has become increasingly clear over the last several decades as a result of

detailed electrophysiological studies that while some receptors can respond to more than one type of stimulus, each is exquisitely sensitive to only one--the adequate stimulus. A receptor may respond to both mechanical deformation and heat but it is orders of magnitude more sensitive to one than to the other.

Generator and action potentials

As in other axons, information is conducted along sensory axons by action potentials. The portion of the peripheral limb at or near its tip is the receptive portion of the neuron. It responds to a stimulus with a graded ("generator") potential, analogous to the graded potentials produced in the dendrites of CNS neurons by synaptic transmitters. If the amplitude of the graded potential is great enough the graded potential reaches the threshold of the axon and generates the action potentials. The amplitude of the generator potential is proportional to the strength of the stimulus, and the greater the amplitude of the generator potential the greater the frequency of action potentials. Thus information about the strength of a stimulus, such as depression of the skin, is coded by the frequency of the action potentials. (See Dr. Baylor's handout.)

Adaptation

When a stimulus is maintained the frequency of action potentials can, depending on the receptor type, diminish; this is adaptation. Different receptors adapt at different rates. Some rapidly adapting receptors discharge only or at a varying rate while a stimulus is being applied and not at all or relatively little while the stimulus is maintained. Some slowly adapting receptors continue to discharge regardless of how long the stimulus is present. The mechanism of adaptation is different for different types of receptors. There is evidence from studies on some receptors that adaptation is a property of the neuron. In the description that follows I point to cases where the tissue associated with the nerve may play a role.

Cutaneous Receptors: The Structure of Different Types and How Their Structure Influences Their Performance

As you might imagine, the receptive portion of cutaneous receptors is situated in the skin itself or in the subcutaneous connective tissue. The skin consists of two distinct layers: epidermis (a stratified epithelium) and the subjacent dermis (a dense connective tissue layer) tightly adherent to the epidermis. There are two sorts of skin: 1) a glabrous (non-hairy) portion that covers, for example, the underside of the fingers and toes and the palms of the hands and soles of the feet; and 2) a hairy portion that covers the rest of the body. Beneath the skin there is a loosely packed subcutaneous connective tissue (superficial fascia or hypodermis). Both the dermis and subcutaneous connective tissue contain a high density of sensory nerve terminals; there are also some nerve terminals within the deeper layers of epidermis.

Based upon their adequate stimulus three general types of cutaneous receptors can be defined: a) mechanoreceptors, which have a very high sensitivity to mechanical stimuli; b) thermoreceptors, which are exquisitely sensitive to small changes in skin temperature; and c) nociceptors, which are insensitive to mild or low intensity mechanical and thermal stimulation but discharge impulses to stimuli that are potentially or actually damaging to the skin.

Mechanoreceptors

PACINIAN CORPUSCLE. These mechanoreceptors have a conspicuous capsule of non-neuronal cells and are situated within the subcutaneous connective tissue. They are rapidly adapting, responding to the onset of an applied pressure and at its removal but not while the pressure is held steady. Moreover they respond best to rapid displacements so that at a frequency of 200 cycles per second, a 1 micrometer deflection of the surface of the skin is readily detected by the axon. (It is thought by some that such vibration detectors in the fingers serve to determine the texture of a material as they are drawn across it.) The response of the nerve ending only to the onset of displacements and to rapid changes in velocity can be accounted for by its surrounding capsule of supporting cells.

The capsule can be several millimeters long and consists of layers of flattened cells concentrically arranged around a single axon which terminates at its center. The axon is moderate in size and loses its myelin sheath as it enters the capsule. The outer layers of the capsule are a continuation of the perineurium and in cross sections present a complete profile around the circumference of the capsule. The layers of cells at the center of the capsule, on the other hand, show a different arrangement; the lamellar profiles are not full circles but semi, with the gaps between the half circles aligned so as to produce a continuous cleft extending from the unmyelinated portion of the axon to the capsule's core. The lamellae of the outer capsule are separated by large spaces (micrometer or more) which, like other tissue spaces are filled with a fluid matrix; the inner layers are more closely apposed (separated by only a few hundred nanometers).

How does it work? Pacinian corpuscles are not found only in subcutaneous connective tissue; they are also situated in connective tissue around joints and in thin mesentery of the gut where, in some animals, they can be seen with the naked eye. Loewenstein exposed the Pacinian corpuscle in the cat's mesentery so that he could stimulate it directly with a stylus (like the one on your phonograph) while recording from the axon. When he pressed on the capsule with the stylus he recorded the same sorts of events seen with displacement of the intact tissue; there was a brief passive depolarization, which is large enough could give rise to an action potential. The appearance of the depolarization occurred only during the dynamic phases of displacement (just as pressure was being applied and just as it was being removed). Moreover, the velocity of displacement needed to be rapid to elicit the response. He then dissected away much of the capsule and found that when the stylus was applied to the axon, the graded depolarization occurred during both the dynamic and static phases of displacement and accordingly it was no longer dependent upon velocity. These findings lead to the following explanation: A slowly applied stimulus delivered to the capsule as a vertical deflection with a stylus results only in the displacement of fluid elements within the capsule so that the force of the stimulus does not reach the terminal. On the other hand, it the stimulus is rapid, owing to the viscosity of the fluid the force is transferred directly to the terminal resulting in a response. Thus the capsule filters out low frequency stimuli or stimuli with a low velocity. It follows that after the dynamic phase of a rapidly applied stimulus reaches the terminal, the fluid elements begin to shift removing force from the terminal so that the static phase has no effect and does not give rise to a generator potential. When pressure is removed from the capsule, owing to the capsule's elastic properties, it snaps back to its resting form and again because of the viscosity of the fluid elements the force is transmitted to the terminal initiating a response. Thus the capsule acts not only to filter out

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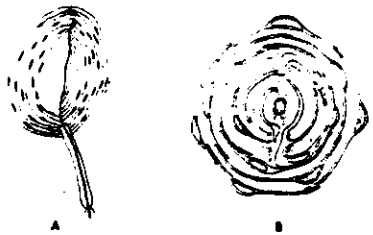
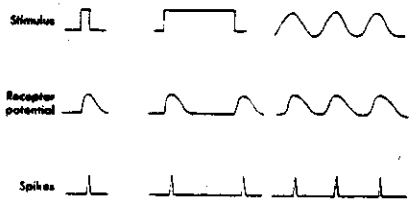
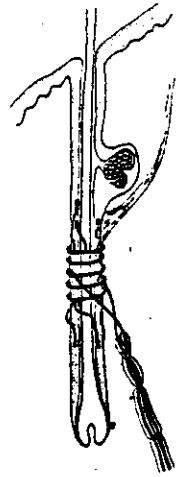


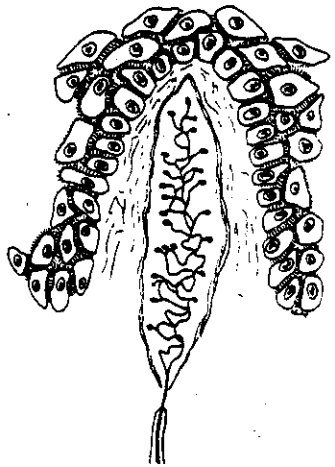
. . Pacinian corpuscle. These are large, encapsulated endings found in subcutaneous tissue, in fascial planes, and in the mesentery. A shows a longitudinal section of a pacinian corpuscle at the light microscopic level. A large, myelinated nerve fiber innervates the ending. The myelin is lost soon after the axon enters the corpuscle. The capsule is composed of numerous concentric layers of connective tissue cells between which are trapped fluid and extracellular fibers. B represents a cross section through the central core region of a pacinian corpuscle at the electron microscopic level. The axon is seen at the center. The connective tissue cells within the core region do not form a complete covering of the axon. Instead, the layers are hemicylindrical, with a distinct gap on each side of the axon. The gap appears to be in continuity with the extracellular space outside the corpuscle. The pacinian corpuscle is extremely sensitive to pressure waves, and in particular to pressure transients, including vibratory stimuli. These receptors are rapidly adapting.



. . . Responses of a pacinian corpuscle. The time course of several mechanical stimuli applied to a pacinian corpuscle are shown in the top line. The receptor potentials and consequent afferent nerve impulses are shown in the lower two lines. At the left, a brief mechanical stimulus is shown to evoke a short-lasting receptor potential, which in turn triggers a single nerve impulse in the afferent fiber. At the middle, a longer-lasting mechanical stimulus produces receptor potentials and resultant spikes at the onset and at the offset of the stimulus. The receptor is rapidly adapting and responds just to transients. At the right, a sinusoidal mechanical stimulus (vibratory stimulus) evokes a series of receptor potentials and spikes at the frequency of the stimulus.



. . . Hair follicle receptor. Finely myelinated nerve fibers are shown terminating on a hair follicle. These would discharge in response to movements of the hair, and so they would signal a form of touch.



. . . Meissner's corpuscle. This is an encapsulated ending found in the dermal papillae of the skin. It is likely that Meissner's corpuscles are involved in detecting fine discriminative touch. (Schwann cells which are situated between terminal branches have been omitted)

low velocity stimuli but also provides an adaptive mechanism. It is of interest, however, to point out that this axon has its own adaptive mechanism. Even when the capsule is removed and the generator potential persists throughout the static phase of stimulation, only a few action potentials are generated at the onset of stimulation.

MEISSNER CORPUSCLE: A receptor with structural characteristics similar to that of the Pacinian corpuscle is the Meissner corpuscle. These corpuscles are situated in the dermal papillae of glabrous skin and are especially numerous in regions such as the finger tips and other sites with tactile sensitivity. In man, they are oval and about 200 x 100 um along major and minor axes. The base of each corpuscle is penetrated by up to 10 axons. These axons are myelinated but upon entering the capsule the myelin is lost so that the axons terminate naked. The terminals spiral and branch among the processes of capsule cells whose cell bodies are at the periphery of the entire structure. As in Pacinian corpuscles, the capsule cells are Schwann and perineurial cells and there are spaces filled with fluid and collagen fibers between processes of the cells. The functional identity of Meissner corpuscles has not been directly established; since the skin is thick the corpuscle cannot be seen in live preparations, which hinders direct correlative structural and functional studies. These corpuscles look much like Pacinian corpuscles, and thus one might predict that they too would be rapidly adapting velocity detectors. In fact, recordings from the foot pads of cats have shown rapidly adapting responses from large, rapidly conducting axons that could not be attributed to receptor endings other than those in Meissner's corpuscle.

LANCEOLATE ENDINGS OF HAIR FOLLICLES: Hair follicles are richly innervated. Several myelinated axons enter the dermal tissue of the hair follicle and each branches into a number of unmyelinated terminal processes; some run up and down the shaft of the follicle, others encircle it spirally. The terminal portion of these fibers lie close to the epidermal basal lamina, sandwiched between sleeves of Schwann cell processes. Fine collagen fibers surround the Schwann sleeves couple the terminals mechanically to the follicle. The terminals are activated by bending the hair shaft and the response is rapidly adapting. You can test its response to stimulation now by bending the hair on the back of your hand with a pencil tip. You will notice sensation while you are bending a hair and when you let go ot it, but not while you hold it steady. The adaptive mechanism of the hair follicle is not known, but certainly proper function of the receptors depends on a non-neuronal element, the hair.

RUFFINI CORPUSCLE: Whereas the capsule of the Pacinian corpuscle dampens stress placed on its nerve terminal, there is a class of receptors where the capsule and its contents are arranged in such a way that they transmit stress to the terminal. Striking examples are the terminals associated with Ruffini corpuscles which are slowly adapting. To establish the structure of these receptors, Iggo and his colleagues recorded from nerve fibers while stimulating the skin until the area of highest sensitivity for an axon was found. Stainless steel wires were driven into the tissue on either end of this area marking the receptor's site for subsequent electron microscopy. Each corpuscle is an elongated spindle up to 2mm long and 0.1mm wide. The capsule consists of several layers of perineurial cells continuous with the shaft around the incoming axon as in the capsules of Pacinian and Meissner corpuscles. The axis of the capsule is formed by longitudinal collagen fibers which are continuous at either end of the capsule with collagen fiber bundles in the surrounding tissues. The nerve fiber penetrates the capsule at its equator or at one pole and breaks up into numerous fine unmyelinated branches which surround and penetrate between the collagen fiber bundles.

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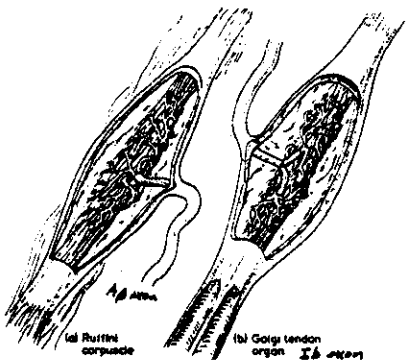
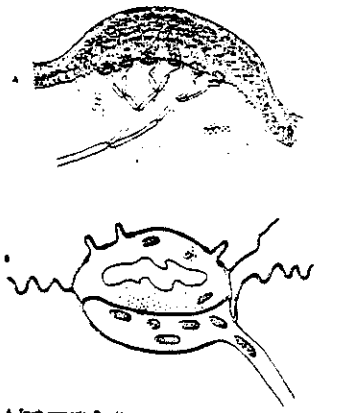
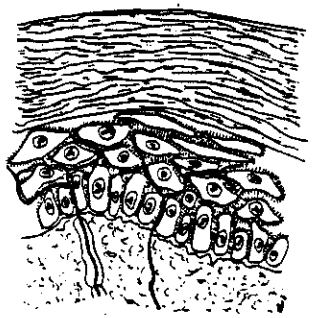


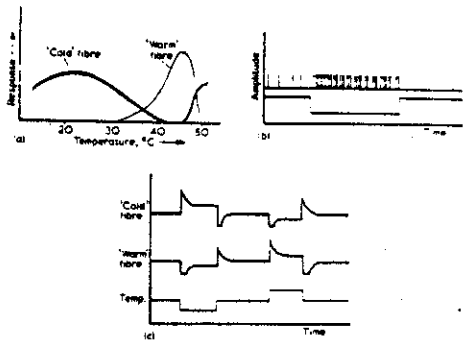
Diagram showing the organization of (a) a Ruffini end organ and (b) a Golgi tendon organ. Note the similar arrangement of terminals around the connective tissue cores, and the outer capsule, diagrammatically cut away to show the interior. ((a) After Chambers *et al.*, 1972, (b) after Schoultz and Swett, 1972).



A, Touch corpuscle. The skin over a touch corpuscle is thickened, as is the dermal core beneath it. A large, myelinated fiber innervates the corpuscle. Branches of the fiber lose their myelin, penetrate the basement membrane, and end on a series of special cells called Merkel cells. The touch corpuscle signals highly localized mechanical stimuli. It has a low threshold and is slowly adapting. B, Merkel cell. The nerve fiber terminal is shown approaching a Merkel cell from below. The Merkel cell contains the usual organelles, but in addition has numerous granulated vesicles. There are specialized areas of contact between the Merkel cell and the nerve ending. These may serve as low resistance paths for current. Alternatively, the granules may represent a chemical transmitter that might be responsible for communication between Merkel cell and nerve fiber. The processes extending from the upper surface of the Merkel cell and underlying epidermal cells (not superficial to it may be distorted by mechanical stimuli. (After Iggo and Muir, 1969)



Free endings. The terminations of two afferent fibers as free endings in the skin are shown. The fiber on the left is a finely myelinated axon, while that on the right is unmyelinated. The endings penetrate the basement membrane and ramify among the cells of the lower layers of the epidermis. Endings of this type may signal touch, pain, or temperature, depending on the nature of the membrane of the particular terminals.



Characteristics of thermoreceptor responses

(a) Static response amplitudes of 'cold' and 'warm' fibres at various temperatures, including the 'paradoxical cold' response at high temperatures. (After Hensel, 1973a.)

(b) Discharge pattern of a 'cold' afferent unit; note that the spikes occur in bursts in the slowly adapting part of the response.

(c) Diagram of the different responses of cold and warm receptors to heating and cooling. (After Hensel, 1973a.)

The way that this receptor is thought to work is that when tension is applied to the collagen fibrils, they tighten upon the the nerve endings, so stimulating them. If this were so then one would predict that the receptor would be most sensitive to stress in line with the capsule's long axis, the direction of the collagen fibers. Iggo and his colleagues have shown that terminals of Ruffini corpuscles are directionally sensitive. If skin is stretched along one axis the Ruffini axon terminals may give a strong response while stretch at right angles to this axis which may pull the collagen fibers apart and loosen tension on the terminals, gives no response at all. Thus the current view is that not only is the capsule and its contents arranged in such a way as to transmit tension, produced by stretch, to the nerve terminal, they aid in making it sensitive to stretch in a particular direction.

MERKEL'S DISCS (TOUCH CORPUSCLES): On the hairy skin of several mammalian species, including man, there are special sense organs, touch corpuscles, which can be seen grossly. Each corpuscle forms a dome-like mound on the skin; the dome has a diameter of several hundred micrometers. The epidermis of the corpuscle is thicker than that of the surrounding skin and the dermal core of the corpuscle contains an especially fine and dense meshwork of collagen fibers. Fingers from the dermal core invaginate the epidermis deeply and are tightly attached to the epidermal basal lamina, providing the touch capsule with a rigidity that hinders mechanical distortion unless a probe is placed directly upon it. Just superficial to the basement membrane of the epidermis are several specialized cells--Merkel cells. One of their obvious characteristics is that they contain a number of granular vesicles which structurally resemble catecholamine containing vesicles in axon terminals of neurons in the sympathetic nervous system where catecholamines are synaptic transmitters. There is but one sensory axon (myelinated) per touch dome. Its unmyelinated terminal branches penetrate the epidermal basal lamina, invaginate the deep surface of the Merkel cells, and form a flat disc-shaped ending that lies in close apposition to the Merkel cells. These mechanoreceptors are exquisitely sensitive to vertical displacement of the dome and are slowly adapting. The stimulus must be applied directly to the dome, however; application several microns away has no effect. It is not clear what roles, if any, the Merkel cells play in reception. An early suggestion was that they may release catecholamines in response to the stimulus and this chemical activates the terminal. Histochemical methods that detect catecholamines in nerve cells have not done so in these cells. This of course does not rule out some other transmitter. The highly localized sensitivity of this receptor is attributed to the structural support of the dermal core of the corpuscle which prevents distortion of the corpuscle when a stimulus is near but not on it. Touch corpuscles are not located only in hairy skin. They are also abundant in glabrous skin, especially in the depressions between friction ridges of the fingers and toes areas of high tactile sensitivity. Because of the ridges they are not seen as domes in these areas.

FREE ENDINGS: Thus far we have talked about receptor terminals as associated with some non-neuronal tissue specialization such as a hair or a capsule. there are numerous fine terminal branches of small myelinated or unmyelinated sensory fibers present throughout the body. They are especially prominent in the superficial protion of the dermis. Terminal branches of some small axons penetrate the epidermal basal lamina and end among the deeper epidermal cells. Recordings from nerves in many regions of the body show that there are slowly conducting, and thus small, axons that respond to mechanical stimulation. The encapsulated receptors, as I have described above, have large diameters with rapid conduction velocities, it is reasonable to associate the small mechanoreceptors with free nerve endings. Such mechanoreceptors are of the slowly adapting variety. That free endings can be mechanoreceptors has been demonstrated by studies on the cornea. The cornea is very sensitive to mechanical stimuli but there are no

encapsulated endings within it.

Thermoreceptors and nociceptors--both of these receptor types are characterized by FREE NERVE ENDINGS. They are terminals of small myelinated or unmyelinated axons, and are slowly adapting.

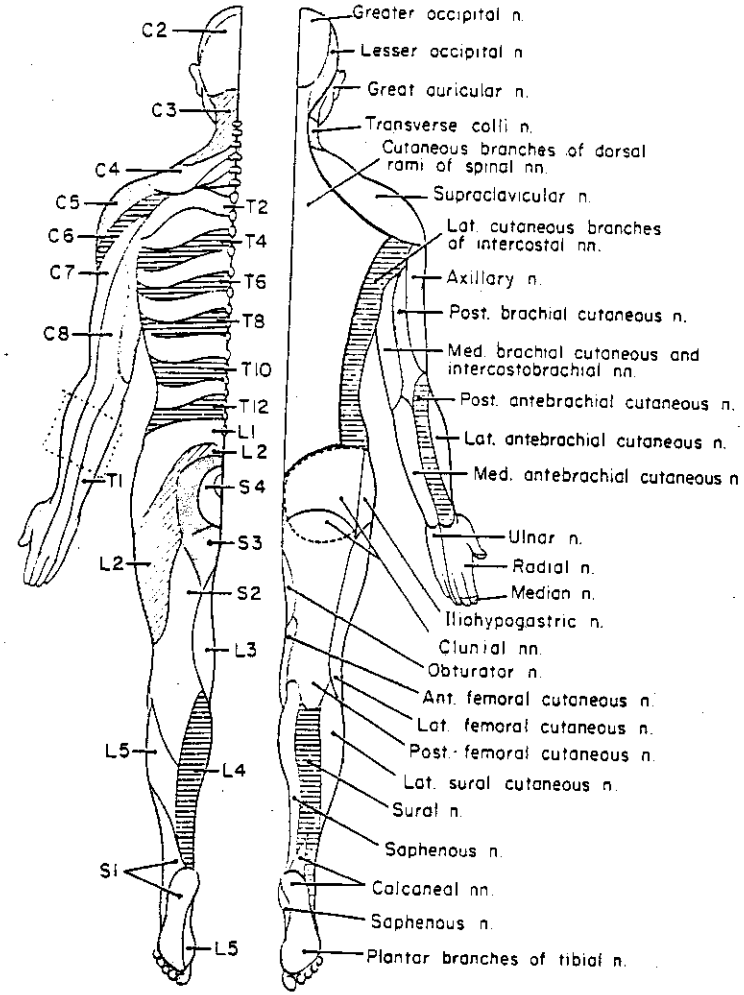
Thermoreceptors are uniquely sensitive to either warming or cooling stimuli. Both warm and cold receptors show a static discharge pattern related to the resting level of the skin temperature. In warm fibers of cats, the discharge frequency is greatest at about 45°C, diminishing rapidly on either side of the optimum. Cold receptors have their highest discharge frequency around 20°C. Cold receptors are also activated strongly at temperatures above 45°C, an anomaly that may be related to our subjective experience of paradoxical cold at high skin temperatures. By determining the thermal diffusion coefficient of epidermis and dermis and recording from axons while analyzing rapid temperature jumps it has been possible to determine how deeply cold receptors are situated in the skin. In the cat they are near the epidermal-dermal boundary. Warm receptors are probably somewhat deeper--well within the dermis. Hensel located cold spots about 100mm in diameter on the cat's nose, marked the spots and sectioned them for elctron microscopy. At these spots he found a small myelinated axon that penetrated the epidermis to terminate (unmyelinated) among the basal layer of epidermal cells. Warm receptors have not been structurally identified.

Axons whose terminals respond exclusively to noxious or potentially damaging stimuli are also distributed throughout the body but are particularly numerous in dermal and subcutaneous connective tissue. Two distinct categories of nociceptors have been described in mammals: a) those that respond exclusively to mechanical stimuli; and b) those that respond to both mechanical and thermal stimuli. The thresholds for either stimulus is much higher than for ordinary mechanoreceptors and thermoreceptors.

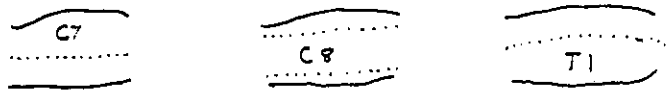
Receptive fields

A single afferent fiber may supply one or more receptor organs. The area that when stimulated causes a discharge in the afferent fiber is said to be its receptive field. In general, single afferent fibers have relatively discrete receptive fields. The receptive fields of several afferents may overlap, since receptor organs supplied by the fibers may be topographically intermingled.

The size of the receptive field of a particular type of receptor may be different, depending on the location of the receptor. For instance, many cutaneous receptors have small receptive fields if they are located on the distal part of an extremity, but the receptive fields of comparable receptors located on the proximal extremity or on the trunk may be much larger. Since sensory discrimination is much better developed on the distal parts of the extremities, there is reason to attribute this in part to the finer sizes of the receptive fields of the sense organs in this part of the body. Another factor, however, is that the density of innervation is also greatest on the distal extremities and so there are many more receptors for a given area of skin on the fingers, for instance, than on the back.



. Posterior view of dermatomes (*left*) and cutaneous areas supplied by individual peripheral nerves (*right*).



Dermatomes

A dermatone is the area of skin which is innervated by a single segment of spinal cord. In the trunk region the dermatomes are simple rings, but in the limbs they are more complex with some odd discontinuities. While there's no need to memorize the dermatomes, it's of great clinical importance to know that dermatome maps are available. For example, in the very common condition of a herniated intervertebral disc, which may compress a single dorsal root, examination of the region of skin whose innervation is affected allows one to determine the position of the lesion in the vertebral column.

Adjacent dermatomes overlap considerably, so that, for example, the edges of dermatomes C6 and C8 meet in the middle of dermatome C7 (see sketch). This overlap is greatest for touch sensation, and minimal for pain sensation, so when looking for evidence of a single-root lesion, it's particularly useful to test for sensitivity to pain--there may be no loss of touch.

Proprioceptors

We now turn to receptors within muscles, tendons, and joint capsules. Clearly, there are pain receptors in and around muscles and joints--any athlete will readily testify to this. I want to focus here on mechanoreceptors--the receptors that provide information necessary for coordinated and effective movements of the body. Again we are interested in the receptors themselves; details of the role they play in movement will be considered in another lecture by Dr. Baylor.

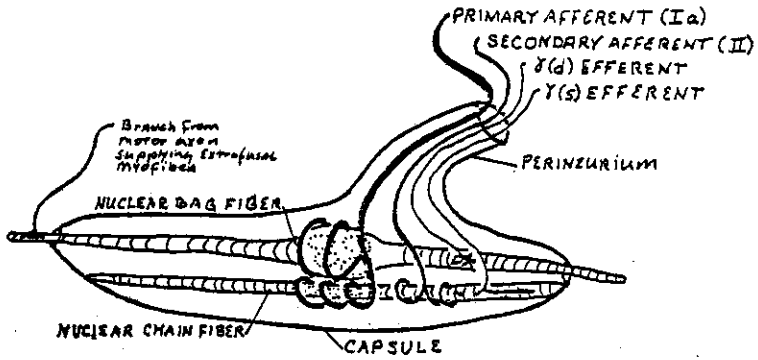
Muscle spindles

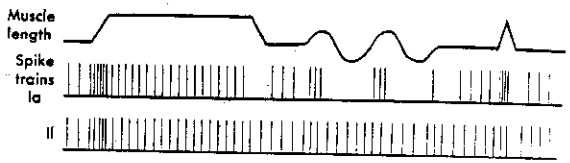
If one records from a nerve to a muscle while pulling on the muscle, axon discharges are clearly detected. These discharges are not derived from distorted motor axon terminals at the neuromuscular junction but from well-defined sensory elements within the muscle and its tendon. These receptors have the job of detecting stretch and tension in the muscle. Measurements on the rate of conduction in the sensory axons and their diameter show that there are 2 distinct axon types. Both are type A fibers and are designated type I and II (see later). Both I and II innervate muscle; only I innervates tendon. As you can see from the Table on page 11, type I afferents are the largest axons in the body. Each of the two types within the muscle respond differently to stretch sending different sorts of information to the CNS. The type I(b) component within the tendon responds differently to the type I(a) component in muscle as discussed below. Here we focus on the muscle afferents.

The two muscle afferents differ in that the type I afferents are sensitive to the dynamic phase of stretch; i.e., when the length of the muscle decreases and increases; type II afferent are relatively unaffected by the rate of stretch but are sensitive to the final degree of stretch, i.e., they respond preferentially to the static component of stretch.

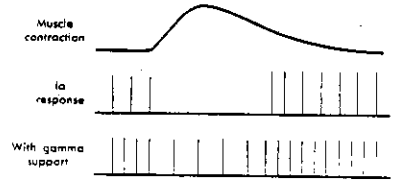
Terminals of both types of axons are closely associated with muscle fibers having special features. The special myofibers are situated in small groups each partially enclosed by a spindle-shaped capsule (continuous with perineurium as usual)--hence the name muscle spindle. There are 2-12 myofibers in a muscle spindle and there may be hundreds of spindles in a particular muscle. Each special intrafusal myofiber possesses the normal contractile apparatus of the

MUSCLE SPINDLE





Responses of afferent fibers from a muscle spindle. The upper trace shows changes in the length of the muscle containing the muscle spindle. The muscle length is changed with various wave forms of stretch and release of stretch. The middle trace shows the response of a group Ia afferent fiber from the primary ending of the spindle. Note the high dynamic sensitivity of this type of ending. The lower trace shows the response of a group II afferent fiber from a secondary ending of the spindle. Note the low dynamic sensitivity.



Response of a muscle spindle afferent to muscle contraction with and without activation of gamma motor fibers. The upper trace shows muscle tension during a twitch produced by stimulation of alpha motor axons supplying the muscle. The middle trace shows the response of the group Ia afferent fiber from the primary ending of a muscle spindle contained within the muscle. Note that the afferent discharge ceases during the contraction because of the unloading effect on the spindle as the extrafusal muscle fibers shorten. In the lower trace, gamma motor axons are excited in addition to the alpha motor axons. The spindle is not unloaded as much by contraction of the extrafusal muscle fibers, because the intrafusal muscle fibers also shortened. This allows the continuation of the discharge of the Ia fiber despite contraction of the muscle

larger extrafusal myofibers situation outside the capsule except in their equitorial region; here the myofibrils are confined to a thin subsarcolemmal layer and the middle of the fiber is occupied by muscle cell nuclei. Two types of intrafusal fiber are distinguishable by a number of features. One obvious difference is in the arrangement of nuclei, which gives the different types their names; nuclear bag fibers in which the equitorial nuclei form several rows, and nuclear chain fibers with a single file of equitorial nuclei. (In both fiber types, myonuclei are situated elsewhere in the usual subsarcolemmal position as found in extrafusal fibers.) The bag fibers are wider and several times longer than chain fibers. The bag fibers leave the poles of the capsule to terminate in the connective tissue binding the extrafusial myofibers while the chain fibers end within the capsule.

Type Ia and II axons enter each capsule where they lose their myelin sheaths. The unmyelinated terminal portions of type Ia end by coiling around the equitorial regions of both bag and chain fibers; these are primary or annulospiral endings. The type II axons terminate on a zone on either side of the equitorial region. These secondary endings occur commonly on chain fibers, rarely on bag fibers. Matthews, who has contributed considerably to our understanding of how the muscle spindle works, has attributed much of the difference in response between type Ia (primary) and II (secondary) endings to specific structural features of the spindle myofibers. When the fibers are stretched it may be that the more viscous regions of the muscle, i.e., the portions in which myofibrils occupy the majority of the cross sectional area will stretch more slowly than the more elastic equitorial regions containing the central nuclei. A stretch will initially result in a lengthening of the equitorial region subjacent to the primary endings, and hence stimulate the endings. After a short period the sarcomeres beneath the secondary endings will stretch stimulating the secondary endings and relax the tension on the primary endings.

Unlike the other receptors we have discussed so far, muscle spindles are designed to enable the CNS to modify or control the activity of the receptors they contain. This influence is exerted by way of nerve fibers that innervate the two types of intrafusal fibers in the spindle. Based upon their conduction velocity they fall into the Aγ (gamma) classification enter the capsule along with primary and secondary axons and terminate near the poles of the spindle. There are actually two sorts of gamma fiber, one that influences responses to the dynamic component of stretch, and one that influences responses to static stretch; these two fibers are known as γd and γs or gamma 1 and gamma 2. From what we have said above one would expect that γd would end on nuclear bag fibers and γs would end on chain fibers. In fact, morphologically each type intrafusal fiber has its own distinct type of neuromuscular junction; bag fibers have small terminals with varicose branches that look like clusters of grapes (en grappe terminals) and chain fibers have a narrow elongated terminal (trail ending). Finally, the bag fibers have a second motor endplate, typical of those on extrafusal fibers, on regions outside the capsule. These are derived from axons that innervate extrafusal fibers. Contraction of the polar ends of the muscle spindle has the same effect as stretching the muscle; the equitorial portion of the intrafusal nerves is stretched resulting in discharge of afferent.

The role of the muscle spindle, as a detector of changes in muscle length during movement will be discussed in other lectures. It is worth pointing out, however, that without the efferent innervation of the intrafusal fibers, the spindle function would be severely limited. The spindle is in parallel with

the extrafusal myofibers. Thus contraction of the extrafusial fibers in muscle movement tends to shorten the spindle. If intrafusal contraction (through its motor innervation) is experimentally prevented the spindle afferents cease firing or slow down during extrafusal contraction. However, intrafusal contraction maintains afferent firing during the extrafusal contraction. Thus the gamma efferents reset the sensitivity of the spindle during extrafusal contraction so that it is capable of sending information about muscle tension during all stages of muscle movement.

Golgi tendon organ (see figure of Ruffini corp. --slowly adapting)

Golgi tendon organs which also respond to tension are situated within tendons near the myotendonal junctions. They are structurally similar to Ruffini corpuscles of the dermis (see above); they consist of a capsule that surrounds bundles of collagen fibers and the terminal processes of the sensory axon that enters the capsule is insinuated between the collagen bundles. These receptors are in series with the muscle and thus they respond best when the muscle contracts. They are slowly adapting as are the terminals of Ruffini corpuscles. The firing of the terminals during tension is attributed to tightening of the nonstretchable collagen bundles upon the nerve terminals.

Joint receptors

Finally we come to the innervation of joints. Much of our sense of limb or joint position is derived from receptors situation in and near joint capsules. Clearly we also receive information about where our limbs and bodies are from our eyes. But if anesthetic is injected into joints a sense of position that does not depend upon vision is markedly reduced, if not abolished. Goldscheider was the first to demonstrate this point by injecting anesthetic into the joints of the fingers. As you recall, muscles that flex and extend the fingers are situated in the forearm and they are connected to the fingers by long tendons. Thus Goldscheider could rule out that anesthetic was influencing muscle receptors and concluded the diminished position sense resulted from inactivation of joint receptors. Conversely, position sense is retained if muscles are denervated or anesthetized while joint innervation is unaltered.

There is no one particular class of joint receptor; instead there are several types of joint receptors in each joint and each receptor is similar to ones that have been described for other tissues. There are Golgi tendon organs--Ruffini corpuscle type receptors that are slowly adapting, Pacinian corpuscles that are rapidly adapting, and free terminals of small myelinated and unmyelinated axons. Among the free terminals there are not only mechanoreceptors but pain receptors as well. In general, each receptor responds to positions only within a small angle of movement rather than the entire range. How information from each of the different receptor types is utilized in sensing position is not known.

Until recently Goldscheider's oft repeated experiments were taken to mean that we receive no sense of position from muscles themselves. The information conducted by afferent from muscle spindle reaches the cerebral cortex but it was thought not to reach the levels of consciousness. However, recent studies on patients who have received an artificial hip joint and thus have had their joint capsule removed, indicate that spindle afferents provide information for determining position, at least at some joints; those patients still had a sense of position of the lower limb. Moreover, Matthews has pulled on surgically exposed muscles to fingers without moving the joints or

distorting the joint capsules; the muscle stretch alone was enough to give the patients a sense of finger movements.

Diameters and conduction velocities of different types of receptor axons

I have indicated that axons of the mechanoreceptors associated with tissue specializations are heavily myelinated and thus conduct action potentials rapidly; axons of mechanoreceptors that have free endings and axons of nociceptors and thermoreceptors are thinly myelinated or unmyelinated and thus conduct slowly. Let's now take a closer look at conduction rates and diameters to see where sensory axons fit in the spectrum found in peripheral nerves.

In an other lecture Dr. Nicholls is discussing the action potential of single axons. If one stimulates a peripheral nerve and records from the same nerve, it is possible to detect the activity of groups of axons. An action potential recorded simultaneously from a number of axons is a compound action potential.

The compound action potential exhibits differences dependent upon experimental circumstances. For example, the size of the compound action potential increases as the stimulus strength increases. Furthermore, the compound action potential is not all or nothing. The explanation for this behavior is straightforward. As we have indicated nerve fibers are not uniform in size. They range from small unmyelinated axons less than 1μm in diameter to large myelinated axons over 20um in diameter. The threshold to electrical stimulation decreases with an increase in diameter. Moreover, the conduction velocity of action potentials in large fibers is greater than the conduction velocities of small fibers. Thus large fibers will be acrivated at lower stimulus strength and conduct faster than smaller fibers. When recording electrodes are placed at some distnace from the stimulating electrode the effect of the range of different sized fibers comes into play. The action potentials in the largest axons will arrive at the recording electrodes first, the action potantials in the progressively smaller fibers will arrive later. If the spectrum of fiber size were uniform, the compound action potential recorded at a distance from the point of stimulation might be expected to resemble a prolonged action potential. However, the spectrum of fiber sizes is not uniform. There are groups of large, intermediate and small myelinated fibers and a group of tiny unmyelinated axons which have more or less distinct conduction velocities. Subgroups have been further detected based on other conduction characteristics which we will not go into here. Myelinated components in most nerves are classified as Type A fibers and the unmyelinated components, Type C. In some nerves that lead to ganglia which innervate viscera (which we will discuss in another lecture) there is a myelinated B component which overlaps in diameter and conduction velocity with small Type A fibers that have other conductive characteristics to distinguish them. Finally Type A fibers can be further subdivided according to diameter, into α, β, γ and δ subdivision. Where sensory axons fall within these classes is shown in the following table.

	Group	Diameter (m)	Conduction Velocity m/sec	Group (see Below)	
Motor axons to extra fusal muscle fibers	Aα	12-20	72-120m	I	Primary muscle afferent (Ia), tendon spindles (Ib)
Pacinian, Ruffini's, Merkel's, Hair Meissner's	β	8-12	48-72	II	Secondary muscle afferents
	γ	4-8	24-48		Muscle spindle efferents
Free mechanical, pain, temperature	δ	1-4	6-24		
	B	≈3	3-15		Preganglionic autonomics
Pain,temp.	C	.1-1	0.5-2		Postganglionic autonomics

We began this lecture with the simple notion that cutaneous receptors and proprioceptors have their cell bodies within ganglia and that all of the cells in these ganglia had the same general appearance varying only in size. It is now clearly apparent that individual cells vary considerably not only in the tissue they innervate but in their sensitivity to different stimuli. Moreover, non-neuronal elements closely associated with the peripheral terminals of the mechanoreceptors play important roles in determining the sort of mechanical stimulation they respond to and in some cases, the sort of responses they generate. Over the next several weeks, the job is to find out the location and connection of pathways within the CNS that convey information generated by the receptor cells.

2. ORGANIZATION OF THE AUTONOMIC NERVOUS SYSTEM

The ANS is involved in the regulation of a wide variety of "visceral" activities: maintenance of appropriate blood pressure and patterns of blood flow, control of body temperature, genital function, certain aspects of metabolism, digestive and excretory activities, certain aspects of respiration, etc. The ANS influences these activities by regulating the heart beat, the contractions of smooth muscle in a variety of tissues, the secretions of certain glands, and the mobilization of fat from at least brown adipose tissue. This talk deals with the structural organization of the autonomic nervous system, first at the level of gross anatomy and then at a microscopic level, the influence of different portions of the autonomic nervous system on different organs, and the identity of neurotransmitters at autonomic synapses.

The nervous system can be divided into somatic and visceral portions. In general, the viscera system deals with the innervation of blood vessels, glands, hair follicles and internal organs (or, more precisely, the smooth muscle, cardiac muscle or gland cells associated with these structures) and, in doing so, regulates the "visceral" activities of the body. The somatic system deals with the rest of the body, mainly skeletal muscles, sensory organs of the skin (but also certain special sense organs such as those of sight and hearing). Traditionally, the term autonomic nervous system applies to the peripheral motor components that regulate visceral functions. As we will see below, this includes: 1) the final neurons (visceral motor neurons) which are entirely outside the CNS and which innervate smooth muscle, cardiac muscle, and relevant glands; and 2) the penultimate neurons which innervate those motor neurons and whose cell bodies are in the CNS. Some people include in the definition of the autonomic nervous system portions of the brain that are heavily involved in the control of visceral activities and which ultimately work through the peripheral neurons; others object to this because such brain areas exert an influence on and are influenced by somatic functions as well. Similarly, visceral sensory fibers are often not included in the definition because they too influence somatic activities and their anatomical relationship to the CNS is generally indistinguishable from that of somatic sensory neurons. For our purposes today we will adopt the traditional definition of the autonomic nervous system as a two-neuron peripheral motor component but keep in mind that these neurons can only do their job on the basis of information received from visceral sensory neurons and from higher centers in the CNS. A note on the course of some visceral sensory neurons will be given at the end of the lecture.

Gross anatomy

One of the most outstanding features of the autonomic nervous system is that its motor axons do not have their cell bodies in the CNS; they lie outside of the spinal cord—indeed, outside the vertebral column—in ganglia. There are four types of autonomic ganglia based upon their location: a) paravertebral, situated alongside the vertebral column; b) prevertebral, in front of the column, usually on or around blood vessels of some of the large visceral organs, c) terminal, situated in the head near the organs they innervate; and d) intrinsic or intramural ganglia, which are collections of scattered nerve cells within the walls of the organs they innervate.

Regardless of which type of ganglion the motor cells are situated in, the pattern of innervation is, according to the classical view, basically the same.

Each motor cell receives input from axons (preganglionic) of nerve cells in the spinal cord and in turn sends its axon (postganglionic) to end upon smooth muscle, cardiac muscle or a gland. Thus, in contrast to the peripheral part of the somatic motor pathway which involves only one synapse (onto the target), the peripheral pathway of the autonomic system involves two (one on the target and one in the ganglion). Recent work indicates that there are exceptions to this rule and some of those will be discussed later in this talk.

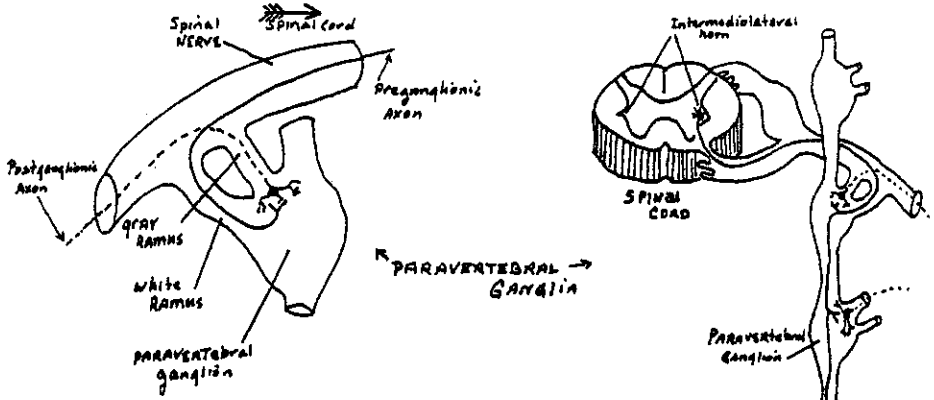
Although the paravertebral ganglia supply axons to some organs, they largely innervate blood vessels, sweat glands and sebaceous glands of hair follicles in the body wall and limbs. There is a paravertebral ganglion for each member of the 31 pairs of spinal nerves. In mammals, groups of the cervical autonomic ganglia are fused so that instead of eight, one finds a superior cervical ganglion formed by the first four ganglia, a middle formed by the fifth and sixth ganglia, and an inferior ganglion formed by seventh and eighth. Sometimes the inferior cervical and first thoracic ganglia fuse to form what is called a stellate ganglion. Because of their large size the superior cervical and stellate ganglia have been very useful for experimental purposes and references to them will be made again later in the course.

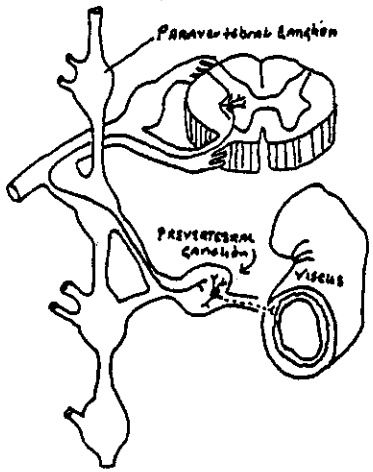
Each ganglion sends postganglionic axons in a distinct bundle to immediately join the spinal nerve of its segment before the extensive branching pattern unfolds. The preganglionic axons to the cells in the paravertebral ganglia travel from the spinal cord via the ventral root. Within the peripheral nerve they pass beyond the entry of the bundle of postganglionics and then leave the nerve to enter the ganglion, also in a discrete bundle. Inspection with the naked eye reveals that the bundle of postganglionic axons is gray while that of the preganglionics is white, hence they are designated as white and gray rami (ramus=branch). The whiteness is due to the presence of a thin layer of lipid-containing myelin wrapped around the preganglionic axons; the postganglionic axons are, in general, unmyelinated.

As you know from previous lectures, unmyelinated axons conduct action potentials much more slowly than myelinated ones. The paucity of myelin around preganglionic axons, and the lack of it around the postganglionics coupled with the synaptic delay in the ganglion, results in the peripheral autonomic pathway being much slower than the peripheral somatic motor system where there is only one, often myelinated, axon involved.

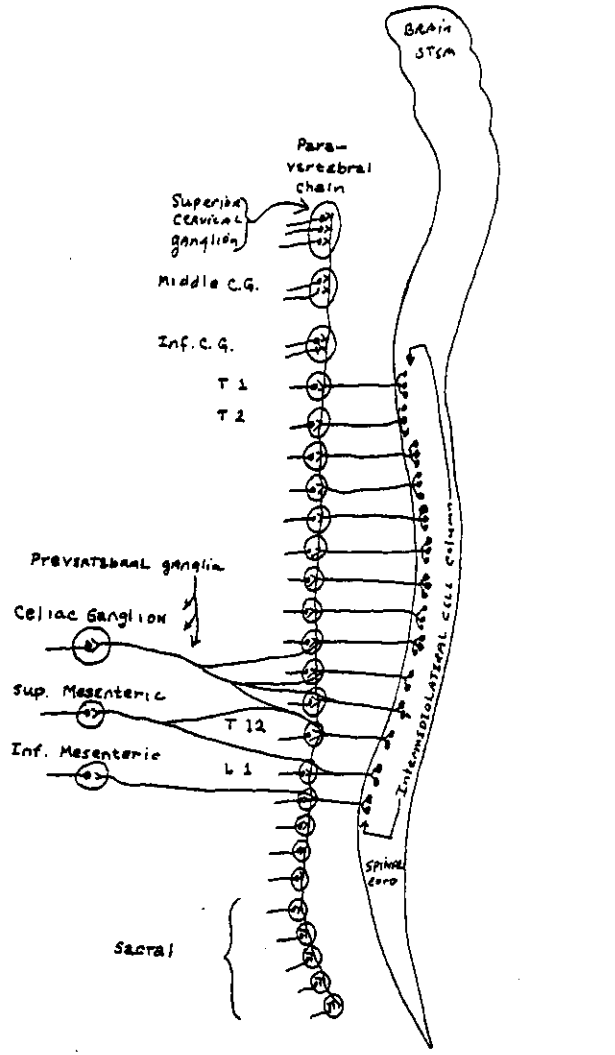
Gross inspection of the spinal cord also reveals gray and white zones. Here the gray matter is centrally located. In cross sections of the cord, it is seen as a butterfly-shaped area and contains cell bodies and dendrites of neurons. The white matter, again, consists largely of myelinated axons running from one part of the CNS to another. The gray matter is divided into "horns" (the wings of the butterly) which have profound functional significance. The dorsal horn contains cells on which many of the sensory axons terminate, while cell bodies of the somatic motor cells lie in the ventral horn. In the intermediolateral horn are cell bodies of the preganglionic axons of the autonomic system.

The ventral horns run the whole length of the cord and their somatic motor cells segmentally give off axons to the spinal nerves. On the other hand, the intermediolateral horns, and hence the cell bodies of the preganglionic axons, extend only from the first thoracic to the second or third lumbar segment. How, then, do the paravertebral ganglia of spinal nerves below the lumbar level and above the thoracic level receive their preganglionic supply? The answer is that preganglionic axons derived from a particular cord segment are not specific for the ganglion of their spinal nerve. Some of the axons arising from the lower

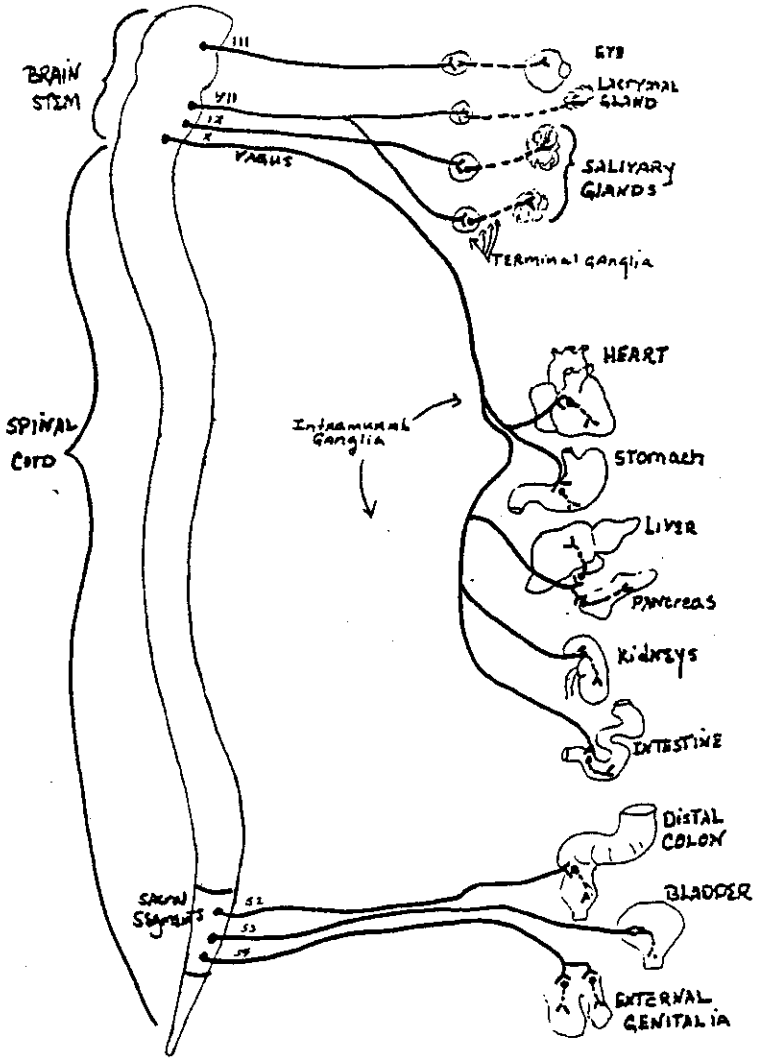




PREVERTEBRAL GANGLION



Sympathetic Division of the Autonomic Nervous System



PARASYMPATHETIC DIVISION of AUTONOMIC NERVOUS SYSTEM

thoracic and upper lumbar segments pass out through the ventral roots and spinal nerves of their segment of origin, enter the local ganglion by the white ramus and then, with or without synapsing there, pass via connectives through succeeding ganglia to reach the appropriate ganglion of termination below the thoracolumbar segments. Similarly, the upper thoracic segments send axons to the cervical ganglia; even in the mid-thoracic regions, axons from a particular segment terminate in ganglia of spinal nerve above and below. Thus, the paravertebral ganglia are not only connected by rami to peripheral nerves, but through connectives, in chain-like fashion, to each other; the paravertebral ganglia are frequently referred to as the chain ganglia.

To sum up: although all of the spinal nerves from cervical to coccygeal segments of the cord have paravertebral ganglia associated with them; all of the preganglionic axons to these ganglia come from the thoracic and upper lumbar segments of the cord. A corollary is that only those ganglia in the thoracic and upper lumbar regions have white rami.

The paravertebral ganglia innervate not only structures in the body wall and limbs but some also send axons to the head. Bundles of postganglionic axons arise from the cervical ganglia and course along the blood vessels of the neck and head to innervate, in addition to the vessels, sweat glands and hair follicles of this region, such structures as the dilator muscle of the iris, and the salivary glands. Thus the thoracolumbar portion of the cord, through its ganglia, influences targets throughout the whole body, from head to toe.

The paravertebral ganglia also send postganglionic axons to certain visceral organs such as the heart and lungs, but most of the viscera of the thorax, abdomen and pelvis are connected to the thoracolumbar segments of the cord through about one-half dozen prevertebral ganglia. These ganglia, unlike the paravertebral, are unpaired and lie near the organs they innervate. There are three main ganglia—the coeliac, superior, and inferior mesenteric ganglia. On their way from the cord to the prevertebral ganglia, the preganglionic axons travel in the ventral roots and pass through the chain ganglia—without forming synapses there.

So far we have been discussing a portion of the autonomic nervous system where ganglia receive preganglionic axons from only a fraction of the CNS—the thoracolumbar segments of the cord. This is the sympathetic division of the autonomic nervous system. There is another division which involves preganglionic axons from the brain and sacral portion of the spinal cord. The craniosacral portion of the autonomic nervous system is also known as the parasympathetic division.

The parasympathetic preganglionic cell bodies in the brain are situated in the mesencephalon and the pons, medulla, and the preganglionic axons are carried by cranial nerves III (oculomotor), VII (facial), IX (glossopharyngeal) and X (vagus). The preganglionic axons of III, VII and IX make synapses in terminal ganglia of the head. The terminal ganglion of III (ciliary ganglion) supplies smooth muscles of the eye (sphincters of the iris and ciliary muscles of the lens). The terminal ganglia of VII (the pterygopalatine ganglion and submandibular ganglion) and the terminal ganglion of IX (the otic ganglion) innervate lacrymal and salivary glands. The vagus nerve descends through the neck into the trunk of the body where it terminates in the intramural ganglia situated in the wall of various organs of the thorax and abdomen. The sacral segments (S2-4) send preganglionics to terminate in intramural ganglia or organs in the pelvis.

Action of sympathetic and parasympathetic nerves on specific effectors

The sympathetic and parasympathetic divisions of the autonomic nervous system innervate a variety of structures either together or independently. In those organs they jointly innervate, the effects of stimulating each division can be antagonistic or complementary to the other or the two affects can be somewhat different but not clearly opposite. The purpose of this section is to point out some of the organs the two divisions innervate, their influence on these organs and how the two systems interact. (for further details see Medical Physiology [XIII Ed] ed. by V.B. Mountcastle, pp. 783-812, 1974).

Stimulation of the sympathetic input to the eye dilates the pupil by contraction of the radial muscles of the iris (letting in light) and relaxes ciliary muscles of the lens (for distance vision). In the salivary glands, sympathetic stimulation brings about constriction of blood vessels but since sympathetic fibers innervate gland cells it also produces saliva. In the skin, blood vessels are constricted, hair follicles erect and sweat glands secrete—altogether influencing temperature (piloerection and vascular constriction increases body temperature; sweat decreases it). In the heart, sympathetic stimulation produces an increase in the rate (chronotropic effect) and strength (iontropic effect) of contraction. In the lungs, the bronchi dilate. In the spleen, the capsule contracts forcing blood into the circulation. In the gut, blood vessels constrict and peristalsis is inhibited. The medulla of the adrenal gland secretes adrenalin reinforcing almost all actions of direct sympathetic stimulation. There is a direct effect of sympathetic innervation which (in addition to the effect of circulating adrenalin) promotes glycogenolysis in the liver and a consequent liberation of glucose. The bladder's sphincter is constricted while the muscles of the wall are relaxed, preventing micturation.

Although the individual activities of the sympathetic division are quite diverse, they are physiologically compatable and can be seen in concert in animals under rage or stress conditions. This is the fight or flight reaction (W.B. Cannon). Under moderate circumstances, the different components of the sympathetic division work selectively, responses being initiated through reflex activity and by centers in the CNS.

The influence of the parasympathetic system in the eye is antagonistic to that of the sympathetic. The pupil constricts (contraction of the sphincter of the iris) and the ciliary body contracts for accommodation or near vision. In these cases the two divisions do not act on a common muscle. In the salivary glands the parasympathetic division dilates blood vessels and induces secretion by the secretory cells. Thus the parasympathetic and sympathetic division can produce secretion; stimulation of both produces a greater output than either one alone. This is a synergistic action. The heart presents an example of antagonism where the two divisions end upon the same muscle cells—the parasympathetic decreases the rate of the heart beat instead of increasing it. Stimulation of the parasympathetic system also constricts the bronchii, increases peristhalsis in the gut, and relaxes the sphincter of the bladder while contracting the bladder's wall, again in each case antagonistic to the sympathetic system. The blood vessels which dilate to produce erection of the penis and clitoris are the only ones to receive parasympathetic innervation (perhaps, too, salivary and certain meningeal blood vessels). All other blood vessels, the sweat glands and piloerector muscles receive only sympathetic innervation. The parasympathetic nervous system, except for highly abnormal circumstances, does not discharge as a whole (as can the sympathetic). Its components behave independently participating

IMPORTANT FUNCTIONS OF SOME AUTONOMIC PATHWAYS

FUNCTION	SYMPATHETIC	PARASYMPATHETIC
Iris	dilates the pupil (mydriasis)	constricts the pupil (miosis)
Lacrimal gland	little or no effect on secretion	stimulates secretion
Salivary glands	secretion reduced in amount and viscid	secretion increased in amount and watery
Sweat glands of head, neck, trunk, and extremities	stimulates secretion (cholinergic fibers) nerve fibers	little or no effect on secretion
Bronchi	dilates lumen	constricts lumen
Heart	accelerates rate, augments ventricular contraction	decreases heart rate
GI motility and secretion	inhibits	stimulates
GI sphincters	constricts	relaxes
Sex organs	contraction of ductus deferens, seminal vesicle, prostatic and uterine musculature; vasoconstriction	vasodilation and erection
Urinary bladder	little or no effect on bladder	contracts bladder wall, promotes emptying
Adrenal medulla	stimulates secretion (cholinergic nerve fibers)	little or no effect
Blood vessels of trunk and extremities	constricts	no effect

in specific reflexes or in specific CNS-directed activities.

Transmitters

The main neurotransmitters in the autonomic nervous system are acetylcholine (ACh) and norepinephrine (NE; noradrenalin). The preganglionic axons in both the sympathetic and parasympathetic nervous system release ACh which always excites the ganglion cell. The postganglionic parasympathetic axons can also release ACh but the postganglionic sympathetics, with few exceptions release NE. One exception is the postganglionic sympathetics that innervate the eccrine sweat glands; here the response to stimulation of sympathetic ganglia is blocked by atropine and potentiated by prostigmine indicating that these sympathetic fibers release ACh. Another exception is found in the blood vessels of skeletal muscles. Here, in addition to the usual vasoconstrictor axons (NE), there are cholinergic vasodilators. Usually stimulation of sympathetic nerves to blood vessels in skeletal muscle gives a complex or mixed response. However, by stimulating certain regions of the brain, one or another effect (dilatation or constriction) can be elicited separately. The vasodilatation has a cholinergic pharmacology while the vasoconstriction is sensitive to adrenergic blocking drugs. The adrenergic sympathetic fibers are constantly (tonically) active, regulating blood pressure. However, the cholinergic fibers seem to act only during emotional stress or in physical exercise, when skeletal muscles require more blood.

Synapses in the autonomic nervous system

Preganglionic axons--ganglion cells. The cholinergic nerve terminals of preganglionic axons are similar in their gross appearance to nerve terminals in the CNS; the terminals consist of a series of varicosities in close apposition to the cell body and dendrites of the postganglionic cell. The varicosities are the release sites of the terminal. Unlike the neuromuscular synapse in skeletal muscle there is no folding of the postsynaptic membrane but otherwise the synaptic fine structure is nearly the same. The varicosities contain synaptic vesicles, some of which are focused upon patches of dense material (active zones) that line the presynaptic membrane. These patches are not linear as at the neuromuscular junction but rather ovoid. Opposite dense material on the presynaptic membrane there is dense material lining the postsynaptic membrane. The terminals, their varicosities and the cell body and dendrites are covered by a blanket of Schwann cell processes.

Postganglionic axons--smooth and cardiac muscle, and gland cells. These synapses differ from the motor nerve terminals on skeletal muscle fibers in several ways. The axonal component of the synapse is usually one in a series of axonal varicosities (1-2μ long and 1-2μ in diameter) which in some cases are 200 Å from the muscle fiber or gland cell but in others may be several microns away. It is assumed that the varicosities release transmitter regardless of their distance from the postsynaptic cell. Although some of the varicosities are covered by Schwann cell cytoplasm as are terminals on skeletal muscle, others are naked. The Schwann cell has a basement membrane and this is continued over the naked varicosities. As in the case of skeletal muscle synapses, the basement membrane of the Schwann cell fuses with that of the smooth or cardiac muscle fiber, but where the presynaptic membrane is separated from the postsynaptic membrane by a space of only 200 Å the basement membrane does not pass through the gap.

Although there are these marked structural differences between the cholinergic synapses on skeletal muscle and smooth muscle, the cytoplasmic constituents of the terminals in both cases are similar, i.e., the varicosities contain synaptic vesicles,

a cluster of mitochondria and in the heart, at least, some of the vesicles are clustered around dense patches of material on the plasma membrane.

The noradrenergic synapses grossly resemble the cholinergic ones in that they consist of numerous varicosities which run at varying distances from the muscle fibers. However, there is a distinct cytoplasmic difference. The vesicles of the noradrenergic varicosities contain a dense core about 200 Å in diameter. Being aware of the vesicle hypothesis, one immediately wonders whether or not the difference in vesicle appearance has anything to do with the difference in transmitter. Once again there is biochemical evidence showing that transmitter within the terminals is contained within vesicles. In addition, when one injects into an experimental animal drugs known to deplete noradrenalin and then serve noradrenergic axonal varicosities in the electron microscope one finds that the dense cores have disappeared from the vesicles; and if one bathes similarly treated tissues in a solution of noradrenalin one finds that the dense cores reappear. So in addition to biochemical evidence, we have correlated pharmacological and anatomical evidence indicating that the transmitter at noradrenergic synapses is contained within vesicles. It is not clear whether or not the transmitter is released from vesicles as a direct result of axonal stimulation, and it is not clear whether the dense osmiphilic cores one sees in the electron microscope are noradrenalin or some other substance to which noradrenalin is bound and which is depleted along with noradrenalin.

In the mid-1950's, Falck and Hillarp observed that formaldehyde condenses with catacholamines to give a highly fluorescent product when viewed with ultraviolet light. Since then this histochemical technique has been extensively used in neurobiology. The reaction is sensitive to excessive amounts of water, so one usually takes a piece of tissue containing noradrenargic axons, freezes it quickly, dries it in a vacuum, and exposes it to formaldehyde vapors. When the tissue is then visualized with ultraviolet light, the noradrenalin containing axons fluoresce a brilliant green. If experimental animals are injected with noradrenalin-depleting drugs, the fluorescence disappears; if depleted tissues are immersed in noradrenalin, the fluorescence reappears. So here we have a technique which permits us to actually visualize the transmitter at known noradrenergic synapses. This method has been helpful in identifying areas in the brain where axons contain catacholamines, thereby suggesting sites where catacholamines might be transmitters.

Modifications of the classical view

As indicated above, the classical view of the peripheral autonomic nervous system is that it is a two-synapse pathway connecting the central nervous system with peripheral targets--one synapse in a ganglion, one synapse on the target. This view also supposes that the ganglion is simply a relay (although not one-to-one--there is some convergence and extensive divergence of preganglionic axons onto ganglion cells). Recent electron microscopy, fluorescence, histochemistry and pharmacological studies indicate that the classical view needs to be modified, in some areas, at least.

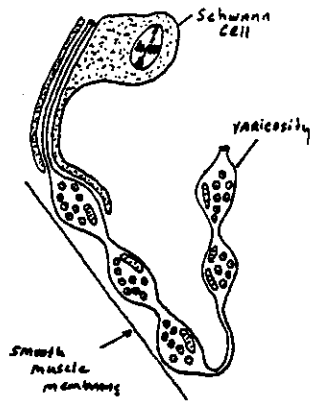
In many sympathetic and parasympathetic ganglia in addition to the usual principal neuron that projects to the peripheral target, there is a second class of nerve cell. They differ from the principal cells in several ways. They have smaller cell bodies (6-12μ in diameter vs. 15-40 um for principal cells), fluoresce much more intensely than sympathetic principal cells after Falck-Hillarp treatment for catecholamines and their cell bodies are packed with granular vesicles (in

sympathetic principal cells the granular vesicles are mainly in terminal varicosities). These cells are called Small Intensely Fluorescent cells or SIF cells. The principal cells greatly outnumber SIF cells--there can be only several hundred SIF cells in a ganglion containing several thousand principal cells. Like principal cells the SIF cells receive input from preganglionic axons. However, unlike principal cells their axons do not leave the ganglion but make contact on the principal cell dendrites. It is not yet clear what catecholamine SIF cells contain (and presumably release); one group claims dopamine while another claims NE. The prevalent view is that the SIF cells are interneurons and on the basis of pharmacological studies using catecholamines and catecholamine-blocking drugs, it is suggested that they are inhibitory. There is also evidence of connections between principal cells in both sympathetic and parasympathetic ganglia. Again, there are few of these connections compared to those made by preganglionics. In the sympathetic ganglia, connections between principal cells are presumably inhibitory. Together these findings indicate that considerable integration occurs in autonomic ganglia.

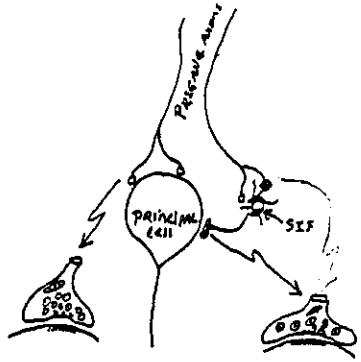
The innervation of the gut is even more complicated than the scheme just described. The intramural ganglia there are clusters of ganglia distributed all along the length of the gut and are embedded in a continuous network of nerve fibers--which altogether are called enteric plexuses. There are two enteric plexuses. One, the myenteric (Auerbach's) plexus, lies between the gut's longitudinal and circular muscle layers which are its target tissue. The other is the submucal plexus which is situated just internal to the circular muscle layer and appears to innervate primarily the exocrine gland cells of the gut. According to the classical view the ganglia would be parasympathetic. The neurons should be cholinergic, innervated only by cholinergic preganglionic axons, and should not be synaptically related to one another. There is now evidence that none of these presumptions is correct. Instead, the plexuses appear to be complicated integrative networks with their own sensory inputs which mediate local reflex activity. For example, reflex activity and even peristaltic waves can be induced by an artificial bolus (inflating a small balloon) in a stretch of intestine removed from the body. Intracellular recordings made from myenteric plexus neurons and muscle fibers while inflating an intralumenar balloon several centimeters away shows that the nerve and muscle are excited--much more so on the anal than oral side of the stimulus. The bolus evidently excites sensory cells which then signal the ganglion's motor neurons through interneurons. When excitatory transmission to the muscle is blocked by atropine, an inhibitory component can be seen. The inhibitory synapses are not blocked by NE inhibitors. There is some evidence suggesting that the transmitter of these inhibitory intramural neurons may be ATP. Finally, based on fluorescence histochemistry it appears that the adrenergic postganglionic sympathetic axons terminate primarily on ganglion cells instead of smooth muscle fibers. In agreement with physiological and pharmacological studies showing the complex nature of the innervation of the gut, four types of axon terminals have been found electron microscopically: 1) terminals packed with mitochondria which look like sensory terminals found in other regions of the body; 2) terminals with small agranular vesicles typical of cholinergic synapses; 3) terminals with small granular vesicles typical of adrenergic synapses; and 4) terminals with large electron opaque vesicles quite distinct from those of the adrenergic terminals and believed to belong to the inhibitory (ATP) nerve cells.

A note on visceral afferent fibers

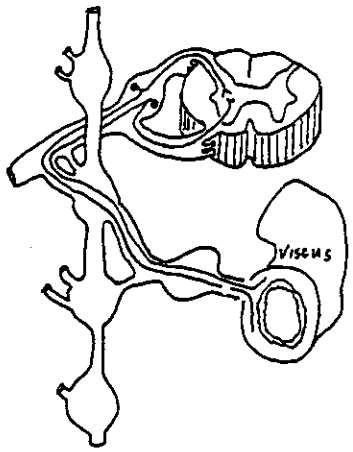
The viscera is heavily innervated by fibers that conduct sensory information to the CNS. Some are mechanoreceptors responding to pressure and tension, some are nociceptors responding to stimuli that tend to damage the viscus and some



Post ganglionic Sympathetic axon terminal



Inputs to principal cells in some Autonomic ganglia



Sensory axons of certain viscera

are chemoreceptors, such as those situated in the walls of the great vessels near the heart and detect changes in the concentration of CO_2 and O_2 and the pH of the blood.

In general, these afferent axons travel within the nerve bundles that carry the efferent axons, passing without synapse through autonomic ganglia (and rami) to reach the craniospinal sensory ganglia where their cell bodies are situated alongside those of the somatic portion of the nervous system. These, as you already know, are T-shaped unipolar cells; their central limb extends to the CNS where it makes synapses upon second order cells. Many of those in dorsal root ganglia reach the spinal cord through the dorsal roots as you would expect. However, in the cat, and perhaps other animals including humans, not all follow this route. Coggeshall and his colleagues, using the electron microscope, recently noted that at least 30% of the axons in the ventral roots of a variety of animal species are unmyelinated. Since all motor neurons are myelinated and since preganglionic (B) axons are thinly myelinated, the unmyelinated axons could not be accounted for. He thus set out to determine where their cell bodies were situated and their function. He fouund that when the ventral root was severed, the peripheral stump continued to contain unmyelinated axons while in the central stump they degenerated--the reverse of what you would expect if the cell bodies were in the CNS and axons left the spinal cord along with the motor fibers. He found no change in the ventral roots when he cut the dorsal roots central to the ganglion or when he cut on the spinal nerve, placing the cell bodies in the peripheral portion of one or both roots. The next step was to remove the dorsal root ganglion; all but a few of the unmyelinated axons in the ventral root degenerated, thus indicating that the axons are sensory cell axons. Coggeshall's groups then examined physiologically the unmyelinated axons of the ventral roots in the sacral region of the cat's spinal cord. They concluded that in the roots the axons are predominantly visceral afferents responding to distension of the rectum, bladder, and vagina.

Lesions to the peripheral sympathetic part of the autonomic nervous system

Most common among lesions of peripheral parts of the sympathetic system are lesions of the upper thoracic ganglia caused by stabbing or gunshot wounds, most commonly seen in wartime, mediastinal tumors, aneurisms of the aorta, glandular absesses of the neck and operative lesions. These leions are followed by abolished sweat secretion, and piloarrection of the head and arm on the side of the lesion, accompanied by vasodilatation. If the inferior cervical ganglion is included so that preganglionic fibers do not innervate the cervical ganglia) Horner's syndrome is usually the most enduring syndrome.

Horner's syndrome:

miosis - small pupil due to loss of functional innervation to dialator muscles in iris.

ptosis - drooping eyelid du' to loss of funct'onal innervation to tarsal muscles.

enophthalmos - sunken eyeball; reason not clear.

The same symptoms can occur when the spinal cord is damaged at segment T1 or above. (Why?)

Most people have an intuitive feeling for the meaning of the word "colour". We name colours in terms of their hues - red, green etc. We also know that by mixing two colours, we invariably end up with a third, totally different, colour. To understand the nature of colour perception and the changes in appearance of colour mixtures, we must first consider what aspect of light carries the colour information, and what receptors are responsible for detection of this information. We must also consider the way in which this colour information is processed at the different levels of the visual system, and how this processing effects the different colour sensations.

Physical Nature of Light

Light is a form of electromagnetic radiation, similar to radio waves, U.V. rays, X-rays, and γ rays. All these radiations differ only in wavelength and hence the energy of their photons. The wavelengths of visible light are between about 400 nm and 700 nm, only a small range in the electromagnetic radiation spectrum. The reason why the human eye can see certain radiations but not others is that the pigments in the photoreceptors absorb radiations significantly only in the 400-700 nm range. The light we see in ordinary life is invariably composed of a broad mixture of wavelengths. The physical composition of a light beam can be described by its power spectrum, which depicts the power carried by its different wavelength components. For example, Fig. 1a might describe the power spectrum of light irradiating from an electric bulb. (The distribution of light in terms of wavelength is also frequently described by its energy spectrum: energy and power spectra have the same shape.)

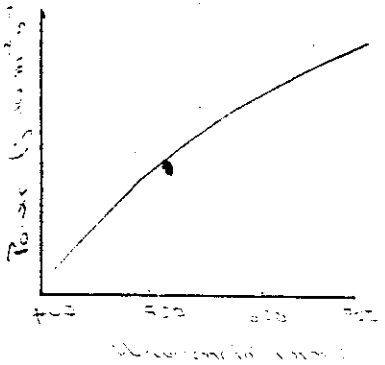


Fig. 1a

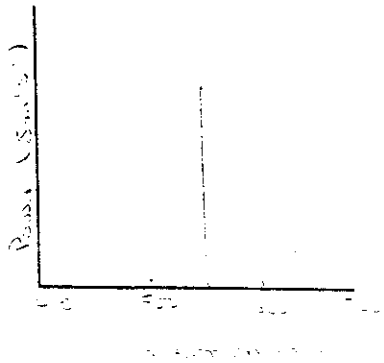


Fig. 1b

By passing light of broad wavelength distribution through special filters which absorb or reflect most wavelengths, one can transmit a band of wavelengths, even down to a bandwidth of the order of a nanometer (Fig. 1b). Since the human eye cannot resolve differences between radiations less than 1 nm apart in wavelength, such light of very narrow bandwidth is for all practical purposes monochromatic light.

Colour

What is colour? Suppose we project two equal-sized rectangular patches of bright light side-by-side on a screen. Suppose the right-hand patch is illuminated by monochromatic light of wavelength 460 nm, the one on the left being illuminated by monochromatic light of wavelength 550 nm at roughly the same intensity. The two patches will appear markedly different to normal observers and we say the patches have different colours. We name the hue of the right hand patch (460 nm) blue, and that of the left hand patch (550 nm) yellow. If we superimpose the two light patches, we can adjust their intensities until the resulting patch appea white. Visual psychophysicists have developed an empirical set of laws which allow one to predict colours which will result from mixtures of lights. Early visual psychophysicists reasoned from the behaviour of mixtures of coloured light that there must be at least three types of light receptor in the eye each of which responds best to a different wavelength region of the light spectrum. Physiologists have confirmed that three types of cone are responsible for colour vision.

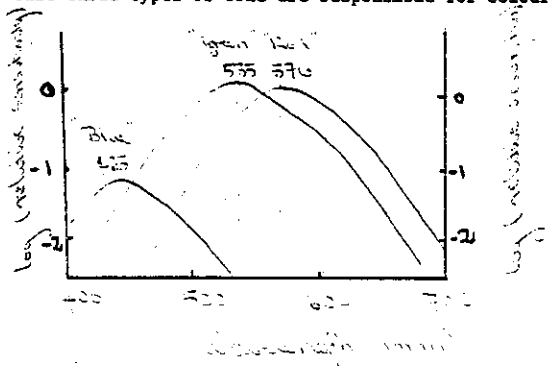


Figure 2. The cone pigment absorption spectra.

Rods and Cones

In the human retina there is one type of rod and there are three types of cone. The rods have a low threshold to light and are responsible for vision in very dim light (e.g. starlight). Such vision, which we call scotopic vision, has poor acuity (i.e. poor resolution of fine detail in the visual world) and no colour sensation. The cones have a higher threshold to light and are responsible for vision in normal daylight conditions, when the rods are completely insensitive.

Such vision, which we call photopic vision, has high acuity and full colour sensation. The gradual transition from scotopic to photopic vision as light level increases is called the Purkinje shift, during which cones gradually take over the role of rods in vision. This transition is characterized by a shift in the wavelength of maximum sensitivity for the human eye from 507 nm (roughly the wavelength of peak absorption for rhodopsin, the rod pigment) to 555 nm (resulting from the combined action of the three cone populations).

The rods and cones are each sensitive to light over a broad range of wavelengths, with their spectral sensitivity curves overlapping considerably (Fig. 2). The rod spectral sensitivity curve peaks at about 500 nm, while those for the three types of cones peak at 445, 535, and 570 nm respectively. They are called blue-sensitive, green-sensitive and red-sensitive cones, or in short, blue, green, and red cones.

When a photon is absorbed, each rod or cone receptor gives a stereotyped response that is independent of the photon's wavelength. In other words, each rod or cone signals only the number of photons which it has absorbed, and wavelength determines only the probability that a photon will be absorbed by a rod or cone (principle of univariance).

The remainder of the following discussion will be restricted to cones, since rods contribute little to colour vision (at the fovea, where rods are absent, colour vision is exclusively cone-mediated. Outside the fovea and at intermediate light levels when both rods and cones are active, rods play a minor role in colour vision.

Colour Matching

Much of our knowledge about human cone pigments was obtained from colour matching experiments. The science of colour matching is known as colorimetry. In colour matching experiments observers are presented with two patches of coloured light side by side. The one patch is illuminated by light of constant intensity; the other contains a mixture of different coloured lights, the intensities of which can be altered by an observer so that the two patches appear identical. (If the fixed light is yellow, normal observers can make a match by mixing red and green lights in the correct proportion.) It will be shown later how measurements of the intensities of the lights in the matching mixture yield information about pigment absorption spectra.

Colorimetry has revealed that the absorption spectra of the three cone pigments of 99% of the female population and of 92% of males are the same. The small group whose pigments differ are called colour blind. Colour blindness is an inherited, sex-linked recessive disorder. There are three categories: monochromats, dichromats and anomalous trichromats. Monochromats have either only rods (rod monochromats) or only rods and blue cones (cone monochromats). Dichromats lack either the red, green or blue cone pigment (protanopes, deuteranopes and tritanopes respectively). In the case of anomalous trichromats, the absorption spectrum of one of the cone pigments differs from that of the normal: in the cases of protanomaly, deuteranomaly and tritanomaly the red, green and blue pigments (respectively) differ. Monochromats can match two coloured lights by adjusting the intensity of one. In general dichromats need a mixture of two coloured lights to match an arbitrary coloured light and anomalous and normal trichromats need a mixture of three lights for the match (hence the term "trichromat"). To see why this is true, consider first the case of a monochromat.

Monochromats

Suppose we present to a monochromat two spots of light put side by side and at different wavelengths λ_1 and λ_2 (Fig 3a). Suppose further that the top patch is at a fixed intensity I_1. The question is, can he adjust the intensity of the bottom patch, I_2, such that the two patches look identical to him?

When the two light patches look identical it means that the same number of photons coming from either patch per unit time is absorbed by the cones (remembering the univariance principle). If the relative fractions of light absorbed by the cone system for the lights at wavelengths λ_1 and λ_2 are f_1 and f_2 respectively (Fig 3b), this condition can always be realized by adjusting I_2 such that $I_1 f_1 = I_2 f_2$. This is true for any wavelength λ_2. Thus for a monochromat, all monochromatic lights can be made to look the same by adjustment of their relative intensities, and monochromats have no wavelength discrimination.

Dichromats

Consider a dichromat with, say, the red and green cone absorption spectra illustrated in figure 4a (tritanope). If the light patches of figure 3a are presented to this observer, he can adjust the intensity of one light patch until

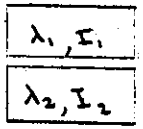


Fig. 3A

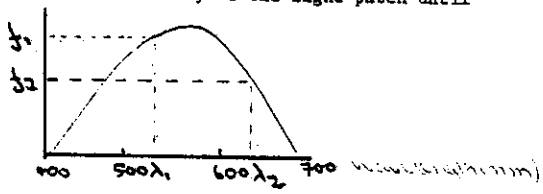


Fig. 3B

the rate of absorption of photons by the green system is the same for both patches of light. In this case, however, the rate of absorption of photons by the red system differs for the two patches, since the absorption spectra for the red and green pigments differ. There is no setting of intensities of the two lights for which the rate of photon capture, in the two patches of retina on which the lights fall, is the same for both the red and green pigments. Dichromats are able to match the two patches if a third light is added to one of them (fig 4b),

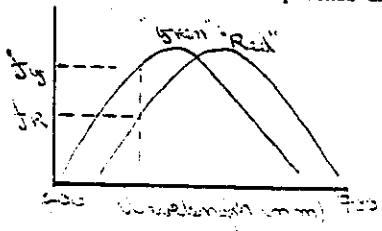


Fig. 4A

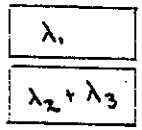


Fig. 4B

and if the intensities of the two lights in the colour mixture are set by the observer. The colour match is unique, and the intensities of the coloured lights needed for the match can be calculated by solving simultaneously the two equations governing the matching condition for each colour system. For each system one equates the total photon capture rates in the two patches of retina. (Photon capture rate is given by the product: monochromatic light intensity x pigment absorption at the wavelength of the monochromatic light. In the general case of non-monochromatic lights with extended spectral distributions one calculates the photon capture rate in small wavelength bands for the whole spectrum. One then adds all these components together to obtain the total photon capture rate.)

Trichromats

This line of reasoning can be extended for three cone systems (trichromatic vision). If a dichromat establishes a match between a coloured light and a mixture of two others, the match will not obtain for trichromats. Trichromats need a third coloured light in the mixture in order to obtain a match. A colour match is established when the total absorption rate is the same in the two patches of retina for each colour mechanism. In this case one must solve three simultaneous equations for the three cone pigments. The absorption spectrum of one cone pigment of anomalous trichromats differs from that of the normal so that, although they still need a mixture of three coloured lights for a match, the amounts of these lights needed for a match is different from those of a normal match.

Colour-matching is commonly achieved using a variable mixture of monochromatic red, green and blue lights. Since the wavelengths of these lights is arbitrary (provided one cannot mix any two of these to match the third), there are an infinite number of ways in which one can choose matching stimuli to achieve a certain colour. Restated, this means that there are an infinite number of power spectral distributions which can be chosen to yield a given colour. By comparison, the auditory system has a far better wavelength discrimination. You will discover

in a later lecture that the ear has many receptors, each tuned to a different small-bandwidth portion of the auditory spectrum. As a result, the auditory system can distinguish between sounds with different power spectra which would appear identical to a three-receptor system.

Dichromacy of Trichromats

Trichromatic observers become dichromatic under two conditions. Blue cones are absent from the central 1/8° of the fovea, so colour-matching at the central fovea is dichromatic ("small-field tritanopia"). The second condition occurs when colour-matching is performed with monochromatic lights in the green-yellow-orange-red region of the spectrum (about 530-660 nm). This occurs because of the low sensitivity of the blue system in this region - the red and green system responses dominate the matches.

Colour-matching laws

An empirical set of laws have been found to fully describe colour matches:

i) If the light mixture A matches the light mixture B and a light C is added to A and B, the resulting lights will still match: A + C $\underline{=}$ B + C. ("$\underline{=}$" meaning 'matches').

ii) If A $\underline{=}$ B and B $\underline{=}$ C, then A $\underline{=}$ C.

iii) Colour matches are independent of the state of adaptation of the eye, provided adapting lights are uniform and to not bleach more than a small fraction of the cone pigments.

These laws are a restatement of the fact that a colour match obtains when the photon capture rates for all the colour mechanisms are equated for two lights. A useful way to represent colour matches on a colour triangle is discussed in the appendix.

Neurophysiology of Colour Vision

We have seen that the wavelength information of a visual scene can be elucidated only through a comparison of signals from the three classes of cone. Electrophysiologically, one finds that the visual system is designed to perform this comparison since there are cells at all levels of the visual system which receive antagonistic inputs from different types of cone. This antagonistic behaviour is found as early as the horizontal cells. Colour-coded horizontal cells (c-type or chromaticity horizontal cells) respond to illumination of their receptive fields by, say, green light with hyperpolarization and by, say, red light with depolarization. Not much is known about the colour-coding of bipolar and amacrine cells. Ganglion, lateral geniculate and cortical cells which have antagonistic center-surround receptive field organizations (center "on" - surround "off" or vice versa) frequently have different chromatic properties in the centre and surround. Consider for example the behaviour of a red-green opponent-colour ganglion cell.

Such a cell might give an "on" response to red light shown on the receptive field center and an "off" response to green light shown on the surround. Green "on" center - red "off" surround cells are also seen, as are red "off" center - green "on" surround and the reverse. The responses of a red "on" center - green "off" surround ganglion cell to white, red and green light are shown in Fig. 5.

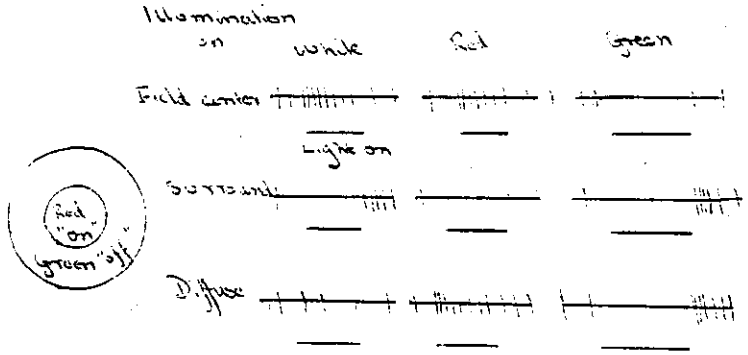


Fig. 5a

Fig. 5b

Since a red "on" center - green "off" surround cell has the red cone system feeding the "on" input and the green cone system feeding the "off" input, the spectral sensitivities of the center and surround overlap broadly, as shown in Fig. 6a, with the resultant response to diffuse light as shown in Fig. 6b. Hence long wavelengths elicit an "on" response and short wavelengths elicit an "off" response. At some intermediate wavelengths the excitatory and inhibitory processes are balanced, and there is no response from the cell at all. Thus for diffuse light this cell crudely codes for long wavelengths with an "on" response and for short wavelengths with an off response. There is, however, ambiguity between spatial and colour contrast. Thus, for example, the cell is equally well excited by either diffuse red light or white light confined to the receptive field center.

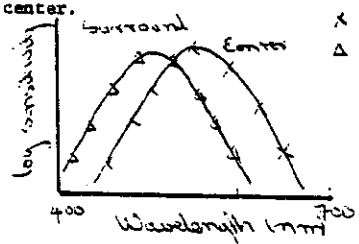


Fig. 6a

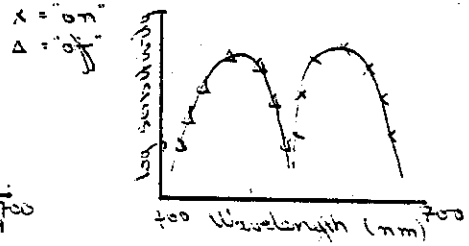


Fig. 6b

The retina also contains a number of dual-opponent cells which have a colour-opponent mechanism in the field center (say red "on", green "off") and the opposite colour arrangements in the surround (green "on", red "off"). Whereas the optimum stimulus for single-opponent cells is a coloured spot (a red spot in the case of a red "on" cell), the best stimulus for the dual-opponent cells is one with colour contrast (a red spot surrounded by a green annulus for the example quoted above). In both cases red-green or green-blue opponency is observed: red-blue opponency has not been found.

Pathways and colour-coding: Retina to Cortex

There is a small group of retinal ganglion cells which are not colour-opponent and which, as a result, cannot convey wavelength information (the so-called "broadband" cells). A small fraction of these have low conduction velocities and appear to feed directly into the superior colliculus. The remainder of

the non colour-opponent cells respond transiently to stimuli (Y-type). They synapse in the superficial layers of the lateral geniculate nucleus (LGN, layers 1 & 2). These LGN layers project into layer 4 of the visual cortex, which in turn projects (via layer 5) to the deep layers of the superior colliculus.

The majority of retinal ganglion cells are colour-opponent and respond in a more sustained manner to stimuli (X-type). Their responses are mediated by one cone mechanism (mostly the red or green, but also the blue mechanism) in the center and by one of two mechanisms in the antagonistic surround. Occasionally the "center" and "surround" overlap completely. The colour-opponent cells project via the deeper layers of the LGN (layers 3-6) to layer 4c of the visual cortex.

The cortex contains cells which respond best either to brightness contrast or colour contrast. The proportion of colour-contrast cells is highest in the region of cortex serving foveal vision. In layer 4 of the cortex there is a large number of dual-opponent cells with concentric receptive fields. These cells have one red-green opponent-colour mechanism in the field center and the opposite colour organization in the surround. These cells appear to receive input either from red-green opponent - colour geniculate fibers without an antagonistic surround or from on-off center-surround fibers driven by one colour mechanism only. These dual-opponent cells respond best to colour-contrast stimuli (for example a red spot surrounded by a green annulus). These dual opponent colour cells synapse with dual opponent-colour simple cells. In most cases the receptive fields of these simple cells consist of a rectangular strip containing one red-green opponent-colour system and two antagonistic flanks with reverse opponent-colour arrangement. The centers of these fields are in register with those of their afferent cells and they have the same colour properties. A second type of simple cell has only one antagonistic flank. Colour-sensitive complex neurons have square or rectangular receptive fields which can only be mapped by moving bars or edges of coloured light. Some respond exclusively to two-colour edge stimuli. Stimulus width and orientation are important but length is not. It appears that dual-opponent simple cells provide input to complex cells which have the same orientation and direction selectivity, stimulus dimension and spectral sensitivity preferences. Hypercomplex cells are excited best by movement of a specifically oriented coloured edge or bar of light. The excitatory area and its surrounding antagonistic flanking areas have the same narrow spectral sensitivity: the wavelength of peak sensitivity varies from 480 to 630nm. It appears that the hypercomplex cells are driven by neighbouring complex cells with similar stimulus preferences.

Pre-Striate Cortex

The striate cortex sends afferents to several distinct regions in pre-striate cortex. In each region the visual world is represented with certain features dominating the responses - ocular dominance, orientation, colour etc. In the region called V4 there is a high incidence of colour-selective cells with large receptive fields and little orientation selectivity.

Conclusion

There is still uncertainty about how cortical cell signals lead to vision and the sensation of colour. Remember too that the electrophysiological evidence quoted above was obtained from primates with visual systems related to that of man. Certain psychophysical experiments lead us to believe that there are orientation and colour-selective cells in the human system.

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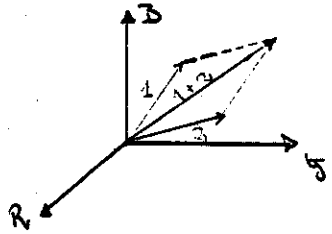
APPENDIX

Colour Triangle

Recall that the colour sensation produced by each light mixture corresponds to a unique ratio of excitation of the red, green and blue cone systems, and that the colour effects of two or more light mixtures add up by summation of their effects on each of the three cone systems. These properties mean that one can represent colour sensation and the light mixtures which produce them by vectors on a 3-dimensional rectangular coordinate system (Fig A1).

Fig. A1



The three coordinates in Fig. A1 represent the red (R), green (G), and blue (B) cone systems, with scales which might depict relative rate of photon absorption. Each vector which projects into space from the origin then represents a unique colour sensation. The colour sensation is constant along a vector, and the length of the vector represents intensity. Blue sensation will be represented by vectors pointing more in the direction of the B-axis because this corresponds to higher excitation of the blue cone system. The same can be said for green and red sensation. White sensation corresponds to a vector pointing diagonally into space because it corresponds roughly to equal excitation of the three cone systems. Addition of colours is represented by vector addition using the parallelgram rule. From Fig. A1 it can be seen that when two light mixtures add (vectors 1 and 2), depending on their relative intensities (represented by the relative lengths of the vectors) the resultant will have a colour sensation that approached one or the other mixture, or anywhere in between.

One can represent colours and their addition more simply by reducing the 3-dimensional representation to a 2-dimensional one, as follows. One can mark off points on the three axes in Fig. A2 that are equidistant from the origin, and by joining the points one obtains a triangular surface which each colour vector penetrates at a unique point (Fig. A2(a)). Addition of two colours is made by marking off the points on the triangle that represent the colours and joining them with a straight line. Any possible colour sensation that can be produced from mixing the two colours then lies on the straight line between the two points.

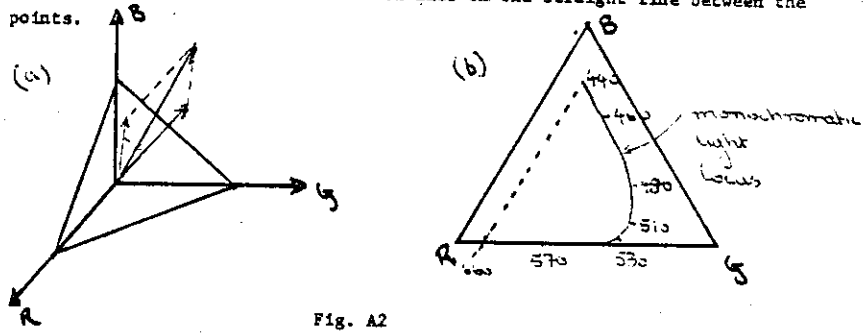


Fig. A2

The locus of colour sensation on the triangle produced by monochromatic light is shown in Fig. A2(b). White sensation is represented by the point W. All the possible colour sensations that can be experienced by the human eye are contained within the area bounded by the monochromatic light locus. The line joining the two ends of the visible spectrum, 400nm and 660 nm, represents all the purples that can be achieved by mixing violet-blue light and red light. (Hence purple is not a monochromatic colour).

With reference to the colour triangle, a few remarks can be made:

1) Two qualities of colour sensation that are often described are <u>hue</u> and and <u>saturation</u>. The hue is the colour name - red, green etc. Colours lying on the lines of monochromatic and purple colours (fig A2(b)) are fully saturated. White is the only completely desaturated colour. Addition of increasing amounts of white to a monochromatic or purple colour decreases the colour saturation progressively.

2) Two colours are said to be complementary to each other if they produce white when mixed in appropriate proportions. Thus any two colours that lie on either side of a straight line intersecting the white point are complementary to each other. Similarly, two monochromatic wavelengths are said to be complementary if they produce white sensation when mixed in appropriate proportions. For example, the complement of 578 nm is approximately 475 nm, and that for 570 nm is approximately 420 nm. Note, however, that there are no complementary wavelengths (except different purples) for the range of wavelengths between approximately 490 nm and 567 nm.

3) One can generate most colour sensations using just a few primary wavelengths. For example, if one chooses 420 nm, 512 nm and 660 nm to be primary colours, then by mixing them in different proportions one can reproduce almost any colour within the colour triangle, including white. This is of practical importance because it means one can reproduce (say in TV, printing, and so on) most natural colours one encounters in the environment with just a few primary colours.

NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Development of the
Central Nervous System

Carla J. Shatz

Development of the Central Nervous System

The nervous system is an enormously complex, exquisitely organized structure and the question naturally arises, how does this elaborate system develop?

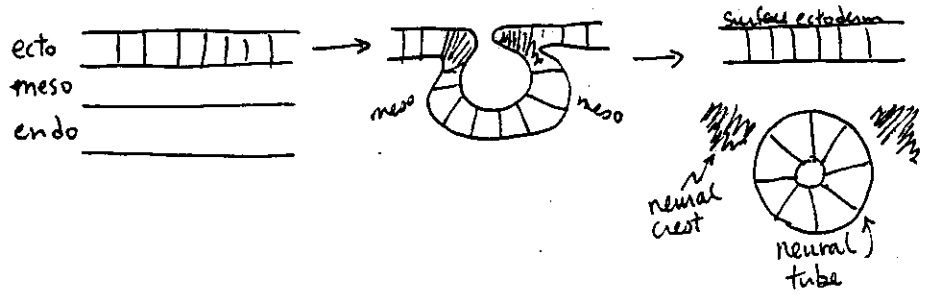
This question will be considered here first by looking at the gross anatomy of development of the central nervous system and next by considering in detail the development of one system, the mammalian visual system. The visual system has been chosen as an example because you have already heard a good deal about its adult organization. Also, many of the fundamental events which occur during the development of the visual system are similar to those occuring during development in general - for example, in the development of the spinal cord, the neuromuscular junction, the cerebellum (all of which are the subjects of excellent developmental studies).

Gross Development

A brief review of embryology:

Recall that the early embryo consists of 3 embryonic layers: ectoderm, mesoderm, and endoderm. During neurulation, the ectoderm invaginates to form the neural tube, as shown in figure 1. The mechanism of neurulation is unknown, but it is clear that the ectodermal cells (which are epithelial cells) change from being roughly cuboidal to pyramidal in shape. This morphological change, incidentally, can be blocked with colchicine an agent which dissociates microtubules.

Figure 1.



During neurulation, ectoderm adjacent to that of the neural tube becomes the neural crest, a germinal cell mass situated to either side of the neural tube. Cells of the neural crest eventually migrate into the periphery to become, among others pigment cells, the postganglionic neurons of the autonomic Nervous System (sympathetic and parasympathetic ganglion cells), and the dorsal root ganglion cells.

Cells of the neural tube eventually become the neurons (and glia) of the spinal cord and brain.

The progressive development of the human brain is shown in figure 2. The brain starts as a tube (not shown) and rapidly develops its characteristic bulges and flexures, presumably due to the differential growth rates (cell proliferation) of various regions. For example, by 4-5 weeks of gestation, the future thalamus and telencephalon are small relative to the midbrain; by 10 weeks the telencephalon has enlarged enormously and almost entirely covers the diencephalon. Note that even at 10 weeks, the cerebellum, which will eventually completely cover the midbrain (mes.) has barely begun to form. As shown in dorsal view, the telencephalon begins as a single vesicle, and the two hemispheres first emerge at about 5 weeks of gestation.

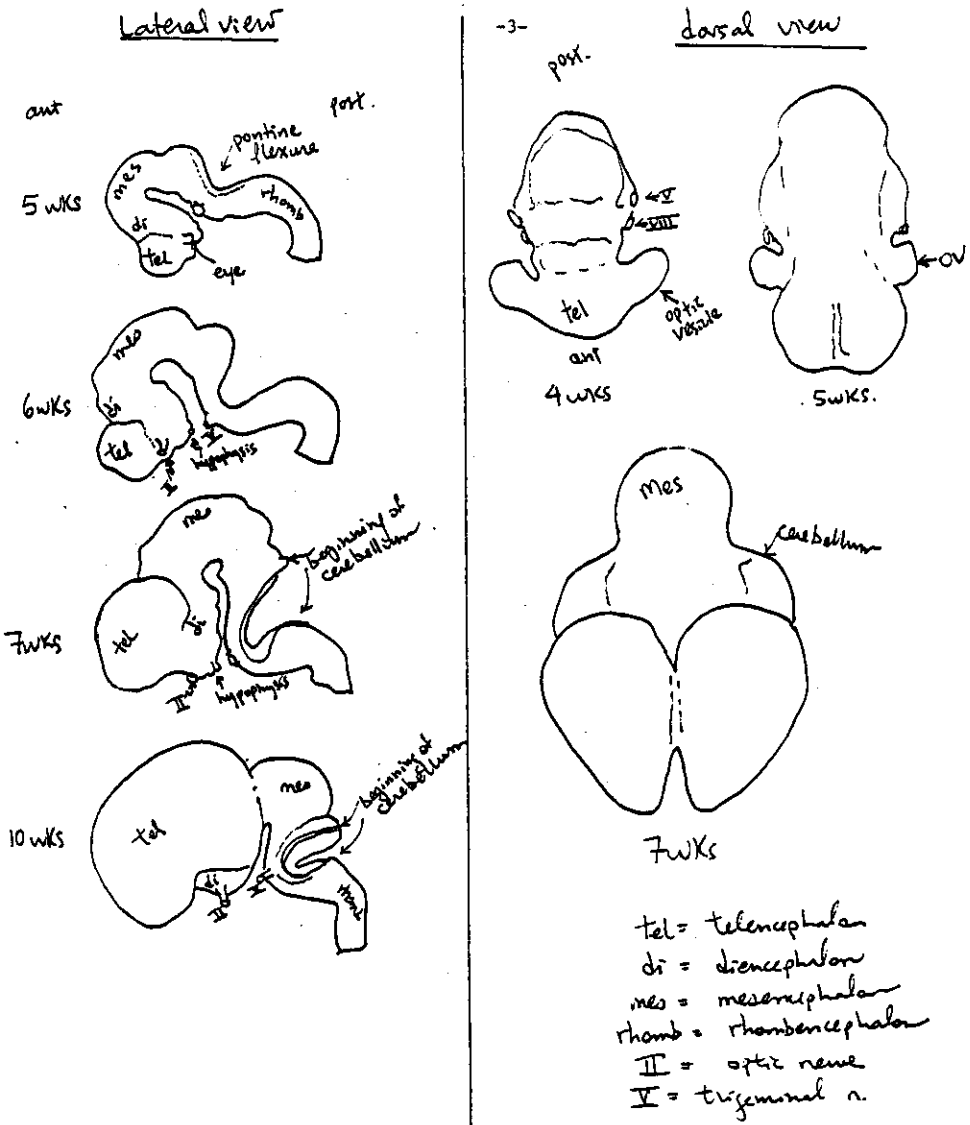
Defects in Gross Development

Most defects in the gross morphology of the brain are lethal, and usually result in spontaneous abortion. For example, if the single telencephalic vesicle fails to subdivide two lobes around the 5th week of gestation the result is holoprosencephaly - e.g. a single hemisphere. Typically the thalamus is also abnormally divided. This defect is frequently accompanied by cyclopia (a single fused eye, with or without a nose).

Another defect (craniorachischisis) arises due to a failure in neural tube closure. Normally the neural tube fuses first near the future neck and fusion proceeds in cephalic and caudal directions. When fusion fails, that unfused portion of the developing nervous system is usually not covered by surface ectoderm (epidermus), but rather is exposed. This defect is accompanied by anencephaly - e.g. there is simply no telencephalon.

Finally, once the neural tube has closed, CSF production and flow must be properly regulated. Hydrocephaly results if CSF accumulates abnormally in the cranial cavity - due for example, to overproduction or an obstruction in the aqueduct of sylvius.

Note that many of these abnormalities are accompanied by facial defects - such defects, then, can indicate underlying nervous system malformations.



development of human brain at various gestational ages. (after Hochstetter)

FIGURE 2

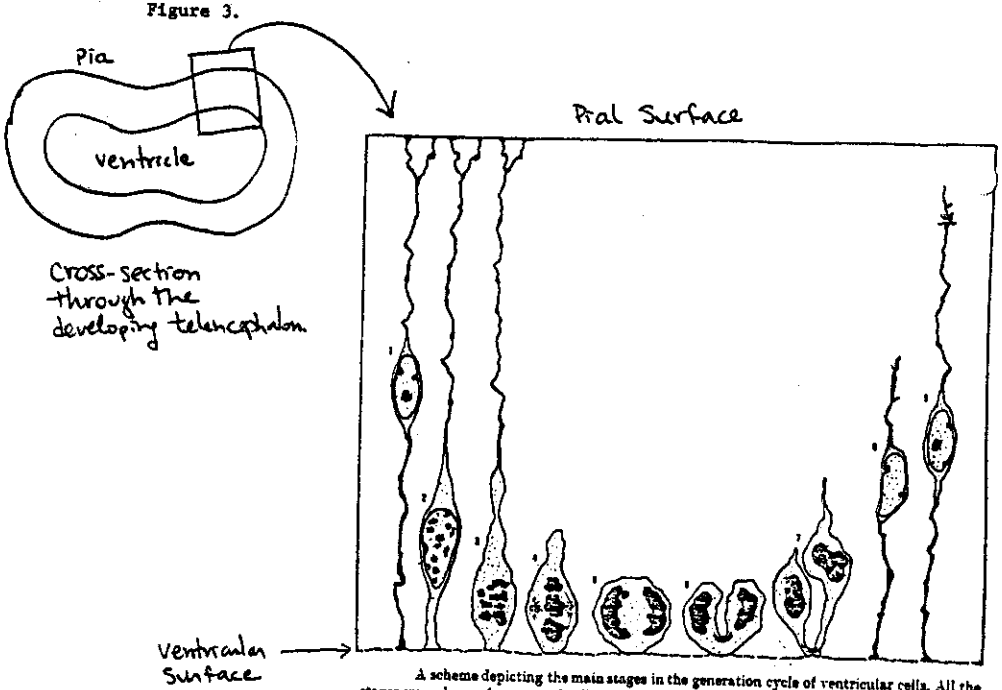
Microscopic Development of the Visual System

The basic questions to be considered here are 1) how you might get a huge multi-layered structure like the cerebral cortex (or retina) from the one layer of cells of the neural tube, and 2) how different parts of the CNS (such as retina, LGN and cortex) might connect up with each other during development.

Cell Proliferation

A cross section of the neural tube at the level of the telencephalon at an early developmental stage (~ 4-5 weeks gestation) is shown in figure 3. The tube consists of a single layer of pseudostratified epithelial cells. Near the ventricular surface is a zone of intense proliferation (mitotic figures can be seen). This is called the "Ventricular Zone" and cells within this zone divide, migrate away and give rise to the entire central nervous system (neurons and glia). (VZ = ventricular zone).

Figure 3.



A scheme depicting the main stages in the generation cycle of ventricular cells. All the stages were drawn from actual cells observed in Golgi impregnations or electron micrographs. The ventricular surface is indicated by the wavy line at the bottom of the picture. *1* mature interphase cell; *2* actively migrating prophase cell; *3* prometaphase cell with 1 external process still connected to the external liming layer; *4* metaphase cell with bulbous external process; *5* spherical anaphase cell; *6* early telophase cell pair; *7* late telophase cell pair with beginning external process still in the ventricular layer; *9* early interphase cell with growth cone of external process approaching the external limiting layer

(from Hinds + Ruffett, 1970)

Golgi studies (Hinds and Ruffett, 1970) have shown that in mammals, nuclei migrate up and down within the processes of the VZ cells, which stretch from the pial to the ventricular surface. When nuclei reach the ventricular surface, the cells round up, lose their pial process, divide and then the two daughter cells extend new processes to the pial surface and the process is repeated. 3H-thymidine studies (Sidman, Sauer) have verified that VZ cells actually synthesize DNA at the top of the ventricular zone; then the nucleus migrates down to the ventricular surface for cell division.

Cell Migration

It is thought that (at least) one of the two daughter cells migrates completely away from the VZ, and in so doing, may eventually lose its attachment to the ventricular surface. As more and more cells migrate, the neural tube thickens, and soon an intermediate zone, filled with the cell bodies of what are presumed to be migrating neurons (based on 3H-thymidine studies discussed below) develops.

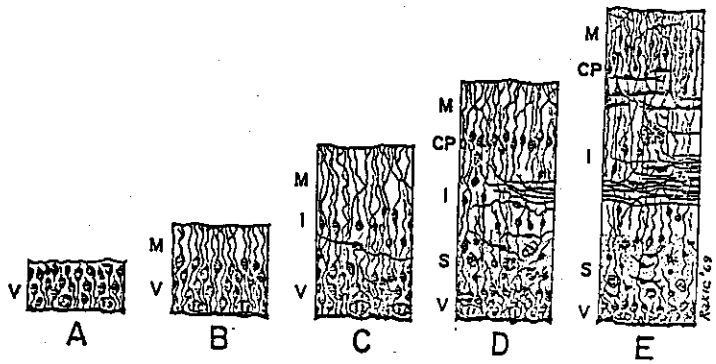
As shown in figure 4, some neurons eventually reach their final destination close to the pial surface. In this case, these give rise to the cortical plate which develops into the familiar 6-layered cortex of the adult.

This basic scheme of proliferation at the ventricular zone and migration away from it is typical of development in any CNS structure: cortex, retina, cerebellum, spinal cord. The details, however, are different, as discussed below.

As time progresses, cortex thickens, underlying white matter (containing afferent and efferent axons) forms and the ventricular zone finally becomes exhausted and differentiates into the adult ependyma.

How do migrating cells find their way, particularly in later stages of development when the distance from the VZ to pial surface is great? One possibility is that they may migrate along the processes of radial glial fibers. Such glia are seen only during the development of the cerebral cortex (and spinal cord), and Golgi studies show that their processes span the entire distance between pial and ventricular surfaces, thereby providing a nice roadway for migration. Recently, it has been possible to identify these radial glia at the electron microscope level by using an antibody to a component of the glia--glial fibrillary protein. EM pictures of the developing cerebral cortex indeed show that cells presumed to be migrating (they have leading and trailing processes) are closely apposed to radial glia.

FIGURE 4

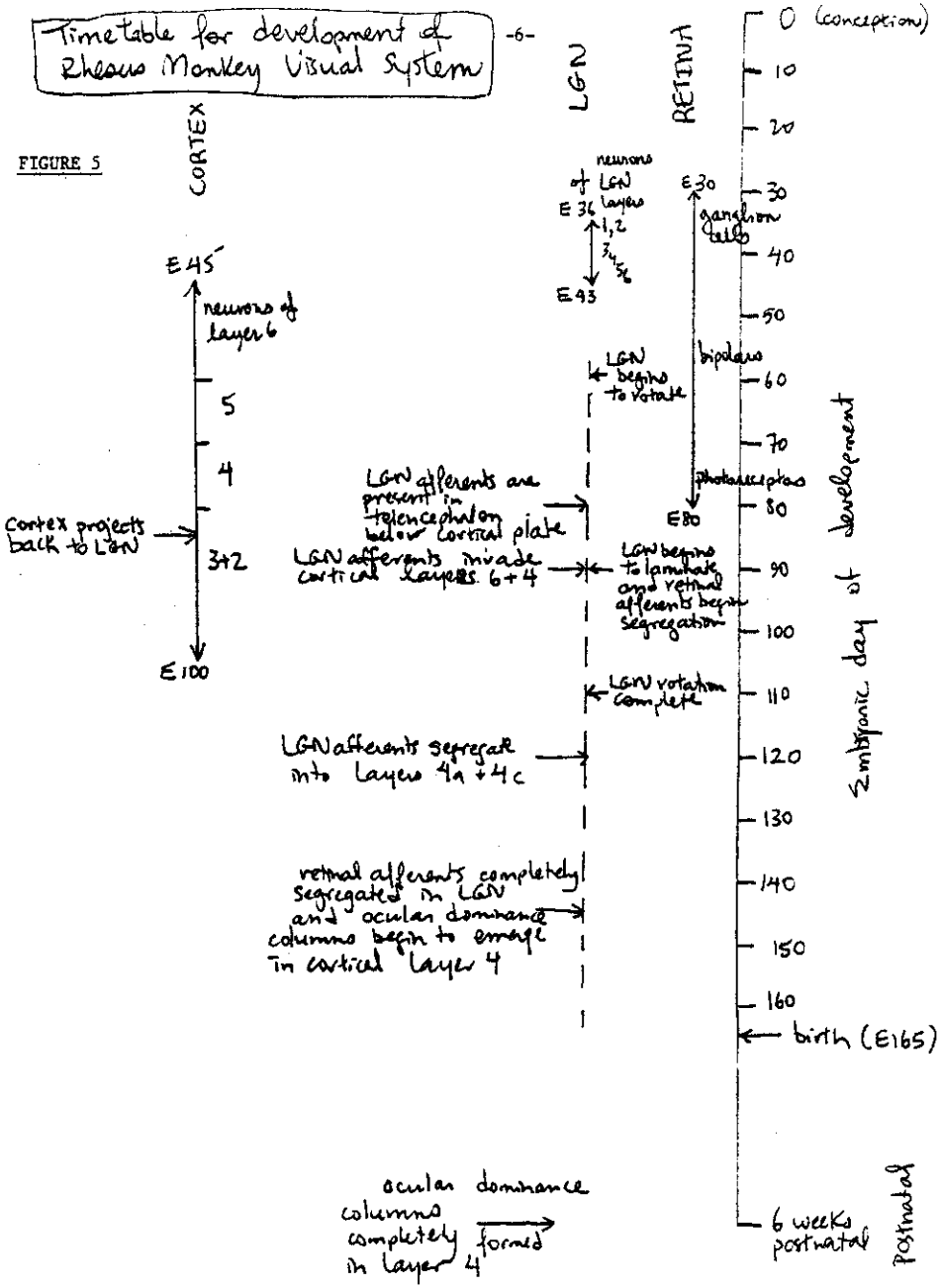


Semidiagrammatic drawing of the development of the basic embryonic zones and the cortical plate. From Boulder Committee[12]. Abbreviations: CP, cortical plate; I, intermediate zone; M, marginal zone; S, subventricular zone; V, ventricular zone.

from Rakic, 1975.

Timetable for development of Rhesus Monkey Visual System

FIGURE 5



(from Rakic, 1977)

Timing and Pattern of Neurogenesis

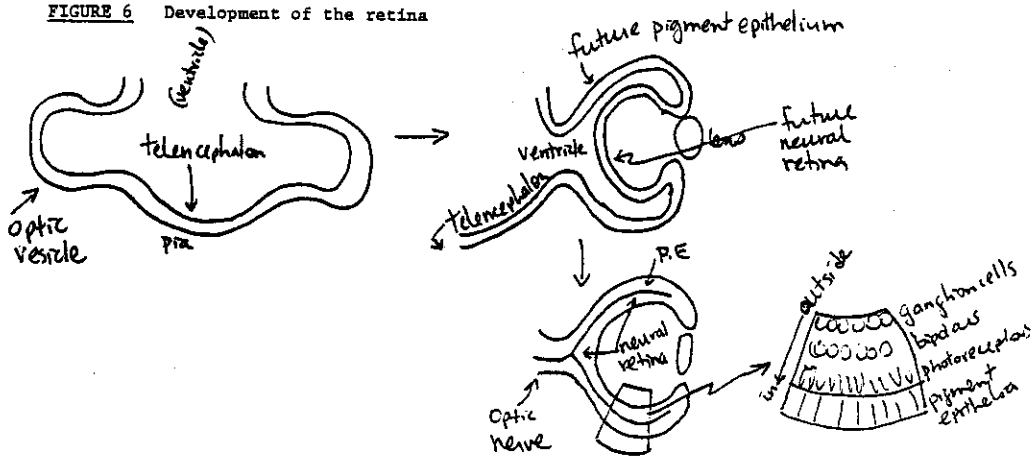
Is there any systematic relationship between the time a neuron undergoes its final round of DNA synthesis--migrates away from the VZ, and its final position in the brain? For instance, the cerebral cortex is a layered structure: are cells belonging to a given cortical layer generated at the same time during development? Recently this question has been answered in the rhesus monkey's visual cortex by means of 3H-thymidine autoradiography (Rakic, 1977). This method makes use of the fact that most neurons, once they have migrated away from the VZ, are forever postmitotic. If a pulse of 3H-thymidine is given during development, then at all subsequent times the most heavily labeled cells seen in autoradiographs will be those neurons which were undergoing their final round of DNA synthesis at the time of 3H-thymidine administration: this time is designated as the birthdate of the neuron. (Cells which go through many cell divisions subsequent to the 3H-thymidine pulse, particularly glial cells, dilute their label and therefore are lightly, or not, labelled).

When Rakic gave 3H-thymidine to a fetal monkey 45 days old (embryonic day 45=E45) and allowed the monkey to be born (gestation is 165 days for rhesus monkeys) and grow up, he found that virtually all thymidine labeled cells in the visual cortex were confined to layer 6. 3H-thymidine given after E60 labeled neurons of layer 5 in the adult visual cortex. Neurons of layer 4 were generated by E70 and finally, the neurons of layers 2 and 3 are generated between E80-E100. These birthdates are summarized in figure 5 (left-hand side).

These results indicate that the deepest cortical layers are generated first, the most superficial layers, last and this sequence of early-to-late development is therefore "inside-out." Note that neurons of the later-formed layers must actually migrate through the earlier-formed layers to assume their final positions within the developing cortex.

For the retina on the other hand, the sequence of neurogenesis is outside-in with the ganglion cells born first, bipolars next, and photoreceptors last. Thus the later-born neurons simply "pile up" in layers inside those of earlier-born neurons, as shown in figure 6. Recall that the ventricular zone giving rise to the retina forms as an invagination of the bulging optic vesicle (see figure 6). The surrounding surface becomes future pigment epithelium.

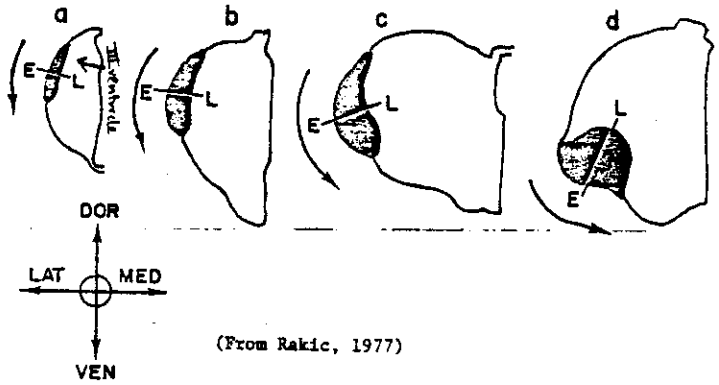
FIGURE 6 Development of the retina



In the development of both cortex and retina, the first neurons to develop tend to be the largest (in the adult), with axons destined for distant points (in the case of retinal ganglion cells, and cells of cortical layers 6 and 5, the targets are the LGN and superior colliculus). This tendency is true of cerebellum (the Purkinje cells are first-born) and spinal cord (motoneurons) development as well.

Neurons of the LGN are also generated in an outside-in sequence during a remarkably short period between E36 and E43 (figure 5). These neurons are generated within the ventricular zone of the thalamus and migrate to the dorso-lateral margin of the developing thalamus. The LGN over the next few weeks is then pushed ventrally and medially due to the enormous growth of other thalamic structures and assumes its adult position (figure 7) by E110. This remarkable transposition of structures during development is another common feature of mammalian CNS development.

Figure 7. Development of the LGN in the Rhesus monkey



(From Rakic, 1977)

Cell Death

During development, the ventricular zone produces all the cells destined for the mature nervous system and one important question is how is cell number regulated? Does the VZ produce exactly the correct numbers of neurons and glia required for each structure initially, or is there overproduction followed by cell death? In the past few years, a good deal of evidence has accumulated in favor of the 2nd alternative: cell death. For instance, simple counts of the number of retinal ganglion cells, or of spinal motoneurons, have indicated that there are about 2 times as many neurons produced initially as are found in the adult. A wave of cell death then eliminates half the population, leaving the remaining half to complete development.

It is not known just what factors determine which neurons will survive and which will not. One possibility is that some neurons simply fail to send their axons to appropriate targets and therefore die. Recent studies of motoneurons by Landmesser and colleagues, however, have shown that target-finding by axons is remarkably precise--mistakes are few and definitely not enough to account for the massive cell death known to occur. Other factors--growth factors, competition for postsynaptic space, neuronal activity, pre-synaptic inputs--are more likely candidates and at present some or all of these may play a role in the survival of neurons.

Formation of Connections

A major miracle of development is the formation of appropriate connections between neurons. Two general steps must be accomplished: (1) axons must find their target structures and (2) within the target, axons must arrange themselves appropriately and make the correct synaptic connections. Virtually nothing beyond the descriptive is known about these events, but it is worth considering briefly these processes even at the descriptive level by examining visual system development as an example.

To study the development of connections in the primate visual system, 3H-proline is injected into one eye of a fetal monkey and the monkey is allowed to survive in utero 24 hours or more while the radioactive label is transported by axoplasmic flow to the terminals of retinal ganglion cells--wherever they happen to be. Then the fetus is delivered by C-section and the brain processed for autoradiography (see Rakic, 1977 for more details).

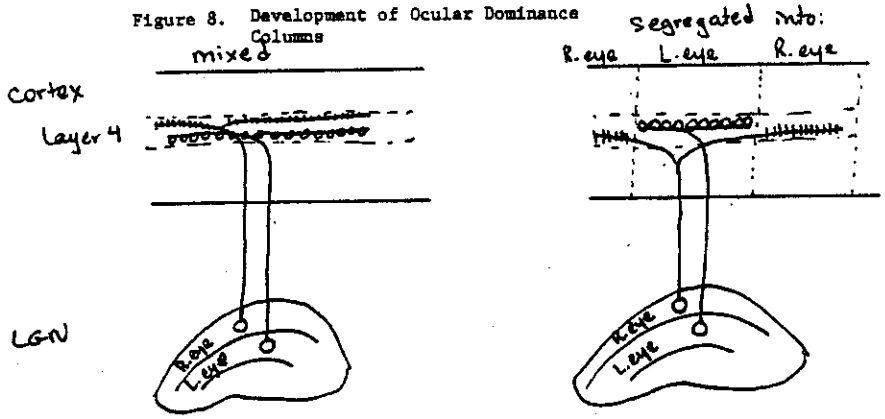
Rakic has shown that retinal ganglion cell afferents are present within the LGN as early as E70, but are distributed throughout the LGN: no layers are evident! Around E90 the afferents begin to segregate into layers (see figure 5), but segregation is not complete until E140, just when cell lamination is also complete. (It is thought that segregation of the retinal afferents slightly precedes cell lamination). If one eye is removed before segregation of the afferents is complete, lamination of the LGN is arrested, indicating that retinal afferents from both eyes are necessary for lamination.

Thus, during development connections from each retina are initially diffusely distributed throughout the LGN, and only assume the adult laminar distribution by a process of segregation.

A similar process of segregation occurs in the development of connections between LGN and visual cortex (see figure 8 and figure 5). Initially, LGN afferents representing each eye are uniformly distributed throughout layer 4 of the visual cortex, and it is only through a process of segregation that they come to occupy the fourth layer patches characteristic of ocular dominance columns. This was shown by means of transneuronal transport of radioactive materials following injection of one eye in fetal monkeys.

Segregation of LGN afferents in layer 4 of the rhesus monkey begins at around E140, (just at the completion of LGN lamination), and is not complete until 6 weeks after birth.

Figure 8. Development of Ocular Dominance Columns



Conclusion

This brief consideration of the development of the mammalian visual system illustrates a number of features common to CNS development in general. First, the developmental process is apparently exquisitely orchestrated--very little appears to be left to chance. Neurons destined for specific psoitions in the adult (spinal cord, cerebellum . . .) are generated during discrete embryonic periods, and these periods of neurogenesis can be closely related to the timing of formation of appropriate sets of connections. Second, it is remarkable that in the formation of connections--for example, between LGN and cortex--the adult columnar pattern emerges gradually by means of the refinement of an initially diffuse set of connections. Such a process of refinement also occurs at the neuromuscular junction--where initially each muscle fiber is innervated by a number of motone..rons. (Recall that in the adult, each muscle fiber is innervated by a single motoneuron.) Just why this process occurs is unknown, but it may provide the opportunity for a fine tuning of connections necessary for the precision seen in the adult nervous system. In the next lecture you will hear about how this process--at least that seen in the visual system--may be altered by the effects of abnormal visual experience.

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NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Visual Deprivation

Carla J. Shatz

The question to be considered in this lecture is to what extent the formation and maintenance of connections in the CNS--in this case the visual system--depend upon experience. Clinicians and experimental psychologists have long known that depriving an animal or man of vision at an early age can lead to profound visual defects. For example, a child who grows up with congenital cataracts will have lasting visual defects after the milky lenses are removed and compensated for by fitting glasses. Cataracts acquired in adult life, on the other hand, lead to no visual impairments even if they are removed after being present for twenty years. Experimental psychologists have attempted to reproduce these experiments of nature by raising animals in darkness or with exposure only to diffuse light: The results have shown that cats and monkeys, like man, require visual experience early in life to avoid sensory deficits. With the knowledge of the visual physiology in the normal cat and monkey, it becomes feasible to learn about the consequences of sensory deprivation at a cellular level, the hope being that such knowledge may provide new insights about the process of normal neural development and the effects of disuse.

Monocular Deprivation

In the normal cat or monkey visual cortex, microelectrode recordings have shown that about 80% of the cells are influenced from both eyes, 10% of the cells receive input only from the right eye, and 10% only from the left. About half of the binocular cells are dominated by the right eye and half by the left. If an animal is visually deprived from birth onwards by suturing closed the lids of one eye, a number of striking changes occur (Wiesel & Hubel, 1965). For example, if the sutured eye is opened after a few months or so, the following observations are made:

1. Pupillary light reflexes are normal in both eyes.
2. The kittens are behaviorally blind in the deprived eye.
3. The large majority of cortical cells respond only to stimulation of the experienced eye (over 80%).
4. Of the few cells influenced by the deprived eye (20%), most give inconsistent, sluggish responses.
5. Some cortical cells, noticed because of their spontaneous activity, cannot be influenced from either eye. Such cells are not seen in the normal visual cortex.

Thus, depriving an animal of normal vision during early life produces striking changes in the normal cortical physiology.

Several questions may now be asked:

Site of lesion?

The fact that cortical cells failed to respond to the deprived eye gives no guarantee that the abnormality is not in the retina or the lateral geniculate body. This can be tested in the same animals by recording also from geniculate cells. One thus finds that cells in the layers receiving input from the deprived eye have roughly normal on- and off-center fields, suggesting that the site of abnormality is in the cortex.

Monocular lid closure produces no gross anatomical changes in the retina or the visual cortex but the lateral geniculate body shows marked abnormalities; the layers receiving projections from the deprived eye are thinner and their cells are reduced in size. Surprising as it may sound, this LGN atrophy is also consistent with the notion that the defect is mainly at the level of the visual cortex. (This will become clear later.)

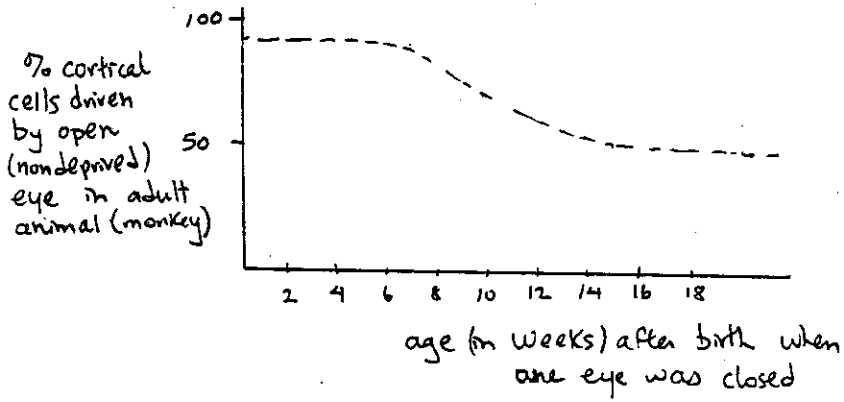
Agent: Light or Form Deprivation?

Lid closure deprives the cats not only of form vision but also to a large extent of light. It is possible to evaluate the relative importance of light versus form deprivation by raising kittens with a translucent occluder over one eye. Physiological recordings in animals deprived of form vision during the first few months of life are similar to those found in lid-closed animals; the kittens appear blind and the large majority of cortical cells respond only to the non-deprived eye. This result indicates that form rather than light deprivation is largely responsible for the cortical changes. (At the level of the lateral geniculate body there is, however, a difference in that the form deprived animals show much less atrophy, suggesting that light deprivation may play a role as well.)

Is There a Critical Period?

It is only during a certain age that cats or monkeys are susceptible to the effects of visual deprivation. By varying the age at closure and the duration of deprivation, one finds that monkeys are susceptible from birth up to about an age of 16 weeks or so after which they become completely resistant to this type of deprivation. An adult animal can have an eye covered for over one year without any discernable behavioral, physiological or anatomical changes. The monkey is particularly susceptible to the effects of monocular closure during the first six to eight weeks, during which time a few days of closure produces clear changes in the cortical physiology. Clinical experiences in children with congenital cataracts indicate that in man the susceptible period to sensory deprivation may extend up to four or six years of age.

Figure 1. Duration of the Critical Period for Eye-Dominance in the Rhesus Monkey



(normally, 50% of the cortical cells are driven by each eye).

Is Recovery Possible?

The effects of monocular deprivation are, in both cats and monkeys (and man), permanent with limited morphological, physiological and behavioral recovery. This is particularly true when the deprivation extends through the entire susceptible period. Variations in the length of deprivation indicate that the recovery is graded with the duration of eye closure.

What Is The Mechanism?

The unresponsiveness of cortical cells to the deprived eye could be due to altered development of cortical connections or to a disruption of established cortical connections (or both). The recordings from the lateral geniculate body have already established that the typical on- and off-center receptive fields develop in the absence of patterned light stimulation. The question whether the more complex cortical connections require visual experience for their development is studied by recording from very young kittens or monkeys. Recordings made in the newborn monkeys show that many cortical cells already have the specific types of response and the binocular interactions seen in the adult. Thus, at least some appropriate connections can be established in the striate cortex in the absence of visual experience. It may be that visual experience is required to maintain these connections. On the other hand, there is also now good evidence that abnormal visual experience can alter the normal development of connections subserving the system of ocular dominance columns--as will be shown below. This should not be surprising, since you have just heard that a good deal of this development occurs postnatally.

At first, disuse seems the most likely reason for the disruption of cortical connections from the deprived dye, but additional experiments indicate that other factors must be important as well.

A. Binocular Deprivation

If kittens are raised with both eyes closed by lid suture during the first few months, one would predict from the results in monocularly deprived kittens that there would be large areas of the cortex containing no responsive cells. This is, however, not at all the case. Instead, responsive cortical cells with completely normal receptive fields and binocular properties can be found. This again confirms our earlier conclusion that many cortical connections are under innate control. Over half of the cells appear, however, abnormal, being either completely unresponsive or giving inconsistent responses and showing no orientation specificity. It is interesting that in spite of a sizeable number of normal cortical cells, the binocularly deprived cat appears blind when the lids are separated.

The presence of a good number of normal cortical cells suggests that the effects of deprivation are less severe if both eyes are closed than if only one eye is closed. In fact, it appears that the effects of right-eye closure upon a binocular cell depends on whether the left eye is open or closed, the right eye connections suffering more if the left eye is kept open.

B. Strabismus

Such interdependence between the inputs from the two eyes is still more striking in kittens raised with artificial strabismus. In strabismus (squint, cross-, or wall-eyedness), the optic axes of the two eyes are not parallel and an object in the visual field does not fall on corresponding points of the two retinas. This leads to double vision. A child with strabismus usually avoids double vision by the mechanism of suppression. Some children suppress one eye which after a long time leads to deterioration of vision in that eye. Other children suppress intermittantly, alternating the suppression from one eye to the other. These children have normal acuity in both eyes, but they have lost normal binocular mechanisms. An adult person lacks the ability to suppress and if, for some reason (nerve palsy, injury, etc.), a strabismus develops late in life, it is often necessary to cover one eye to avoid double vision.

Kittens and monkeys made strabismic shortly after birth by sectioning the medial or lateral rectus muscle appear, as they grow up, to develop good vision in both eyes, but seem to alternate and fix with one eye at a time. The results from cortical recordings are that cells respond entirely normally, except that there are very few cells which receive inputs from both eyes.

The cellular activity is very rich in these animals suggesting that binocular cells have not simply dropped out. If the majority of binocular cells had disappeared, one would expect large cortical areas devoid of responsive cortical cells. Instead, cells appear to have changed their ocular affiliation because in cortical penetrations, cells driven by different eyes are found aggregated together in a columnar fashion. This gives the impression that the normal ocular dominance columns have become accentuated so that they are now made up of cells completely controlled by one eye instead of just being dominated by a given eye. It appears in other words as if a binocular cell normally dominated by a given eye has lost its input from the non-dominant eye.

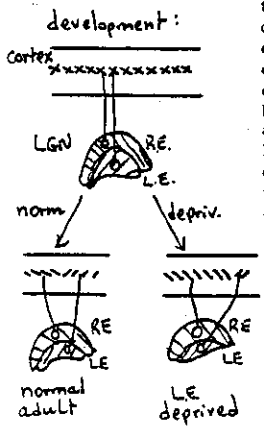
C. Alternating Monocular Occlusion

The dramatic cortical changes in strabismic animals are surprising since the overall input from the two eyes has been normal. It is only the time relationships between impulses from the two eyes that has been abnormal. The strabismus makes a given stimulus fall on non-corresponding points and a binocular cell is not synchronously activated from the two eyes, which presumably occurs in the normal animal. The simplest explanation of these results may be that this deficit is caused by the lack of synergy between the two eyes. This can be tested directly by raising kittens with an opaque occluder placed over one eye one day and over the other eye the next, alternating eyes each day. After two or three months, the animals seem to have normal vision in both eyes, but they have grown up with no binocular stimulation. The effects on binocular cells in the visual cortex are similar and perhaps more extreme than those produced by the strabismus operation: very few cells have inputs from both eyes, and the large majority respond only from the right or the left eye. Again cells responding to a given eye are aggregated in a columnar fashion.

The mechanisms of this change in ocular affiliation are not understood, but the results suggest that there is a definite interaction between the inputs from the two eyes. This interaction somehow affects the efficacy of synaptic input from the different eyes in such a way that the dominant input to a given cell succeeds in gaining complete control. Thus in examining effects of sensory deprivation we may not only consider disuse but also interaction (competition) between various inputs to individual cells.

Anatomical Basis

Figure 2

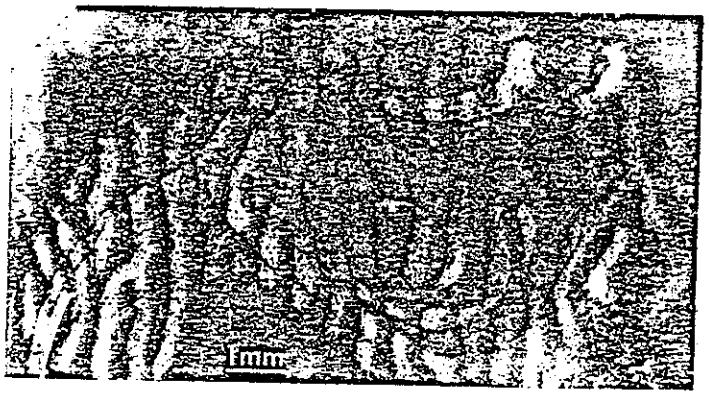


Is there some anatomical correlate for the loss in binocularity, and the consequent dominance of the non-deprived eye, in the visual cortex? Recall that in normal monkeys (cats), the 4th layer of the visual cortex is shared equally by geniculate afferents from the two eyes, which are segregated into the patches corresponding to ocular dominance columns. In animals deprived of vision in one eye from birth, the patches of LGN afferents belonging to the deprived eye are much smaller, and those belonging to the non-deprived eye, much larger than normal. This has been demonstrated by Hubel, Wiesel and LeVay (1977) using the transneuronal transport autoradiographic method, as shown in Figure 3. As can be seen in Figure LGN afferents from the non-deprived eye occupy almost all of the fourth layer, thereby providing an anatomical basis for the physiologically observed dominance of cortical cells by the non-deprived eye.

How do afferents representing the non-deprived eye come to occupy such a large portion of layer 4? As mentioned earlier and shown here in fig. 2, during development the LGN afferents representing the two eyes are initially intermixed in layer 4 of the visual cortex, and these segregate out into the right- and left-eye patches by about 6 weeks postnatally. This 6-week period is also when the cells of the visual cortex are most susceptible to the effects of eye closure (Figure 1). Hubel et al. (1977) have suggested that closing one eye may place its afferents at a competitive disadvantage during the segregation process, thereby giving the open eye's afferents the opportunity to occupy more space in layer 4 than normal. The closed eye's afferents, on the other hand,

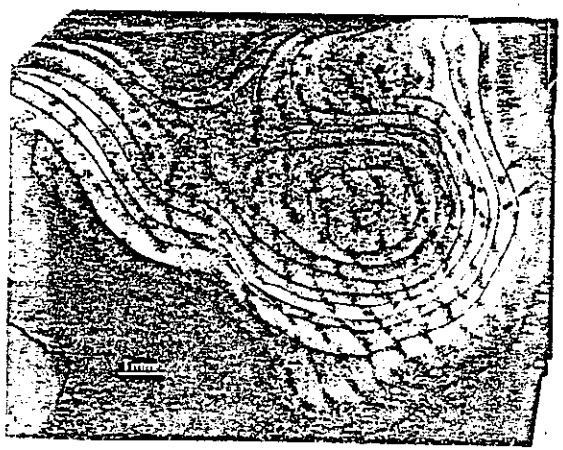
would consequently occupy less space than usual as shown in figure 2. This restriction of axonal arborization might also be responsible for the atrophy of cells seen within the deprived laminae of the LGN.

Figure 3. Ocular Dominance Columns in the Fourth Layer of the Monkey's Visual Cortex, as demonstrated autoradiographically.



A. NORMAL

Area of layer 4 occupied by afferents representing the injected eye appears white.



B. MONOCULARLY DEPRIVED

The non-deprived eye was injected in this case. Note that almost all of layer 4 is occupied by the non-deprived eye, with only small patches of the deprived eye (black) remaining.

From Hubel, Wiesel & LeVay, 1977

This hypothesis, that the right- and left-eye afferents compete with each other for space in layer 4, and that competition can be tipped in favor of one eye or the other by uneven visual experience is supported by the observation that when both eyes are simultaneously deprived of vision during the critical period, the fourth layer ocular dominance columns are roughly normal in size and neither eye manages to physiologically take over a large majority of cortical neurons. Thus it appears that balanced visual input from the two eyes is required for the normal development of ocular dominance columns in the visual cortex.

Concluding Remarks

The existence of a period of susceptibility to the effects of visual deprivation indicates a certain plasticity of the central nervous system early in life. Such a flexibility may obviously not only be harmful but may provide a useful mechanism for modification of the system with use. For example, a child with strabismus due to a muscular anomaly is obviously at an advantage in being able to suppress and thereby avoid double vision, even if the price is the loss of normal binocular vision. One can also imagine that the critical period is useful in making fine adjustments in neural connections from the two eyes relative to each other. Such adjustments once made early in life might then become firmly established and unchangeable.

The period of life during which the nervous system is more or less flexible appears to vary from animal to animal and no doubt from system to system in a given species. In fact, some plasticity seems to persist since many of us are able to learn new facts and skills all through life.

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NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Pumps and Channels

Bruce G. Wallace

I. Mechanisms of Ion Permeation

A. Structure of membranes.

1. Cell plasma membrane consists of a phospholipid bilayer and associated membrane proteins.
2. If lipids are extracted and reconstituted into artificial membrane, it is permeable to lipophilic substances and water but not to hydrophilic low molecular solutes or ions.
3. Obviously the movements of ions that occur during action potentials and synaptic transmission must be mediated by specific proteins; knowledge of the properties of these proteins is important in understanding neuronal function and dysfunction.

B. For convenience we can consider two basic mechanisms for ion transport.

1. Carriers: bind and move a small number of ions across the membrane.
   a) Small mobile proteins or polypeptides
   b) Larger proteins that span membrane, conformational change translocates bound ions from one side of membrane to other.
2. Channels: proteins that traverse the membrane creating a pore through which ions can pass.
3. Characteristics of ionophores
   a) Hydrophobic groups outside; hydrophilic interior.
   b) Stabilization of ions (with or without water of hydration) in ionophore interior by coordination bonds between ion and oxygen atoms
   c) Ion selectivity determined by charge density and size.
   d) Voltage dependence can arise from net charge of carrier - ion complex, charged subunits or charged portion of channel protein.
4. Model systems for study of ion transport.
   a) Carrier - valinomycin and nigericin, K-carriers.
   b) Channel formers - amphotericin (anion) and alamethicin (cation-K).
   c) Experiments show steps in conductance in lipid bilayers and muscle membranes corresponding to opening and closing of single channels.

II. Channels

A. Difficult to purify and study biochemically, no adequate assay systems.

B. Na-channel consists of three distinct components.

1. Channel itself, or selectivity filter

a) Blocked specifically and with high affinity by tetrodotoxin- (TTX) (K_d = 3 nM) and saxitoxin ($K_d \sim$ 1 nM)

i. No effect on K channels

ii. Effective only on outside of membrane

iii. Binding of tritiated toxins allows estimate of channel number; non-myelinated axons: 20-300 per sq. micron, occupy a very <u>small</u> fraction of total membrane, i.e. they are far apart; mammalian Node of Ranvier: 3000-10,000 per sq. micron, occupy perhaps 20% of total membrane.

b) Dimensions can be estimated using voltage clamp and measuring organic cation permeation, ≈ 3 x 5 Å.

c) Pore surrounded by oxygen atoms, one strong negative site to account for Na preference (P_{Na}/P_K = 12). Reduce pH, permeability falls as if titrating site with pK = 5.2.

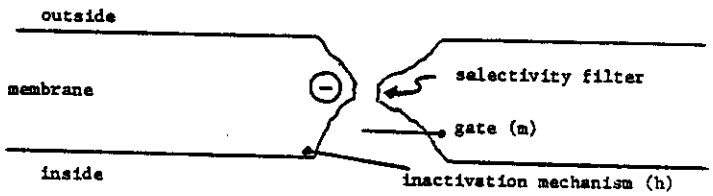
2. Gate or voltage dependent mechanism.

a) Can see "gating current" without any ion flow.

b) <u>Centruroides</u> scorpion venom specifically affects the activation process of the Na channel without affecting TTX binding or inactivation.

3. Inactivation mechanism can be removed in Pronase perfused squid axons or slowed by <u>Leiurus</u> scorpion venom without affecting TTX binding or channel activation.

4. Model for Na channel



C. K-channel less thoroughly characterized

1. Blocked by TEA

2. By organic ion permeation studies probably consists of 3 Å diameter pore surrounded by oxygen atoms, weak negative charge.

a) K may have to lose all water of hydration to get through
b) Permeation can be blocked by reducing pH, pK = 4.4.

c) Ratios of K^+ influx to K^+ efflux indicate that K^+ channel is a long, narrow pore containing several K^+ ions crossing the membrane in single file.

D. ACh-Channel at frog neuromuscular junction

1. Allows Na^+ and K^+ ions to cross membrane with nearly equal ease, impermeant to anions

2. Molecular models of permeant ions suggest open pore is at least as large as a square 6.5 Å x 6.5 Å.

3. Ionic selectivity determined more by access and friction than by specific chemical factors. Walls of channel not highly charged, anions probably excluded primarily by the negative surface potential of the membrane.

III. Sodium Pump

A. Need to maintain Na and K gradients over life time of cell. Mechanism in neurons (and elsewhere) is sodium pump.

1. Transports Na out and K in, both ions moving against their electrochemical gradients and therefore energy is required.

a) Relative numbers of Na and K ions moved will determine if pump is <u>neutral</u> or <u>electrogenic</u>.

b) Movements of Na and K can be <u>coupled</u> or <u>uncoupled</u>.

c) In best studied examples of sodium pump, Na and K movements are coupled; 2 K enter for every 3 Na removed, and so pump is electrogenic.

2. Source of energy is ATP.

a) Perfused squid axons demonstrate ATP as energy source.

b) Under intracellular concentrations of reactants and products, ATP hydrolysis supplies sufficient energy.

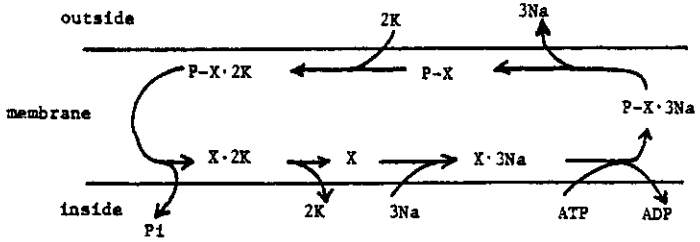
c) As much as 2/3 of a neuron's ATP production can go to provide energy for the pump.

3. Pump is asymmetric: Na and ATP must be present <u>inside</u>, K must be present outside, ouabain only works from outside.

4. Indirectly the sodium pump controls and powers many other processes through coupling with Na movement.
   a) Ca transport
   b) amino acid transport
   c) maintains constant cell volume
   d) regulates other activities influenced by Na and K concentrations (e.g., mitochondrial oxidative phosphorylation)

B. Evidence that Na-K ATPase is the pump.
   1. General correlation of level of active Na-K transport and ATPase activity.
   2. Best evidence comes from red blood cells.
      a) Corresponding changes in K transport and Na-K ATPase sites/cell.
      b) Dependence on Na, K and ATP and inhibition by ouabain is the same for both transport and ATPase activity.

C. Mechanism of action determined by studying red blood cell Na-K ATPase.



   1. Phosphorylation is Na (and Mg) dependent and is associated directly with outward movement of Na^+ and change in affinity from Na to K.
   2. Dephosphorylation of enzyme is associated with inward movement of K and return to high Na affinity and is blocked by ouabain.

D. Sodium pump in snail neuron most thoroughly analyzed physiologically.
   1. Inject Na without passing current across cell membrane, see long lasting <u>hyperpolarization</u>.
      a) No change in conductance of membrane (other than passive changes caused by hyperpolarization)
      b) Hyperpolarization blocked by ouabain and removal of external K

   2. Determine coupling ratio by comparing <u>net current flow</u> produced by pump with <u>net transport</u> of <u>Na</u>.
      a) Voltage clamp cell to measure net current
      b) Use Na-sensitive electrode to measure change in intracellular Na.
      c) Net current equalled 1/3 the amount of Na transported, therefore for every 3 Na ions pumped out 2 K ions must have been pumped in.
   3. Electrogenic pumps will have consequences for signalling.

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PHYSIOLOGICAL PROPERTIES OF SINGLE ION CHANNELS

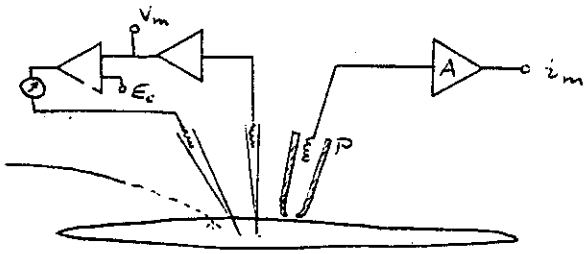
Earlier lectures in the course have described how neural signals are generated by the selective opening and closing of specific ion channels in the membrane. You have heard how the action potential is generated by the sequential activation of sodium and potassium channels. Similarly, synaptic excitation and inhibition result from the activation of still other sets of ion channels when chemical transmitters combine with postsynaptic receptor molecules.

Given that there are ion-selective permeation sites whose conductance is controlled by membrane potential or the receptor-transmitter complex, how does an individual site behave? What is its conductance, and how large a voltage change does it produce? Is a single channel an all-or-none device with only two conductance states - open or closed - or might it have a multiplicity of conductances? How does a single channel respond to changes in membrane potential or to the synaptic transmitter substance? These questions are basic for understanding nervous action, but they cannot be tackled with the conventional voltage recording or voltage clamp techniques which we have talked about up to now in the course. This is because both methods, in their usual form, provide information averaged over a large number of channels - perhaps 10^3-10^6.

Observations on the properties of single channels have been made over the past 9 years with two different approaches. The older of the methods, noise analysis (or fluctuation analysis), examines the statistical fluctuations in the signals generated by a large number of channels to derive the properties of the single channel. In the more recent and direct method of single channel recording, currents generated by only one or a few channels are observed in isolation by the use of certain experimental "tricks". This lecture summarizes some of the properties of the single channels and the experimental methods. Single channel recording is presented first because of its simplicity.

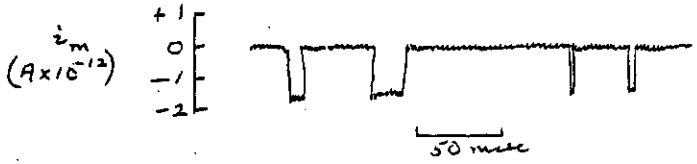
Single Channel Recording

By using several ingenious experimental innovations Neher and Sakmann (1976; reference at end of handout) succeeded in measuring currents through single ACh channels in frog muscle fibers. The arrangement is diagrammed below.



The frog muscle fibers had been denervated 4-6 weeks previously so that extrajunctional ACh receptors were present over their entire surface. The intracellular pipettes were connected in a conventional voltage clamp circuit for controlling the membrane potential. Pipette P in the first diagram was a fire-polished external electrode with a tip lumen about 2 μm in diameter. It contained the cholinergic agonist drug suberyldicholine. Amplifier A, connected to this electrode, recorded the current passing through the patch of membrane just under the pipette. The fiber was "cleaned" with proteolytic enzymes, allowing the pipette to be pressed very close to the surface of the fiber so that it could record efficiently. In order to obtain channel openings of longer than usual duration, making the events more readily observable, the experiments were done at low temperature. Denervation also helped in this regard (extrajunctional ACh receptors have longer open times than junctional receptors), as did the use of suberyldicholine, which causes the channel to open for a longer time than ACh does. By working on a site away from the end-plate region, it was possible to study a patch with only a few functional receptors. Finally, by electrically hyperpolarizing the fiber to -120 mV with the voltage clamp circuit it was possible to obtain channel currents larger than usual.

The current through the membrane patch showed a random series of rectangular inward-going current pulses, as diagrammed below



Each pulse occurs when a single channel is opened by the agonist drug. The pulses have an amplitude of about 1.5×10^{-12}A and a variable duration. They occur randomly and independently in time when the drug concentration is low. (No such pulses are seen when no drug is present).

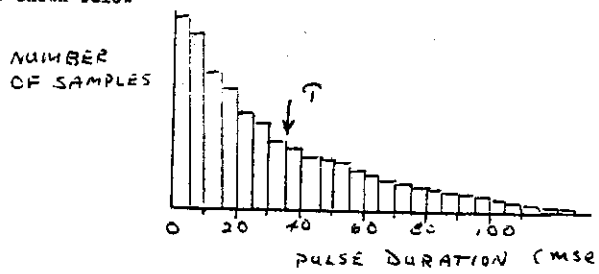
Several important conclusions can be drawn by analyzing the pulses:

1) The channel has only two conductance states: open and closed. This follows from the stereotyped size of the pulses, which is always constant at a given membrane potential. The open state conductance, g, obtained from the ratio of pulse amplitude to driving force (membrane potential minus reversal potential) is 2×10^{-11}S and is independent of potential.

2) Transitions between the open and closed states are instantaneous within the time resolution of the measurements. This follows from the abrupt rise and fall of the pulses.

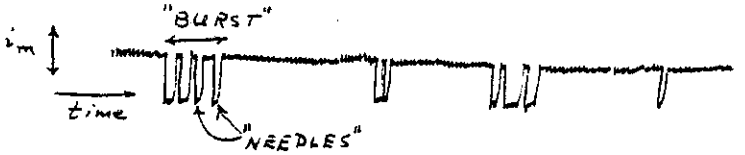
3) Analysis of the pulse duration shows that an open channel exhibits unpredictability in when it will close. Sometimes closing occurs very soon after opening, sometimes much later. The behavior is exactly like that of decay of a radioactive atom, where one cannot specify exactly when an individual atom will disintegrate, but only give the mean lifetime or half-life.

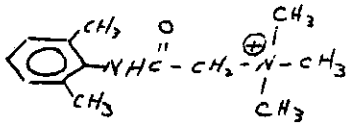
For single channels, the analogous parameter is called the mean open time, τ. A histogram of channel open times from a very large number of pulses would look like that shown below



In Neher and Sakmann's original experiments τ was about 45 msec at -120 mV membrane potential and 8°C. The mean open time was shown to depend on potential, increasing with increasing hyperpolarization of the membrane, as expected from previous work on end-plate currents. It also depended on the nature of the agonist drug, being 45 msec for suberyldicholine, 26 msec for ACh, and 11 msec for carbamylcholine. There is also a difference between the open times of the end-plate receptor channels and the extrajunctional receptors that appear after denervation: noise analysis (see later) has demonstrated that in a given fiber under the same conditions the value of τ for the extrajunctional receptors is about 3 times longer. This finding supports other evidence indicating subtle differences in the two kinds of receptors.

A striking example of how single channel recording can clarify molecular events is provided by more recent work on the effect of local anesthetics on currents through ACh channels. Neher and Steinbach found that certain lidocaine derivatives modify the single channel currents in an interesting way. Whereas the normal single channel current is a rectangular pulse of variable duration, the anesthetics caused the pulses to be chopped into bursts consisting of a series of "needles", as shown below





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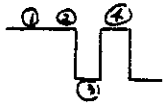
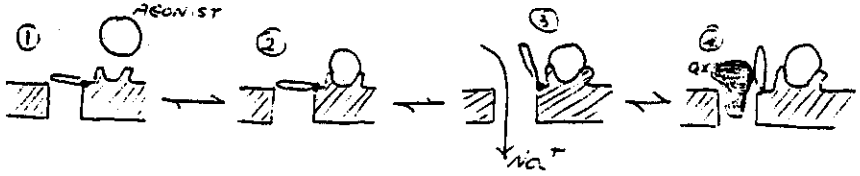
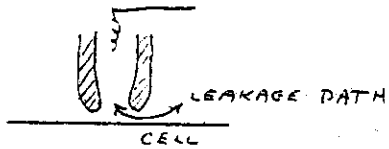
EVENTS IN A BURST:





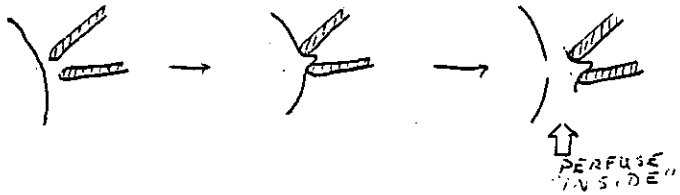
The burst could be quantitatively explained by a simple model in which it is assumed that an open channel can be blocked by the entry of a single anesthetic molecule from the outside. When this occurs, the anesthetic binds transiently, "sticking in the throat" of the open channel and preventing entry of other ions. When the anesthetic unbinds, conduction begins again. Repetitive binding and unbinding gives the chopped appearance. It is interesting that the channel cannot close while it is blocked, as indicated by the fact that the total open time during the bursts is, on average, the same as the mean open time without anesthetic. Blocking of the open channel is favored by hyperpolarization, which drives the positively-charged anesthetic molecule into the channel more frequently, while unblocking is promoted by depolarization. It has been suggested that a similar blocking process, mediated by a "tethered ball" acting from inside the membrane, may produce inactivation of the voltage-sensitive Na channels in axons.

In its original form single channel recording could resolve only very large and/or long-lasting single channel currents in the instrumental noise. This noise is generated by thermal agitation of ions in the "leakage" resistance path between the membrane and the pipette (see below).

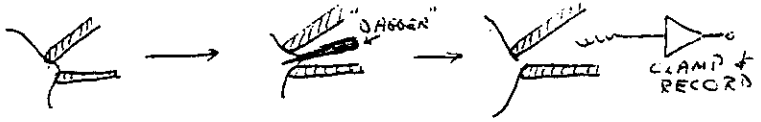


The "gigohm seal" procedure of Neher, Patlak and Sigworth improves the situation by making an extremely high resistance seal between pipette and cell (1 gigohm = $10^9\Omega$). A very clean patch of membrane is sucked forcefully against the mouth of a fire-polished glass pipette with a 1 μm hole. The membrane suddenly forms a tight bond to the glass all around the circumference of the tip, lowering the instrumental noise to a level 100 times less than that previously attainable. The small and brief currents through voltage-sensitive Na channels have been recorded in this way from tissue culture cells by Neher and Sigworth (reference at end). The seal is so tight that the patch of membrane can be avulsed from the cell, leaving the patch still attached to the tip and with the channels functioning! This allows one to change the composition of the fluid contacting the "inner" surface of the patch. Some variants of the procedure are shown below

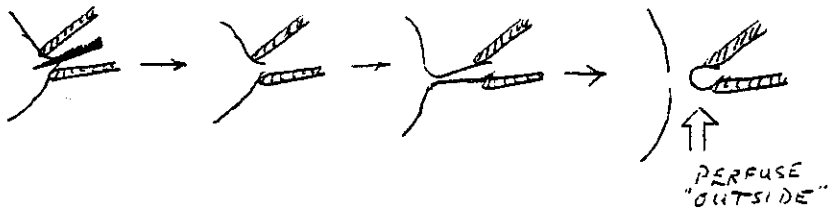
1) Gigohm seal and avulsion



2) Gigohm seal, puncture, intracellular recording and voltage clamp



3) Gigohm seal, puncture, reversal of membrane ("inside in")

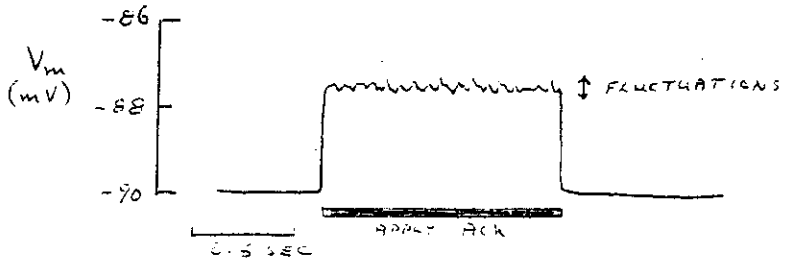


Coming years will see widespread use of these procedures for characterizing channels, receptors, and drug actions.

Noise Analysis

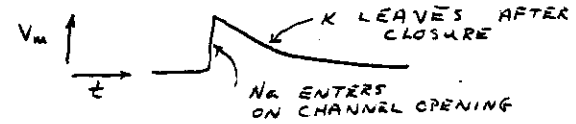
A less direct but still very useful method for determining single channel behavior is to analyze the fluctuations in a cell's voltage or current. When certain conditions are satisfied, these fluctuations give information about the small elementary events that summate to produce the average response. This approach was first used by Katz and Miladi (1972 - reference at end) in an imaginative and elegant study of ACh channels in frog muscle fibers.

The basic experiment was to record the membrane potential of a fiber while ACh was iontophoresed onto it from an external electrode positioned at the end-plate region. The depolarization produced by a steady dose of ACh was found to be accompanied by very small voltage fluctuations, as diagrammed below



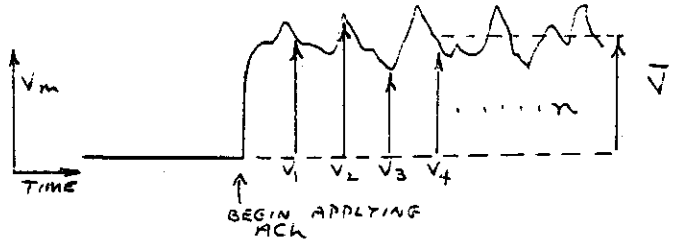
The fluctuations in the ACh response were a genuine feature of the molecular action of the transmitter, as shown by several controls. Thus, when the fiber was depolarized by current injected intracellularly, there was no noise. Furthermore, the noise was present when ACh was applied diffusely in the bath or when the injecting pipette was backed away from the fiber, ruling out fluctuations in the rate of release of ACh from the iontophoretic pipette as the source of the noise. Finally, the noise elicited by carbachol and ACh showed characteristic differences (now known to be due to the different channel open times mentioned earlier).

In analyzing the noise, Katz and Miledi assumed that it represented the superposition of many tiny transient depolarizations occurring in a random sequence in time. Each tiny depolarization was assumed to be triggered by the opening of a single ACh channel. This allows Na to enter for a brief time, and the excess positive charge on the muscle is then dissipated after channel closure by leakage of K out of the fiber:



Many such events were assumed to add up with one another, in a manner analogous to the way that raindrops on a roof produce a continuous noise.

The size of the events that underlie the noise was estimated by quantifying it and finding the ratio of the noise variance to the mean size of the signal. These parameters were measured as diagrammed below



The signal generated by ACh was measured at a large number n of regular intervals. The mean depolarization, $\bar{V}$, was determined from the values V_i at each sampling point by

$$\bar{V} = \frac{\sum_{i}^{n} V_i}{n}$$

The variance σ_V^2 was found from

$$\sigma_V^2 = \frac{\sum^{n} (V_i - \bar{V})}{n}$$

The variance may be thought of as the average value of the (deviation of the signal from the mean) squared. It is thus a measure of the "wiggleyness" of the signal.

Katz and Miledi applied Campbell's Theorem to find the event amplitude from these parameters as follows. (The treatment below is not "obligatory" but is presented for the interested or skeptical reader. Others may accept the result that the ratio $\sigma^2/\bar{V}$ gives the approximate size of the event). According to the theorem, if a noisy signal is made up of a random series of linearly additive unitary events with peak size a and shape f(t), occurring at a mean frequency ν then the mean value $\bar{V}$ and variance σ^2 are given by

$$\bar{V} = \nu \int af(t)dt$$

$$\sigma^2 = \nu \int a^2 f^2(t)dt$$

taking the constant a outside the integrals and solving for it gives

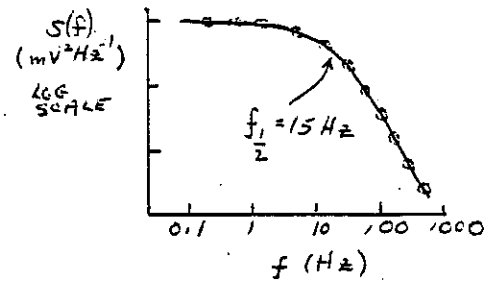
$$a = \frac{\sigma^2}{\bar{V}} \times \frac{\int f^2(t)dt}{\int f(t)dt}$$

The integral term at the far right is a "shape factor" which depends on the shape of the underlying event. In practice it has a value of 0.5-1. Clearly, even without knowing the exact value of the shape factor one can estimate the order of magnitude of the event amplitude, a, from the ratio of variance to mean. When this was done for the ACh potential, a was found to be about 0.3μV. This corresponds to a tiny charge movement of about 10^{-14}C, and about 3000 such events occurring synchronously would produce a miniature end-plate potential of 1 mV.

But what about f(t), the shape of the events? Just as the magnitude of the noise, its variance, gives information about the size of the underlying events, so also the temporal properties of the noise give information about the shape of the events. Now naturally the noise signal itself is random, which means that no two stretches of record will ever be exactly the same. Nevertheless the average speed with which the record "wiggles" is connected with the shape of the events. Fast events give a noise record that fluctuates rapidly, while slow events give slow fluctuations. These differences are independent of the average frequency at which the underlying events occur.

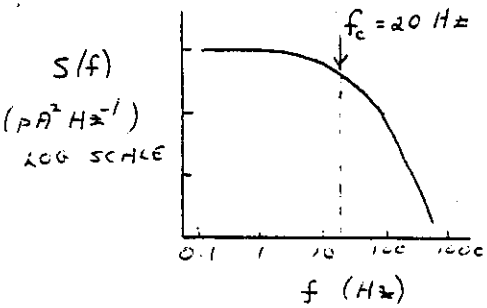
Katz and Miledi quantified the temporal properties of the noise by determining its power spectral density, or power spectrum. In this procedure one feeds a computer with a long stretch of noise record. The record is measured at a large number of points and then is decomposed into the fundamental sinusoidal components

that make up the record (its "Fourier transform"). The final power spectrum is a graph of the magnitudes of the sinusoidal components of the noise as a function of their frequency. For the acetylcholine voltage noise the spectrum had the form:



The ordinate, power spectral density or S(f), gives the contribution to the total noise variance in a narrow band of frequency centered on each of the frequencies plotted on the ordinate. The spectrum is flat below about 5 Hz and then falls at higher frequency, declining to half at about 15 Hz. It can be shown that this spectrum is exactly what would be expected for noise generated by the process we talked about earlier: a random sequence of brief inward current pulses, each causing a rapid depolarization which slowly decays as the charge spreads along and leaks across the membrane. The main factor determining the voltage decay is the membrane time constant of about 20 msec. When the spectrum is plotted in this way, on a log ordinate, the form of the spectrum depends only on the shape of the underlying events. The mean frequency of occurrence of events determines its vertical position on the ordinate. There is a reciprocity in the description of an event in the time and frequency domains. A brief event gives a spectrum which extends to relatively high frequency before declining; a long-lasting event gives a spectrum which begins to decline at lower frequency. Here the spectrum of the event is dominated by the passive cable properties of the muscle membrane and contains almost no information about the time-course of the underlying conductance change.

Anderson and Stevens determined the time course of the conductance change by analyzing voltage clamp records of the inward current flowing in response to ACh. The spectrum of these current fluctuations was not distorted by the muscle fiber's cable properties. When plotted as before, the spectrum had the form



The cut-off or half-power frequency f_c was at about 20 Hz. It was shown that the form of the spectrum was exactly that expected for a random series of events having an average form

$$f(t) = e^{-t/\tau}$$

namely a jump followed by an exponential decay. The time constant τ of this decay is related to f_c, the half-power frequency of the spectrum, by

$$\tau = \frac{1}{2\pi f_c}$$

Now Anderson and Stevens interpreted this average event shape to characterize a population of rectangular events with a variable event duration (open time), as we have discussed earlier. Their analysis and assumptions were confirmed by Neher and Sakmann, who showed directly that the half-power frequency of the spectrum of a noise record gave the same τ as that obtained by direct measurements on an ensemble of single channel currents.

A variety of other properties of the single channel events were determined by analyzing the power spectra of the noise. For instance, hyperpolarization of the fiber shifted the value of f_c to lower frequency; this indicated that the open time of the channel was prolonged. The conductance of the channel could be estimated as about 2×10^{-11}S. Can you guess how ACh and carbamylcholine spectra would differ? How junctional and extrajunctional ACh spectra would differ?

Further Reading

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Neher, E. and Sakmann, B. (1976). "Single channel currents recorded from membrane of denervated frog muscle fibers." Nature 260: 799-802.

Neher, E. and Steinbach, J.H. (1978). "Local anaesthetics transiently block currents through single acetylcholine-receptor channels." J. Physiol. 277: 153-176.

Sigworth, F. and Neher, E. (1980). "Single Na+ channel currents observed in cultured rat muscle cells." Nature 287: 447-449.

NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Synaptic Chemistry I

Bruce G. Wallace

An important concept in our understanding of the operation of the nervous system is that specificity in the synaptic interactions of neurons arises not from a proliferation of highly specific chemical and electrical signals, but rather from the very precise way in which neurons become interconnected during development. Thus, only a handful of relatively simple chemicals appear to be required as neurotransmitters. Looked at in this light, the metabolism of those compounds released as neurotransmitters may seem of secondary importance. However, if we are to understand how the efficacy of synaptic transmission can be modified by use, disuse or through the action of neuropharmacological agents, then we will want to know as much as possible about the chemistry of synapses. In addition, as our understanding of synaptic chemistry increases, it is becoming possible to interpret dysfunctions of the nervous system in terms of specific biochemical deficits and thus prescribe more appropriate therapeutic measures.

Our consideration of synaptic chemistry will encompass five general topics:

1) the identification of transmitters
2) their biosynthesis and its regulation
3) the mechanism of release of neurotransmitters
4) the interaction of transmitters with the postsynaptic membrane
5) the termination of transmitter action

I. Identification of Transmitters

A. This is an extraordinary difficult task, primarily because of the minute quantities released during synaptic transmission and the complexity of the arrangement and interconnections of cells in the nervous system.

1) Perhaps the best criterion by which the neurotransmitter at a particular synapse can be identified is: that substance which, when applied to the postsynaptic cell in the same amount as is liberated by a nerve impulse, produces the same postsynaptic effect as nerve

stimulation. It is technically difficult to achieve quantitative agreement. One can come close for acetylcholine at the vertebrate neuromuscular junction.

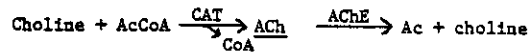
2) If one relaxes the criteria slightly, requiring that a substance be released in response to nerve stimulation and have the same effects on the postsynaptic cell, then both norepinephrine and γ - aminobutyric acid (GABA) can be included with acetylcholine as identified transmitters.

3) Several other compounds, such as 5-hydroxytryptamine (5-HT, also called serotonin), glutamate, glycine, dopamine, octopamine, and peptides (such as Substance P), have either been shown to be concentrated in some nerve terminals or to have physiological effects similar to nerve stimulation, or both, but in none of these cases has it been shown satisfactorily that the compound is released when the pre-synaptic nerve is stimulated.

II. Biosynthesis of Neurotransmitters (see Appendix)

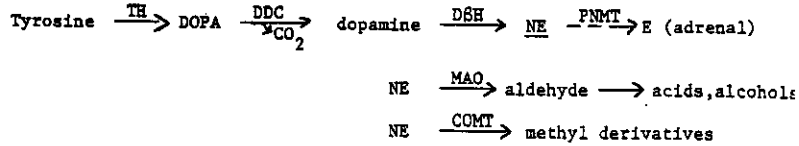
A. Pathways.

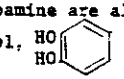
1) Acetylcholine (transmitter at vertebrate neuromuscular junction and sympathetic ganglion):



2) GABA (transmitter at lobster inhibitory neuromuscular junctions):

3) Catecholamines (released by postganglionic sympathetic neurons [NE] and adrenal medulla [E]):



4) A few notes on nomenclature. Neurons that release norepinephrine often are called "adrenergic" since sympathetic nerve stimulation affects the organs and viscera of the body in a manner that's very similar to "adrenalin" (epinephrine). Epinephrine, norepinephrine and dopamine are also called "catecholamines" (derived from catechol,  , plus amine, $-\overset{+}{N}H_3$.

III. Regulation and Transmitter Pools

A. Two general considerations are important to the understanding of how nerve terminals accumulate and maintain their stores of transmitter.

1) Transmitters are accumulated until a steady state is reached, at which the rate of synthesis equals the rate of degradation; at rest there is a continuous turnover of transmitter.

2) The rate of synthesis is determined by the level of transmitter, often by feedback inhibition on the rate limiting step in transmitter synthesis.

B. Control of ACh synthesis:

$$\text{AcCoA + choline} \underset{}{\overset{\text{CAT}}{\rightleftharpoons}} \text{ACh + CoA} \xrightarrow{\text{AChE}} \text{Ac + choline + CoA}$$

1) At rest the acetylcholine content of nerve terminals is in a steady state, the rate of synthesis by choline acetyltransferase (CAT) is exactly balanced by the rate of degradation (inside the nerve terminal) by acetylcholinesterase (plus a small leakage of acetylcholine).

2) Feedback inhibition is not important, acetylcholine does not inhibit the activity of CAT.

3) Rate of synthesis and the steady level to which acetylcholine accumulates appear to be determined at least in part by the Law of Mass Action. This means that the reactants (choline and AcCoA) and products (ACh and CoA) of acetylcholine synthesis are in chemical equilibrium.

4) Several difficulties arise from this simple scheme.

a) This is clearly not a particularly efficient way to maintain a constant store of acetylcholine, since the position of the equilibrium also will depend on the concentration of choline, acetylcoenzyme A and coenzyme A.

b) Much of the acetylcholine (probably greater than 80%) is not free in the cytoplasm where it can interact with CAT (a cytoplasmic enzyme) but rather is sequestered in vesicles.

c) The location of intraterminal acetylcholinesterase and the contribution of turnover to regulating stores is unclear; intraterminal acetylcholinesterase can be demonstrated by incubating a preparation containing cholinergic nerve terminals in solutions containing an inhibitor of AChE.

i) If the inhibitor is uncharged, like eserine, so that it can diffuse across cell membranes, then the content of acetylcholine in the preparation rapidly increases to about twice the normal resting level. Apparently eserine diffuses into the terminal, blocks ongoing degradation of acetylcholine by AChE, and so "excess" acetylcholine accumulates.

ii) This accumulation of excess acetylcholine is not seen when a charged inhibitor of AChE, such as neostigmine, is used, apparently because neostigmine cannot cross the cell membrane.

iii) AChE is present inside the terminals, but is apparently not found inside the vesicles, since stable acetylcholine-filled vesicles can be isolated.

C. The accumulation of GABA, at least in crustacean inhibitory nerve terminals where it has been best documented, is limited by direct feedback inhibition.

1) Consider the overall reaction:

$$\text{glutamate} \xrightarrow[-CO_2]{\text{GAD}} \text{GABA} \xrightarrow{\text{GGT}} \xrightarrow{\text{SSDH}} \text{succinate}$$

a) The enzymes that degrade GABA, GABA-glutamate transaminase (GGT) and succinic semialdehyde dehydrogenase (SSDH), are present in all cells at comparable levels.

b) Cells that accumulate GABA also contain high levels of glutamic acid decarboxylase (GAD), the enzyme catalyzing the synthesis of GABA from glutamate. In lobster inhibitory nerve terminals the maximum activity of GAD exceeds that of the degradative enzymes by 3 to 1, therefore GABA accumulates.

2) GABA inhibits the activity of the synthetic enzyme, GAD, so the accumulation of GABA continues until its concentration in the nerve terminal is so high that GAD activity is inhibited by 70%. At this level, the rate of synthesis is equal to the rate of degradation and the concentration of GABA is maintained in this steady state.

D. Control of norepinephrine biosynthesis.

1) Norepinephrine is synthesized from tyrosine:

$$\text{tyrosine} \xrightarrow{TH} \text{DOPA} \xrightarrow[-CO_2]{DDC} \text{dopamine} \xrightarrow{D\beta H} \text{norepinephrine}$$

2) Control must be exerted at the step in the pathway that is rate-limiting, in norepinephrine biosynthesis this step is the conversion of tyrosine to DOPA by tyrosine hydroxylase (TH).

a) the activity of DOPA decarboxylase (DDC) and dopamine β-hydroxylase (DβH) exceeds that of TH by at least two orders of magnitude.

b) the level of tissue tyrosine is relatively high, while there is very little DOPA or dopamine (except in those few places where there are neurons that apparently release dopamine as a transmitter). By analogy with other pathways, the substrate of the rate-limiting step tends to build up whereas metabolic intermediates tend to be present in very low concentration.

c) the activity of DDC or DβH can be inhibited up to 95% without affecting the rate of synthesis of norephinephrine. Inhibition of tyrosine hydroxylase greatly reduces the rate of norepinephrine synthesis.

d) using radioactively labelled tyrosine and DOPA as substrates, the synthesis of labelled norepinephrine from DOPA is more than three times faster than that from tyrosine.

e) in perfusion experiments with organs such as the heart, the K_m for the overall process of tyrosine $\longrightarrow$ norepinephrine is 2×10^{-5}M, about the same as the K_m for tyrosine hydroxylase itself.

3) The activity of tyrosine hydroxylase is regulated by <u>feedback inhibition</u>.

a) as norepinephrine accumulates in nerve terminals it gradually inhibits the activity of the tyrosine hydroxylase, until the rate of synthesis is just balanced by the rate of destruction and spontaneous release.

b) Cytoplasmic norepinephrine is degraded by monoamine oxidase (MAO), an enzyme located on the outer membrane of mitochondria, and the deaminated products are removed from the cell and eventually appear in the urine.

4) The subcellular distribution of the enzymes involved in norepinephrine biosynthesis suggests that this schema for regulating synthesis is oversimplified.

a) Tyrosine hydroxylase is apparently soluble in the cytoplasm, as is DOPA decarboxylase.

b) Most dopamine β-hydroxylase activity is associated with synaptic vesicles, approximately half firmly bound in the vesicle membrane and the remainder free inside the vesicles.

c) Most of the norepinephrine is sequestered inside the vesicles, protected from degradation by monoamine oxidase but also prevented from interacting with tyrosine hydroxylase.

d) Apparently, for feedback inhibition to operate, there must be a small cytoplasmic pool of norepinephrine, in equilibrium with the supply of norepinephrine stored in vesicles, that interacts with tyrosine hydroxylase to regulate synthesis.

5) The activity of tyrosine hydroxylase and/or dopamine β-hydroxylase is subject to modification by various other factors, including unidentified endogenous inhibitors, intracellular calcium concentration and phosphorylation.

IV. Coupling Synthesis to Activity

A. Obviously once transmitter is being released, either the pools must be rapidly replenished or eventually the amount released will be reduced as the supply is exhausted. Under most physiological conditions, the rate of synthesis is sufficiently high so that pools of transmitter are maintained and thus do not become a limiting factor in release.

B. At cholinergic nerve terminals (experiments done by Birks and MacIntosh using the superior cervical ganglion of the cat) the rate of net synthesis of acetylcholine increases in response to nerve stimulation by greater than 100 fold.

1) If a ganglion is removed from the animal and perfused with plasma, the store of acetylcholine is maintained for several hours, even if the preganglionic nerve is stimulated at a rate of 20 impulses/sec.

2) In the presence of eserine to inhibit endogenous cholinesterases, acetylcholine can be detected in the perfusate in the absence of nerve stimulation .

a) Release from the resting ganglion is equal to about 1/1000th of the total acetylcholine content/min. Since the total acetylcholine content of the ganglion is constant, this release represents the net acetylcholine synthesis of the ganglion at rest.

b) When the ganglion is stimulated at 20 impulses/sec, the rate of acetylcholine release increases 100 fold; each minute about 10% of the total acetylcholine content of the ganglion is released. This rate of release can be maintained for as long as 60 minutes. Thus, during one hour of stimulation, the ganglion can release six times the original amount of acetylcholine and still end up containing as much as was originally present.

3) If eserine is added to the perfusion fluid the amount of acetylcholine present in the ganglion doubles, indicating that acetylcholinesterase plays a role in determining presynaptic acetylcholine levels.

a) the rate of accumulation after the addition of eserine can be considered a measure of the rate of synthesis at rest and is initially quite rapid, a 25% increase in the acetylcholine content

in the first 5 min. At this rate, a resting ganglion would synthesize and degrade 3 times its initial acetylcholine content in one hour. The contribution that changes in the rate of degradation of transmitter makes to the control of presynaptic stores has not been explored.

b) the "surplus" acetylcholine that accumulates in the presence of eserine does not affect the rate of acetylcholine release from the ganglion at rest or in response to nerve stimulation. Thus, changes in the levels of transmitters in tissues do not necessarily have any effect on the amount released.

4) Cholinergic nerve terminals require an exogenous source of choline to maintain release.

a) If a ganglion is perfused with Locke's solution (a buffered salt solution) instead of with plasma, the level of acetylcholine in the ganglion and the amount released decreases very rapidly.

b) acetylcholine synthesis can be maintained if choline is added to the perfusion fluid.

c) If hemicholinium HC-3, a compound that inhibits choline uptake, is added to the perfusion fluid, then even if choline is present the content of acetylcholine in the ganglion and the amount released by nerve stimulation fall rapidly to low levels.

d) The choline uptake mechanism is specific for choline and similar compounds, acetylcholine is not taken up; if the hydrolysis of acetylcholine is prevented by an anticholinesterase, the rate of release falls rapidly during prolonged stimulation in Locke's solution.

e) Demonstration of uptake as the source of choline for acetylcholine synthesis comes from experiments in which the preparation is bathed in fluid containing ^{14}C-choline and stimulated. The rate at which ^{14}C-choline is incorporated in the acetylcholine stores is enhanced when AChE is inhibited, because this inhibition removes a competing source of choline. Put the other way around, under normal conditions, cholinergic nerve terminals recapture and reuse nearly half of the choline produced by transmitter hydrolysis.

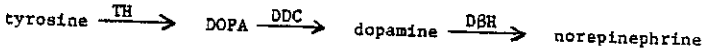
5) Newly synthesized acetylcholine is preferentially released

a) If sympathetic ganglia are incubated in fluid containing ^{14}C-choline so that all the intracellular acetylcholine pools become labelled and then perfused with Locke's solution, the rate at which the stored ^{14}C-acetylcholine is released, both at rest and in response to nerve stimulation, is reduced if unlabelled choline is included in the perfusion fluid. Unlabelled acetylcholine, which must have been newly synthesized from unlabelled choline, is released twice as fast as labelled acetylcholine.

b) These data are compatible with the concept that a small readily releasable part of the transmitter stores in cholinergic nerve terminals turns over more rapidly than the remainder. This biochemical finding must be kept in mind when attempting to interpret other data on release from cholinergic nerve terminals, such as the vesicle recycling hypothesis suggested by Heuser and Reese.

C. Regulation of norepinephrine synthesis.

1) These experiments have been done using tissues that receive sympathetic innervation, such as the heart, spleen, and vas deferens. The pathway for norepinephrine biosynthesis is:



2) The rate of synthesis is regulated by feedback inhibition of tyrosine hydroxylase by norepinephrine.

3) There is a steady turnover of norepinephrine at rest, the rate of synthesis balanced by the rate of degradation.

4) Nerve stimulation increases the rate of norepinephrine synthesis as measured by the formation of radioactively labelled norepinephrine from labelled tyrosine

a) stimulation does not increase the uptake of tyrosine by the tissue or affect the production of metabolites from norepinephrine.

b) the rate of synthesis of norepinephrine from ^{3}H-DOPA is the same in both control and stimulated preparations and about three-fold higher than that seen in unstimulated preparations with ^{3}H-tyrosine as precursor (Table I).

TABLE I

RATE OF NOREPINEPHRINE SYNTHESIS

Precursor	tyrosine	DOPA	tyrosine + NE
Control	100%	325%	42%
Stimulated	170%	320%	38%

c) These experiments verify two important hypotheses.

i) The hydroxylation of tyrosine is the rate-limiting step in norepinephrine synthesis: norepinephrine is formed more rapidly when DOPA is used as a precursor.

ii) Control is exerted at the rate-limiting step, that is, stimulation increases the rate of synthesis from tyrosine but not from DOPA.

5) We might speculate that as norepinephrine is released from sympathetic nerve terminals during stimulation, the concentration of norepinephrine in the cytoplasm is lowered, reducing the feedback inhibition of tyrosine hydroxylase and thus increasing the rate of synthesis of norepinephrine

a) If so any treatment that increases the level of norepinephrine in the cytoplasm should increase feedback inhibition and slow synthesis.

b) Incubation of tissues in exogenous norepinephrine and administration of reserpine, a drug that blocks uptake of norepinephrine into vesicles, increase the level of norepinepherine in the cytoplasm and reduce the rate of conversion of tyrosine to norepinephrine (see Table I).

c) Maintaining high cytoplasmic norepinephrine concentrations by these means also blocks the increase in the rate of synthesis of norepinephrine from tyrosine seen with nerve stimulation (Table I).

6) Newly synthesized norepinephrine is preferentially released.

a) If the spleen is perfused with a solution of ^{14}C-tyrosine and the splenic nerve stimulated the specific activity of norepinephrine in the perfusate rapidly increases and exceeds the specific activity of norepinephrine remaining in the preparation by eight fold.

b) If stimulation is continued and the perfusion fluid is changed to one containing unlabelled tyrosine, the specific activity of released norepinephrine rapidly declines below that of the norepinephrine remaining in the preparation.

7) These effects on tyrosine hydroxylase activity are all short term, occurring with a time course of seconds or minutes. They involve changes in the activity of existing enzymes and do not involve protein synthesis or changes in the actual number of tyrosine hydroxylase molecules in the nerve terminal. This is always tested by grinding up the preparation, assaying for tyrosine hydroxylase activity in a test tube, and finding no change in the amount of enzyme, i.e. no change in activity at saturating levels of substrates.

D. Long-term Control

1) The sympathetic nervous system has proved particularly fruitful as a system in which to study the influence of activity, hormones, and other "factors" on enzyme levels over periods of hours, days, or weeks.

a) treatments that require the sympathetic nervous system to be abnormally active for extended periods of time increase the ability of nerve terminals and the adrenal medulla to synthesize norepinephrine from tyrosine by increasing the number of tyrosine hydroxylase molecules.

i) the stress of prolonged exposure to cold or forced immobilization

ii) Administration of certain drugs, such as reserpine, which depletes vesicles of norepinephrine, α-methyltyrosine, which blocks the synthesis of DOPA, or phenoxybenzamine, which blocks the effect norepinephrine by blocking some of the postsynaptic receptors.

b) Other enzymes, such as lactic acid dehydrogenase, monoamine oxidase, and DOPA decarboxylase, are not affected. (Some increase is seen in dopamine β-hydroxylase)

c) Inhibitors of protein synthesis block the increase in tyrosine hydroxylase levels, suggesting that new enzyme molecules are being synthesized. Also, by immunological techniques you can show that the number of enzyme molecules has increased.

d) The signal that triggers sympathetic neurons and adrenal medullary cells to synthesize more tyrosine hydroxylase molecules appears to be at least in part mediated by neural input. The induction is abolished by either decentralization, i.e., cutting the fibers that provide input to the sympathetic neurons or adrenal from the central nervous system, or by blocking transmission between the CNS and the sympathetic neurons pharmacologically.

2) One can speculate that the trans-synaptic induction of tyrosine hydroxylase functions continually to keep the synthetic capability of the neurons and adrenal medullary cells at appropriate levels via a peripheral feedback loop.

a) Sensory neurons in the periphery monitor the response of target organs to sympathetic activity.

b) If the effect of sympathetic activity on peripheral organs is insufficient (for example, blocked pharmacologically with phenoxybenzamine) the CNS responds by increasing the rate of firing of cells supplying input to the sympathetic neurons and adrenal medulla

c) this increased activity in some way signals the sympathetic cells to gear up and improve performance by synthesizing more of the important enzymes.

3) Other factors have long term effects on catecholamine synthesis in the sympathetic system.

i) Insulin increases adrenal tyrosine hydroxylase; apparently the hypoglycemic stress put on the sympathetic system by the insulin reaction increases neural input to the adrenal which in turn causes enhanced synthesis of tyrosine hydroxylase.

ii) Nerve growth factor (NGF) apparently can influence the levels of enzymes involved in catecholamine synthesis in the adult and during development.

In summary, we have seen that the complex synaptic interactions of cells in the nervous system may be mediated by a relatively small number of simple chemical compounds. The best studied neurotransmitters are acetylcholine, GABA, and norepinephrine. The accumulation of these transmitters is controlled by balancing the rates of synthesis and degradation. In neurons, a significant fraction of the stored transmitter is liberated in response to stimulation and stores are maintained by enhancing the rate of synthesis by mechanisms such as removal of feedback inhibition. Long-term controls operate to maintain the synthetic capability of the nerve terminal at a level sufficient to meet the demands of release.

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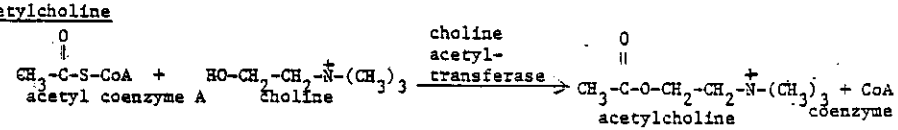
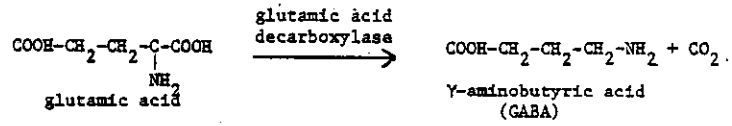
APPENDIX

BIOSYNTHETIC PATHWAYS

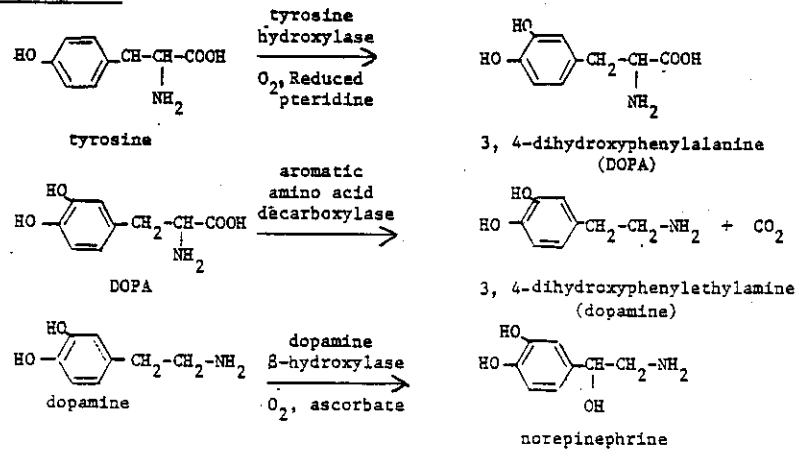
Acetylcholine



GABA

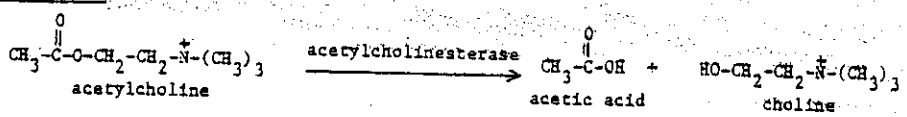


Norepinephrine

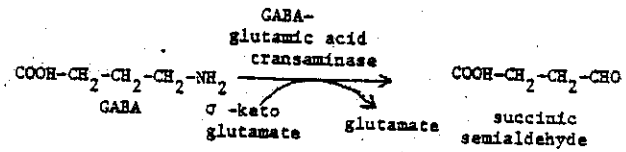


DEGRADATIVE PATHWAYS

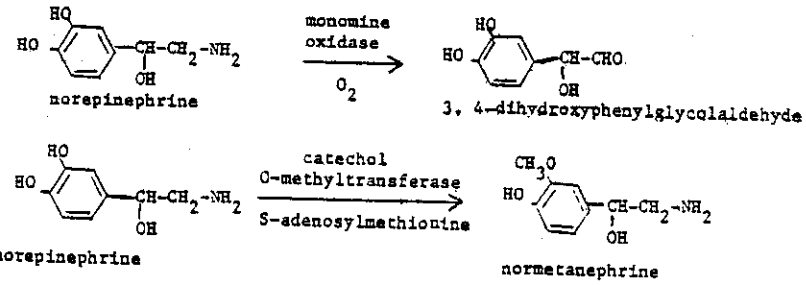
Acetylcholine



GABA



Norepinephrine



NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

Synaptic Chemistry II

Bruce G. Wallace

In the first lecture we discussed factors regulating the accumulation and maintenance of transmitter stores in presynaptic terminals. In this lecture we will consider biochemical studies of the mechanism of transmitter release, the interaction of transmitters with the postsynaptic membrane and transmitter inactivation. Again, we will confine our discussion to results from a small number of synapses at which acetylcholine (ACh), norepinephrine (NE), and γ-aminobutyric acid (GABA) are released as neurotransmitters.

I. Mechanism of Transmitter Release.

A. Morphological and physiological evidence indicates release by exocytosis

1) Physiological evidence at vertebrate NMJ indicates that ACh is released in multimolecular packets, or quanta, each producing a "miniature e.p.p."

2) In electron micrographs, nerve terminals are packed with vesicles, especially clustered near presynaptic release sites.

3) There is also biochemical evidence consistent with release by exocytosis, in which a vesicle fuses with the external membrane of the cell and flattens out, releasing its entire soluble contents. This consists of comparing the contents of vesicles with what is released in response to nerve stimulation.

B. Release from the adrenal medulla - a model for release from sympathetic nerve terminals.

1) Isolate vesicles by differential centrifugation. They are large (200 nm in diameter), have dense cores, and contain most of the epinephrine present in the adrenal.

2) Lyse vesicles by hypo-osmotic shock and determine soluble contents and composition of membrane (Table I).

3) Perfuse adrenal, stimulate release by activating splanchnic nerve or by perfusing with ACh or carbachol. Collect perfusate and analyze for release of soluble vesicle contents (Table I).

	CA (μ mole)	ATP (μ mole)	DβH (units)	Chromogranin A (mg)
in granule	4	1	29	0.40
in perfusate	4	1	37	0.34

4) Since ratio of catecholamines to ATP, DβH, and chromogranin A (the major soluble protein in adrenal vesicles, identified immunologically) in the lysate of the vesicles is the same as that released in response to stimulation, this is strong evidence for release of the entire soluble contents of vesicles by exocytosis.

5) Further evidence: soluble cytoplasmic proteins are not released (e.g. lactic acid dehydrogenase, phenylethanolamine N-methyltransferase); phospholipids and cholesterol, constituents of vesicle membrane, not released; electron micrographs show profiles fused to cell membrane and open to extracellular space, as well as entire dense cores outside of the cell, but never an entire vesicle outside the cell or a "hole" in the cell membrane.

6) Lipid composition of vesicle membranes differs from that of other membranes, has an abnormally high proportion of lysolecithin (lyso lipids are missing one of the usual two fatty acids). The ratio of lysolecithin to total phospholipid is ≈15%, other subcellular organelles have ≈1%. Lysolipids have been shown to cause membrane fusion in several in vitro systems. However, synaptic vesicles isolated from brain or electric organ have only ≈ 1% lysolipids.

7) Retrieval of vesicle membrane from surface of cell after fusion must be very specific, both appropriate proteins (DβH, ATPase) and lipids must be retrieved or destroyed and replaced.

C. Release from sympathetic neurons

1) There are both large (100 nm) and small (50 nm) dense core vesicles in sympathetic neurons, large predominate in cell bodies, small predominate in terminals.

2) Large vesicles, isolated from postganglionic nerves, are known to

contain NE, ATP, DβH, and chromogranins, and all constituents are released together.

3) In spleen, amounts of DβH and chromogranin recovered in perfusate are 6% and 1%, respectively, of the amounts present inside large preterminal vesicles. However, the contents of large preterminal vesicles apparently differ from those of the smaller terminal vesicles, therefore it is difficult to make a quantitative comparison.

4) In hypogastric nerve-vas deferens preparation it has been observed that NE/DβH in vesicles (isolated from terminals) is the same as that in perfusate during stimulation.

D. Release from cholinergic terminals.

1) Best morphological evidence for 1 vesicle = 1 quantum comes from vertebrate skeletal neuromuscular junction, experiments of Heuser and Reese.

2) Good physiological data for number of molecules necessary for quantum from Katz "noise" analysis (approximately 1000 ACh-receptor interactions) and Kuffler's iontophoresis experiments (5000-10,000 molecules applied).

3) Isolate ACh vesicles from electric organ of the marine rays Torpedo or Narcine. Vesicles contain ACh and ATP in a molar ratio of approximately 2.5 to 1. Although it has been reported that cholinergic vesicles contain one major soluble protein, vesiculin, no soluble proteins are found in highly purified vesicle preparations.

4) ATP is released along with ACh when nerves are stimulated; no information on release of vesiculin from cholinergic nerve terminals is available.

E. How do vesicles accumulate transmitters?

1) Concentration of transmitters in vesicles is apparently greater than that in cytoplasm, therefore they must be pumped or somehow held against a concentration gradient.

2) Vesicles isolated from sympathetic nerves take up catecholamines in vitro by a process requiring Mg^{++} and ATP, but have not been shown to accumulate NE against a concentration gradient. The active uptake of catecholamines into chromaffin granules appears to be driven by a transmembrane proton concentration gradient which is maintained by an ATP-powered proton pump.

Vesicle uptake is not specific for norepinephrine or epinephrine,

a variety of related amines are also transported. Transport is blocked by reserpine, with the result that vesicles become permanently leaky to catecholamines.

3) Isolated cholinergic vesicles slowly lose their stores of ACh. No active uptake of ACh into cholinergic vesicles has been demonstrated.

F. Cholinergic and adrenergic vesicles appear to contain transmitter, ATP, and proteins that have no known enzymatic function. It has been postulated that ATP serves to neutralize the charge of the transmitters and that, at least for catecholamine-containing vesicles, the proteins provide a matrix for binding the transmitter-ATP complex so that the stores are not hyperosmotic.

II. Interaction of transmitters with receptors.

A. Acetylcholine receptors.

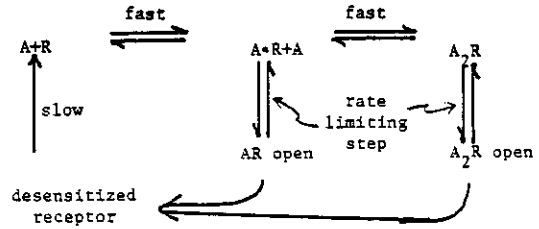
1) Different classes of ACh receptors can be distinguished pharmacologically.

a) Nicotinic: agonists include nicotine and carbachol, antagonists include hexamethonium, curare, and specific snake venoms (e.g. α-bungarotoxin). Found at skeletal NMJ, effect of ACh is to increase permeability to Na and K.

b) Muscarinic: agonists include muscarine and acetyl-β-methylcholine, antagonists include atropine and scopolamine (hyoscine). Are not inhibited by α-Bungarotoxin and similar snake venoms. Found at parasympathetic postganglionic terminals, e.g., onto heart muscle, effect of ACh is to increase permeability to K.

2) Combination of transmitter and receptor rapidly opens ionic conductance channels in membrane, receptor must include both an ACh binding site and an ionophore.

3. Reaction scheme for nicotinic receptor.



a) A = acetylcholine, R = receptor, AR and A_2R are receptor sites combined with 1 or 2 ACh molecules.

b) In continued presence of ACh receptor will desensitize and no longer produce an ionic conductance change.

4. Isolation of nicotinic receptor made possible by two discoveries: an especially rich source of material in electric organs of rays (Torpedo) and eels (Electrophorus) and high affinity, highly specific ligands.

a) α - bungarotoxin, basic polypeptide of 8000 daltons, binds almost irreversibly to receptor.

b) α - cobra toxins (Naja naja species) also very specific but binding is more readily reversible.

c) Isolation of receptor by affinity chromatography of detergent solubilized extract

5. Properties of nicotinic receptor from Torpedo.

a) Native protein is large (250,000-500,000 daltons).

b) Sodium dodecyl sulfate polyacrylamide gels show four subunits with molecular weights of approximately 64K, 60K, 50K and 40K. They are present in a molar ratio of 1:1:1:2.

c) 40K subunit has binding site for acetylcholine.

d) Reconstitution experiments introducing purified receptor back into lipid vesicles and planar bilayers demonstrate that this complex contains both the acetylcholine binding site and the ionophore.

6. Receptors are densely packed at neuromuscular junction.

a) I^{125}-α-bungarotoxin and quantitative EM autoradiography demonstrate receptors are concentrated on upper portions of synaptic folds.

b) Assuming two α-bungarotoxin binding sites per receptor, there are 13,000 receptors per μ^2. This density leaves little room for any other proteins.

c) By comparison, there are 2600 ACh esterase sites per μ^2 and they are uniformly distributed along synaptic folds. Most of the ACh esterase is attached to the basal lamina.

7. Myasthenia gravis, a disease characterized by skeletal muscle weakness and easy fatigability, appears to be an auto-immune disease in which patients produce antibody against the acetylcholine receptor.

a) Disease can be mimicked in rabbits by injection of purified receptor or γ-globulin fraction from myasthenic serum.

b) Biopsy samples from myasthenic patients show reduction of α-bungarotoxin binding sites to about 20% of normal.

c) Anti-receptor antibodies can be detected in serum from myasthenic patients but not in control sera or sera from patients with other neurological diseases affecting skeletal muscle.

B. Catecholamine receptors.

1) There is an extensive pharmacology by which classes and subclasses of receptors for catecholamines have been distinguished. We need only mention two main classes: α-receptors, which are blocked by phenoxybenzamine and β-receptors, which are blocked by propranolol.

2) Little information on the structure or localization of postsynaptic receptors for catecholamines is available. There are no highly specific toxins for catecholamine receptors nor has a particularly rich source been found.

3) The postsynaptic effects of catecholamines are mediated by at least two general mechanisms. In the smooth muscle of the guinea pig vas deferens, for example, norepinephrine released from adrenergic nerve terminals activates α receptors, increasing Na conductance and causing muscle action potentials in a manner exactly analogous to the action of acetylcholine at the skeletal NMJ. At many other sites, such as cardiac muscle, the interaction of norepinephrine with postsynaptic β-receptors does not lead directly to any significant change in ionic conductance, but rather activates adenyl cyclase and causes accumulation of cyclic AMP in the post synaptic cell. In such cases application of cAMP analogues often mimic the effects of adrenergic stimulation, giving rise to slow, long lasting electrical signals or changes in the responsiveness of the postsynaptic cell. The mechanism by which changes in cAMP produce many of these effects remain obscure, although other activation of a protein kinase leading to phosphorylation of a protein(s) is hypothesized.

III. Mechanism of Transmitter Inactivation.

A. At cholinergic synapses the action of ACh is terminated by hydrolysis by acetylcholinesterase, AChE.

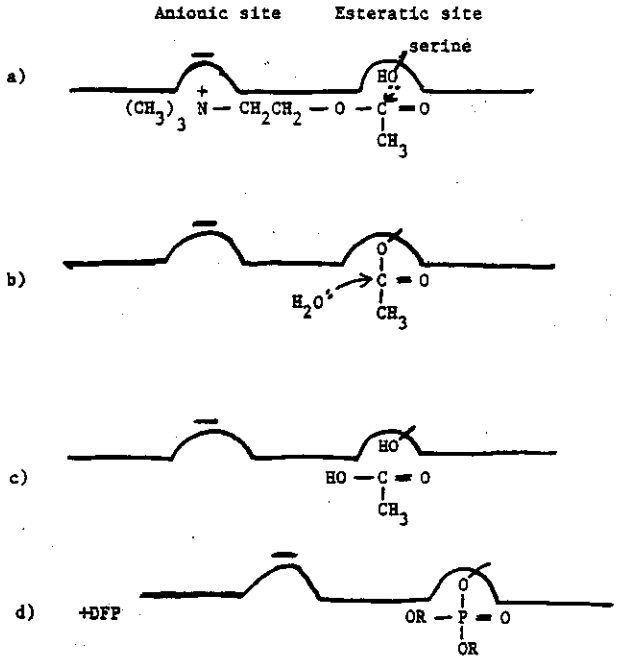
1) After inhibition of AChE with eserine or neostigmine, the response to a single impulse is potentiated and prolonged.

2) During a long train, this initial potentiation and prolongation is followed by a reduction and eventual abolition of the postsynaptic response as the ACh receptors become desensitized. Thus, AChE inhibitors can block neuromuscular transmission.

3) This is the mechanism of action of particularly potent poisons, the organophosphorus compounds such as DFP (diisopropylfluorophosphonate). They are the infamous "nerve gases" stockpiled by the government, but are also used as insecticides.

4) The detailed studies of the mechanism of action of acetylcholinesterase led to an antidote for DFP poisoning, pyridine-2 aldoxime methiodide (2-PAM).

5) Active region of acetylcholinesterase has two sites, an anionic site that binds the positively charged choline portion of ACh and the esteratic site capable of donating electrons to the acetate portion of ACh (see Fig. a). An acetyl-enzyme intermediate is formed, then hydrolyzed by water (b,c). DFP is also a substrate for AChE, but the alkylphosphoryl-enzyme intermediate is not hydrolyzed by water (d). By knowing the configuration of the active region, it was possible to design a molecule (2PAM) that would bind to the anionic site and have a ready pair of electrons to hydrolyze the phosphoryl-enzyme intermediate.



B. Inactivation of norepinephrine occurs by uptake into the presynaptic terminal.

1) MAO and COMT are not involved in transmitter inactivation:

a) They are intracellular.

b) Inhibition does not potentiate the effect of nerve stimulation.

c) They are important in inactivating amines in the circulation.

2) Uptake can be blocked by drugs such as cocaine and amphetamines. They potentiate the actions of amines. Uptake into terminals is by a different mechanism than uptake into vesicles, e.g., it is not blocked by reserpine.

3) Uptake is into presynaptic terminal:

a) Transmitter taken up can be released by nerve stimulation

b) No transmitter is accumulated by denervated tissues

c) Radioactively labelled transmitters can be localized in nerve terminals by EM autoradiography.

C. Inactivation of GABA at the lobster NMJ occurs by diffusion from the synaptic cleft and uptake into glia cells. Glia cells in the mammalian CNS also have a high affinity uptake mechanism for GABA, but here neurons also may recapture GABA after its release.

D. High affinity uptake (10^{-8} M) mechanisms for choline and norepinephrine may be distinguishing features of nerve terminals releasing ACh and NE, but cannot be considered conclusive.

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NEUROBIOLOGY 200. The Nervous System

Fall Quarter 1981

CNS Transmitters: Amines, Peptides, and Neuromodulation

Bruce G. Wallace

CNS TRANSMITTERS: AMINES, PEPTIDES, AND NEUROMODULATION

I. Identification of transmitters in CNS.

A. Technically difficult because of inaccesibility, multiple inputs, difficulty in separating pre-and postsynaptic elements, identification of terminals and widespread occurrence of amino acid transmitters (glycine, glutamate).

B. Some neurons may not form punctate synaptic specializations but liberate their transmitter more diffusely.

1. Could interact with specific postsynaptic receptors causing conductance change and thus a relatively slow synaptic potential.

2. Or could act as neurohormone, interacting with receptors on pre- or postsynaptic cells to modulate efficacy of synaptic transmission. For example:

a) Might affect Ca^{+} levels in presynaptic terminal and thereby regulate amount of transmitter released.

b) Might interact with postsynaptic cell to increase input resistance and so increase amplitude of synaptic potentials.

c) Specificity is determined not by precise release site but by localization of receptors and the response they mediate.

3. Modulation of transmission in cerebellar cortex

a) NE released from terminals of locus coeruleus slows spontaneous activity of Purkinje cells and reduces resting membrane conductance.

b) Apparently NE interacts with β-receptor on Purkinje cells activating an adenyl cyclase, increasing the intracellular concentration of cAMP, which leads to the decrease in conductance and hyperpolarization.

c) In many other instances the effects of stimulation of β-receptors appear to be mediated by changes in cAMP.

4. Modulation of transmission in gill-withdrawal reflex in Aplysia

a) Transmission from identified sensory to motor neurons is facilitated by stimulation of a 5-HT-containing interneuron that makes presynaptic endings on the terminals of the sensory neuron.

b) Stimulation of this interneuron or application of 5-HT activates an adenyl cyclase, increasing intracellular cAMP in the nerve terminal.

c) cAMP activates a protein kinase that apparently phosphorylates some protein in the membrane. As a result the input conductance of the cell is lowered, the action potential is altered so that more Ca^{++} enters during an impulse, and, as a result, more transmitter is released.

d) Effect can be mimicked by intracellular injection of cAMP or the catalytic subunit of protein kinase and may result from a decrease in K^{+} conductance.

e) Increase in transmitter release is long-lasting.

5. Actions of neurohormones may be mediated by complex mechanisms including changes in Ca^{++} concentration, levels of cyclic nucleotides, and protein phosphorylation.

a) Many effects of intracellular Ca^{++} may be mediated by calmodulin, a ubiquitous Ca^{++}-binding protein. Ca^{++}-calmodulin can activate protein kinases leading to phosphorylation of specific proteins.

b) Ca^{++}-calmodulin levels regulate the activity of a Ca^{++}-dependent adenylate cyclase and phosphodiesterase, both isolated from brain. Thus levels of cyclic nucleotides can be influenced by Ca^{++} influx.

C. In the CNS many amines and peptides may primarily be acting as neurohormones rather than neurotransmitters.

Peptides.

A. Hypothalamic releasing factors.

1. Hypothalamus and pituitary are major regulatory areas for various visceral activities: water balance, internal secretions, sugar and fat metabolism, temperature regulation, sleep.

a) Control is exerted by neurons in hypothalamus that send processes into hypophysis (pituitary).

b) These hypothalamic cells release peptides into the portal circulation within the hypophysial stalk which carries these "releasing factors" or "hormones" to secretory cells in the hypophysis which, in turn, release peptide and protein hormones into the circulation.

2. Releasing factors are peptides from 3 to 14 amino acids long: the simplest is thyrotropin releasing hormone (TRH): pyroglutamyl-hystidyl-proline amide.

3. Major amounts of releasing factors are located outside the hypothalamus.

a) When injected into animals these peptides produce profound behavioral changes.

b) Many neurons in various areas of CNS are inhibited by releasing factors.

4. There is considerable functional interaction between amines and hypothalamic peptides.

   a) Hypothalamus and median eminence receive input from NE, dopamine and serotonin containing cells.

   b) Depleting catecholamines from certain hypothalamic regions stimulates prolactin secretion and supresses release of follicle stimulating hormone and luteinizing hormone.

   c) Release of melanocyte stimulating hormone is enhanced by reserpine and melanocyte stimulating hormone release inhibiting hormone accumulates in the hypothalamus.

B. Substance P.

1. First isolated as hypotensive substance (lowered blood pressure) that also caused contraction of intestinal tissue.

2. Later a "sialogenic" peptide (causes massive salvation) was discovered while isolating releasing factors from hypothalamus. It turned out to be identical to substance P.

3. Substance P is an undecapeptide:
   H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

4. Substance P found in substantia nigra, hypothalamus, dorsal roots (but not ventral roots), and substantia gelatinosa.

5. Substance P implicated as sensory transmitter, perhaps specifically for those neurons involved in perception of pain, but may act as modulator of transmitter release from sensory terminals.

   a) Depolarizes spinal motoneurons (although response is very slow); seems to excite selectively cells in dorsal horn that respond to noxious stimuli.

   b) Ligate dorsal roots, Substance P accumulates on peripheral side of ligation and decreases in spinal grey. Cut dorsal roots, Substance P disappears from dorsal horn.

   c) Ca^+ dependent release of Substance P from spinal cord with sensory nerve stimulation.

   d) Immuno fluorescence shows Substance P in small cell bodies in sensory ganglia (approximately 20% of cells stain), in terminals in the outermost layers of spinal cord (substantia gelatinosa), and in free nerve endings in the skin. Substance P is also found in the nucleus caudalis of the trigeminal, that region generally believed to receive nociceptive input. It disappears from the nucleus after sections of the trigeminal nerve.

   e) Terminals of sensory neurons are depolarized by Substance P.

   f) Capsaicin, a derivative of homovanillic acid, produces intense pain when administered acutely and causes release of Substance P from spinal cord. Chronic treatment renders animals insensitive to pain and depletes Substance P in the substantia gelatinosa.

C. Enkephalin.

1. Radioactive opiates selectively bind to membrane fragments from brain homogenates, suggesting the presence of opiate "receptors".

   a) There is high degree of correlation between binding of agonists and antagonists to isolated opiate receptor and the pharmacological potency of drugs.

   b) Opiate receptors are primarily found in CNS regions concerned with pain.
      i. Regions receiving direct input fron pain pathways: Periaqueductal gray in midbrain reticular formation and thalamic regions.
      ii. Regions probably involved in emotional perception of pain: hypothalamus and amygdala.

2. Since brain does not <u>normally</u> contain opiates, what are the normal substrates for these receptors?

   a) Enkephalins (pentapeptides: H-Try-Gly-Gly-Phe-Met [or Leu]-OH) Isolated from brain, have same regional distribution as receptor.

   b) Act as agonists in bioassays and binding to receptor preparations: blocked by opiate antagonists.

3. Enkephalin sequence found in β-lipotropin, a large pituitary hormone.

   a) Residues 61-65 are Met-enkephalin.

   b) Residues 61-91 more active than enkephalin, normally present in pituitary, called β-endorphin.

4. Synthesis of various peptides appears to occur by cleavage of large precursor protein.

   a) A 31,000 dalton protein is precursor for adrenocorticotropic hormone (ACTH) and β-lipotropin.

   b) β-lipotropin contains sequences for β-melanocyte stimulating hormone, enkephalin, endorphins.

   c) In stress situations, when ACTH is released, there is also a well known analgesic response.

   d) If peptide is released as transmitter, there is a problem: in mammals peptides longer than 3 amino acids are invariably synthesized on ribosomes, but there are no ribosomes in nerve terminals. May be overcome by releasing very small amounts and having receptors with very high affinity (e.g. 10^{-8} to 10^{-10}M rather than 10^{-6} or 10^{-8} as for acetylcholine and catecholamines).

5. In spinal cord enkephalins apparently bind presynaptically to inhibit the release of Substance P from sensory nerve terminals.

   a) There is a striking similarity in the anatomical distribution of opiate receptors, enkephalin and Substance P; by immunohistochemistry, enkephalin-containing interneurones and Substance P containing sensory terminals are abundant in substantia gelatinosa.

b) In experiments on spinal cord slices, opiates and enkephalin block the release of Substance P.

c) After dorsal root section Substance P and the number of opiate binding sites in the substantia gelatinosa fall dramatically, but there is no change in enkephalin content.

6. A number of neurons have been found that contain both a neuropeptide and norepinephrine or serotonin, suggesting that a single cell may release more than one neurotransmitter or neuromodulator.

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NEUROBIOLOGY 200: The Nervous System

Fall Quarter 1981

Brainstem II: Reticular Formation,

Raphé Nuclei, Locus Coeruleus,

Substantia Nigra

Bruce G. Wallace

BRAINSTEM II: RETICULAR FORMATION, RAPHÉ NUCLEI, LOCUS COERULEUS, SUBSTANTIA NIGRA

I. Amine-containing cells in the brainstem.

A. Much of the mass of the brainstem is not organized in discrete nuclei and tracts but consists of a diffuse network of neurons and processes, called the Reticular Formation.

1. Phylogenically old portion of brain, remained as more rostral regions developed preferentially during evolution.

2. Receives input from most sensory systems and has efferent connections to all levels of the neuraxis.

3. Primitive "reticular" (forming a network) character is retained in multineuronal, polysynaptic nature of its intrinsic pathways.

B. Prominent feature of brainstem, especially the reticular formation, is the presence of cells containing biogenic amines.

1. Catecholamine and 5-HT (serotonin) cells and processes localized using formaldehyde-induced fluorescence technique of Falk-Hillarp.

2. In mammalian CNS there are relatively few amine containing cells, their cell bodies lie in the brain stem.

3. Processes of these cells appear to innervate almost all areas of the brain, although often there are no obvious synaptic specilizations seen.

II. Locus coeruleus - cluster of NE-containing cells just beneath floor of rostral fourth ventricle.

A. Locus coeruleus nucleus in rat contains some 1400 cells, all of which contain NE.

1. Nucleus accounts for about 45% of NE-containing cells in rat brain.

2. Nucleus innervates cerebral cortex, cerebellum and thalamus, single cell may project to both cerebral and cerebellar cortices.

3. Profiles containing dense-core granules form "normal" synaptic terminals (e.g. on dendritic spines on cerebellar Purkinje cells) and also are seen without obvious pre- or post-synaptic specializations.

4. Stimulation within locus coeruleus inhibits spontaneous firing of targets such as the Purkinje cells, and exogenously applied norepinephrine produces a similar effect. This inhibition is slow in onset, prolonged, and the hyperpolarizing response of the target cell is accompanied by <u>increased</u> membrane resistance.

   a) Response is mediated by β-adrenergic receptors and appears to involve activation of adenyl cyclase.

   b) Actions of NE on Purkinje cells blocked by prostaglandins of the E series, by nicotinate and by cerain heavy metals, like Pb and La, which block NE activation of the adenylate cyclase.

   c) Effects of NE or locus coeruleus stimulation mimicked by iontophoretic application of cyclic AMP and both are potentiated by phosphodiesterase inhibitors.

   d. Stimulation of locus coeruleus or application of NE increases level of cyclic AMP in Purkinje cells.

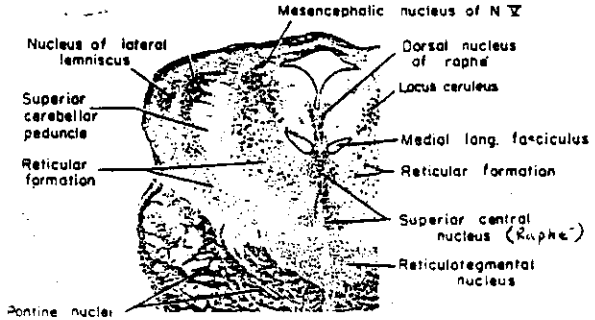
B. Behaviorally the locus coeruleus is implicated in the onset and maintenance of REM sleep.

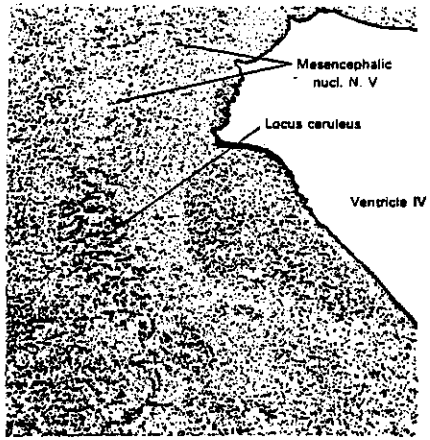
C. NE released from terminals of the locus coeruleus in the cerebral cortex may play a role in promoting or allowing longterm changes in signalling between cortical cells.

   1. In kittens, the pattern of inputs to cells in the visual cortex can be altered by visual deprivation.

   2. If NE-containing terminals are destroyed by injecting kittens with a relatively specific chemical neurotoxin, 6-hydroxydopamine, visual deprivation no longer causes changes in inputs.

   3. If NE is replaced in a small region of cortex in 6-hydroxydopamine treated animal by microperfusion, inputs to that region can once again be altered by deprivation.





III. Raphé nuclei can be considered part of reticular formation. Consists of 4 to 9 nuclei along midline from medulla to mesencephalon. Many cells contain 5-HT, most 5-HT-containing cells are in raphé nuclei.

   A. Groups in medulla project to spinal cord and affect pain perception, perhaps by blocking release of Substance P.

   B. Groups in pons and mesencephalon innerative essentially entire brain; including projections to caudate nucleus and a dense innervation of hypothalamus.

   C. According to the prevalent monoaminergic theory of sleep, activation of 5-HT-containing neurons in Raphé nuclei produces non-REM or "slow" sleep, while activation of NE-containing cells in locus coeruleus (see above) produces REM sleep. Although both 5-HT and NE containing cells are probably important in controlling sleep, conflicting results suggest that this theory is oversimplified, at least.

   D. LSD (lysergic acid diethylamide) may produce hallucinogenic effects by interaction with CNS serotonin receptors.

IV. Reticular Formation

   A. Extends throughout medulla, pons and midbrain, contains 9 nuclei (see Fig. I for locations on schematic cross-sections)
      1. Medulla:
         a) lateral reticular nucleus
         b) paramedian nucleus
         c) ventral reticular nucleus

d) gigantocellular (magnocellular) reticular nucleus

e) parvicellular reticular nucleus

2. Pons

a) caudal pontine reticular formation (nucleus reticularis pontis caudalis), rostral extension of medullary gigantocellular nucleus

b) Rostral pontine reticular nucleus (nucleus reticularis pontis oralis)

c) Parvicellular reticular nucleus, rostral extension of corresponding medullary nucleus

3. Midbrain

a) Mesencephalic reticular nucleus

B. Reticular nuclei can be divided into 3 functional groups

1. Nuclei with predominantly cerebellar connections: lateral ret. n. and paramedian nucleus.

2. Lateral nuclear group - parvicellular nuclei of the medulla and pons - can be thought of as an association area

a) Receive sensory input from essentially all sensory modalities, except dorsal column discriminative touch and proprioception, via spino-recticular fibers and collaterals from spinothalamic and secondary sensory cranial nerve nuclei fibers.

b) Projects primarily to medial group of reticular nuclei

3. Medial nuclear group - medullary ventral, giganto-cellular, pontine caudal and rostral, and mesencephalic reticular nuclei - can be thought of as output area

a) Receives sensory input both directly from spinoreticular fibers and indirectly from lateral nuclear group.

b) Motor functions: projects to spinal cord, ends directly or indirectly on both α and γ motor neurons; γ reflex loop arises in reticular formation. Descending output also essential in control of visceral functions.

i. Lateral (or medullary) reticulospinal tract arises in ventral and gigantocellular nuclei of medulla, (nuclei are medial tract is lateral) acts to inhibit myotactic reflexes, reduce muscle tone, lower blood pressure, slow heart.

ii. Medial (or pontine) reticulospinal tract arises in caudal and rostral pontine nuclei, acts to facilitate myotactic reflexes, enhance muscle tone, increase blood pressure, elicit expiration.

c) Ascending Reticular Activating system - ascending projections of medial nuclear group appear crucial in arousal, attention, and maintaining consciousness.

i. Fibers ascend primarily in central tegmental tract to intralaminar nuclei in thalamus and from there to other thalamic nuclei and to essentially all areas of cortex. Ascending projections from medial regions of the caudal midbrain reticular formation project to hypothalamus.

ii. Barbituates and general anesthetics probably produce tranquillizing effect or unconsciousness by impeding transmission through this polysynaptic pathway. Serious damage to reticular formation produces coma.

V. Substantia Nigra

A. There are four prominent dopamine-containing cell groups in the brainstem.

1. One cell cluster in arcuate nucleus sends processes to median eminence of hypothalamus, an area rich in peptide releasing hormones.

2. Three other dopamine cell groups lie in mesencephalon (midbrain) and innervate mainly the basal ganglia (striatum).

a) Each dopamine neuron innervates large areas within these structures.

b) Of particular clinical importance is the "nigro-striatal" pathway from cells in the pars compacta of the substantia nigra to the neostriatum (caudate nucleus and putamen).

B. Degeneration of nigro-striatal dopamine neuron system implicated in Parkinson's disease, a basal ganglia disorder involving akinesia (difficulty in initiating motor acts and a paucity of movement), rigidity of skeletal muscle, and tremor at rest.

1. Electrophysiological experiments show that stimulation within substantia nigra inhibits firing of cells in caudate nucleus and facilitates firing in putamen.

2. Patients with Parkinsonism have decreased dopamine in striatum.

3. Symptoms can be relieved by large oral doses of DOPA. (Dopamine will not work since it does not cross the blood brain barrier).

a) During therapy dopamine levels can return to near normal.

b) If dopamine nerve terminals have indeed degenerated, dopamine must be synthesized, accumulated and released by other cells.

C. Symptoms of Huntington's Chorea may arise from a relative overactivity in the dopaminergic nigro-striatal systems produced by a global degeneration of striatal interneurons releasing acetylcholine, GABA, and neuropeptides.

D. Dopamine neurons implicated in schizophrenia.

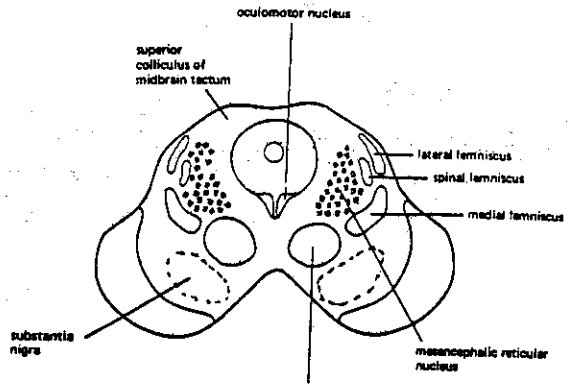
1. Phenothiazine drugs used to treat schizophrenia block effects of dopamine applied iontophoretically to CNS neurons.

2. Phenothiazines cause increase in dopamine metabolites in urine, apparently resulting from dramatic increase in firing rate of dopamine neurons, an action presumably mediated by a neuronal feedback loop trying to overcome blockade.

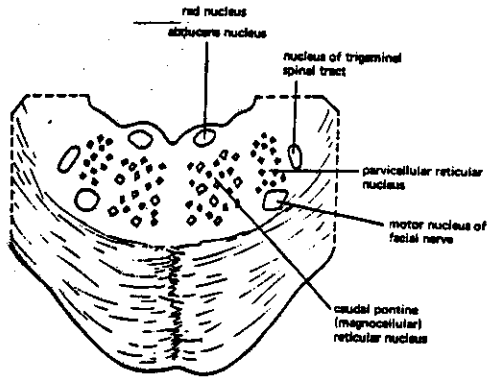
3. Dopamine receptors in homogenates or membrane fragments isolated from the caudate nucleus are blocked by phenothiazine drugs, competition with dopamine binding parallels pharmacological effectiveness.

4. Treatment with phenothiazines often produces stereotyped Parkinsonism-like motor side effects that could be related to blocking dopamine receptors in striatum.

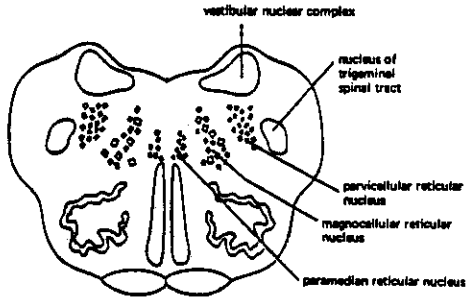
FIG. 1 RETICULAR NUCLEI



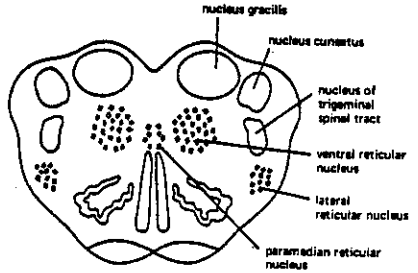
ROSTRAL MIDBRAIN



CAUDAL REGION OF THE PONTINE TEGMENTUM

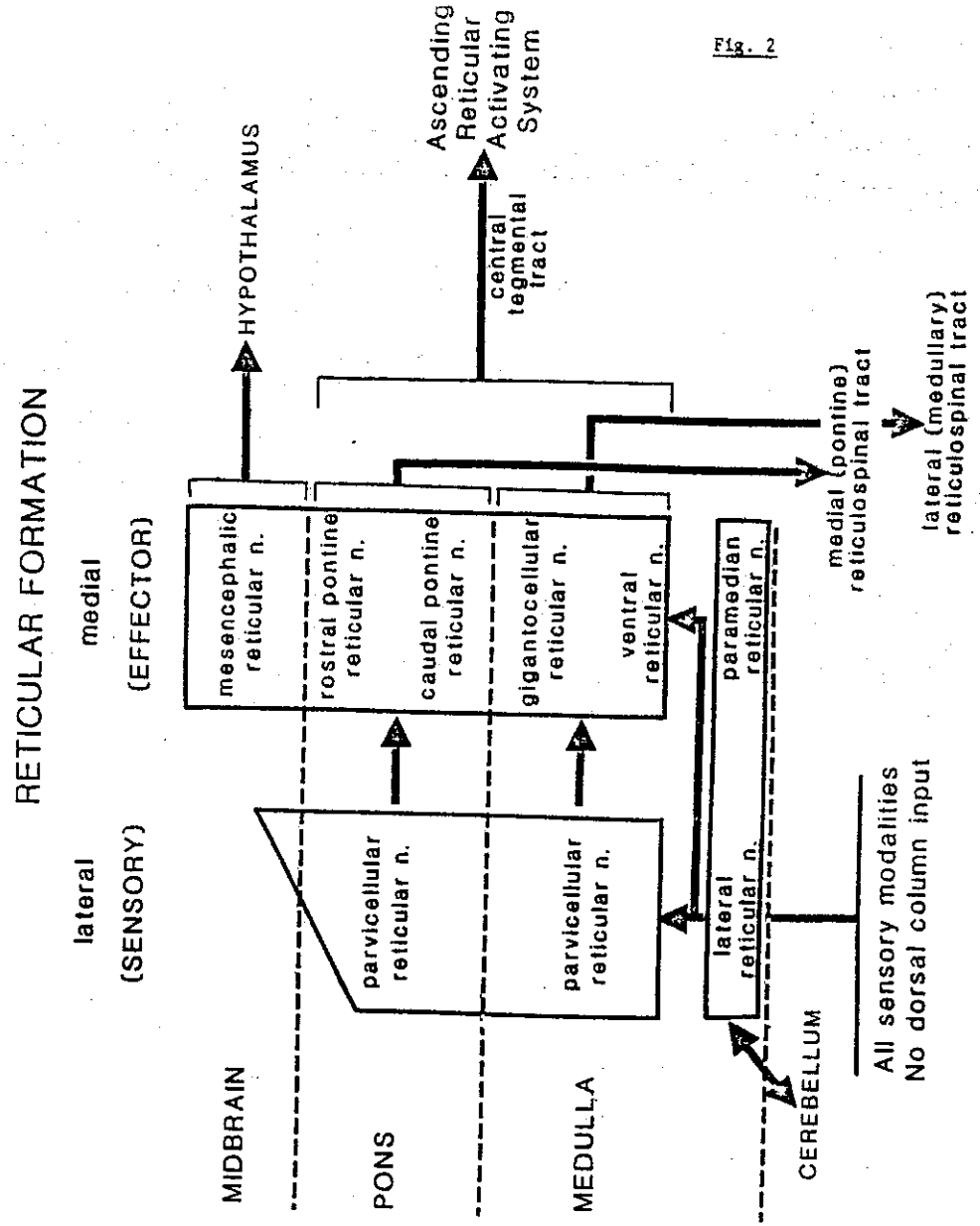


MEDULLA AT THE LEVEL OF THE ROSTRAL PORTION OF INFERIOR OLIVARY NUCLEUS



MEDULLA AT THE LEVEL OF THE CAUDAL PORTION OF THE INFERIOR OLIVARY NUCLEUS

Fig. 2



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NEUROBIOLOGY 200. The Nervous System

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Sensory Receptors

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SENSORY RECEPTORS

You are familiar with the idea that nerve cells can be excited or inhibited by synaptic action, in which a post-synaptic cell converts chemical signals, consisting of the local concentrations of neural transmitter substances, into changes in membrane potential which are transmitted to other cells. In this lecture we consider the different but somewhat analagous situation in which a stimulus in the external or internal environment generates electrical signals in a sensory receptor. This process, in which a sensory receptor absorbs a particular kind of energy from the environment and converts it into a neural signal, is called sensory transduction. Since the central nervous system can only analyze neural signals, we expect that our perception of the world outside and inside our bodies should depend directly on the transduction processes in the sensory receptors. Thus, no matter how potentially interesting a stimulus might be, we will not be able to perceive it unless we have a receptor which can transduce it to a neural signal. Along the same lines, since the neural signals are all that the brain can perceive, we expect that sensory coding should have an important influence on the way in which we perceive stimuli.

Today's lecture will be concerned with the form of the sensory code and some of its implications for perception as well as the mechanisms by which transduction occurs in the receptors. We can immediately ask several questions about these sensory processes: 1) How does the quality of a sensation (or its modality) arise, and how is it represented neurally? Why are there the qualitative differences between sensations like touch, pain, sound, warmth, light? 2) Given only stereotyped electrical signals to work with, how does a receptor encode quantitative dimensions of a stimulus, such as its strength and duration? 3) By what mechanisms does the stimulus come to generate the electrical signals?

Let's examine first the neural basis for the quality of sensation.

A specialized receptor for each kind of perceived stimulus.

Perhaps the most useful generalization which can be made about sensory receptors is that a given cell is terribly choosy about stimuli and will respond well to only one form of energy, the adequate stimulus for the receptor. Thus, for a retinal rod or cone receptor the adequate stimulus is light, for a touch receptor a touch to the skin, for an olfactory receptor a particular kind of odorant molecule, etc. Most man-made transducers have a similar specificity. For example, a photocell may be highly sensitive to light but generally will be very insensitive to mechanical vibrations. A pressure transducer is completely different and gives large electrical outputs to pressure changes at its input but no response even to very intense lights. Naturally in man-made transducers the requirement for a specific type of energy at the input arises as a direct consequence of the construction of the device, whether for example it contains a substance with electrons which can be knocked free by a photon or an electrical resistance whose value depends sensitively on its length. Similarly, we believe that the specificity of biological transducers, the sensory receptors, obtains for the same general reason, although in many cases the exact basis of the specificity cannot yet be satisfactorily explained. One instance of a receptor whose specificity can be explained at a molecular level is that of the rod and cone receptors in the retina, which you will hear more about in

a later lecture. These cells contain light sensitive visual pigment molecules which reside in the membranes of the cells and which undergo a configuration change on absorbing a photon.

Several prescient individuals in the last century, including Hermann Helmholtz and Johannes Müller, suggested that different receptors might all use the same kinds of stereotyped electrical signals for coding the stimulus but have different requirements for specific activating stimuli. The first direct experimental evidence for this view came from work by Adrian and his colleagues in the 1920's and 1930's. Their approach was to record electrically from single sensory fibers while delivering a variety of natural stimuli to the peripheral endings of the receptors. In a flurry of brilliant investigations they obtained recordings from a wide range of different types of receptor and uncovered the main features of their behavior. The action potentials which they recorded were found to be essentially of the same form in all nerve fibers, whether they occurred in motor fibers innervating muscles or sensory fibers activated by touching the skin, pulling on a muscle, distending the lungs, or burning the skin with acid. Each sensory fiber was found to have a sharply delineated receptive field, or region of the innervated structure from which a stimulus could elicit a response, and more importantly each fiber was found to respond to only one kind of stimulus. Thus, some fibers innervating the skin of a frog responded to very gentle touches applied to the skin but gave only a poor response when the skin was penetrated by a needle. Another group of fibers gave a vigorous and long-lasting discharge of impulses when the skin was penetrated but did not respond to light touches. Since a stimulus giving a brisk steady discharge in the cutaneous touch receptors did not make a frog jump, but a prod with a needle did, Adrian concluded that the animal distinguished between touch and pain by recognizing which fibers were active rather than by analyzing some other feature of the sensory message such as the pattern of impulses. These results and conclusions have stood the test of all later work, although a large number of new types of receptors have been found. There is also now a firmer basis for understanding how the central nervous system is able to recognize which receptors are activated. Although this will be taken up in detail in later lectures on central sensory mechanisms, we can say generally at this point that the recognition is made possible by a precise pattern of central connections which, in spite of widespread convergence and divergence, does not jumble messages from different kinds of sensory fibers.

To give more impression of the kinds of specialization for receiving the stimulus which can exist even in a closely intermingled population of receptor endings we can consider briefly some of the types of sensory elements present in a monkey's skin; fuller accounts are available in all the standard texts. One kind of receptor innervates the base of hairs and is excited by small movements of a hair but does not continue to respond to steady displacements. A given receptor of this sort usually supplies about 5 hairs and thus has a receptive field of the order of 1 cm^2 on the skin. The axons of these cells are myelinated and conduct relatively rapidly. Another type of touch receptor ends in small domes in the skin situated between hairs and visible with a dissecting microscope; a given receptor innervates one or a few domes. These fibers respond vigorously to small indentations of the domes, the threshold indentation being of the order of

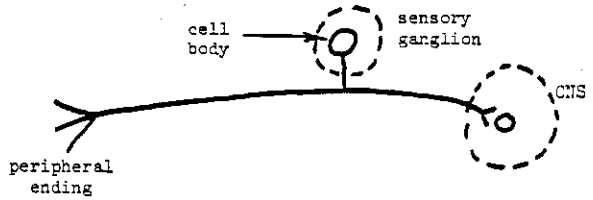
10 μm. Interestingly, these touch receptors signal steady indentations, in contrast to the hair receptors mentioned above. Try on your own skin to see if there is a corresponding difference in the way you perceive touches delivered by bending a single hair or indenting skin between hairs. Situated deeper, in the dermis, are the endings of another type of mechanoreceptor, the Pacinian corpuscles, which we will discuss more later in this lecture. Fibers ending in these structures respond to vibrating stimuli applied to the skin but poorly to single indentations. In addition to these three types of mechanoreceptor (of which there are also others) there are two types of temperature receptor. One sort is specifically activated when the skin is cooled slightly below the neutral skin temperature of 32°C whereas the other sort is activated by mild warming above the neutral temperature. Each temperature receptor ends in a small free ending and thus has a small receptive field. Finally there are at least two types of afferents which are only activated by noxious stimuli which damage the skin. One variety has a small diameter myelinated axon which conducts at about 18 m/sec and probably mediates the initial rapidly perceived flash of pain which follows a painful stimulus. The other kind has a smaller unmyelinated axon which conducts more slowly and probably mediates the slower burning pain which comes on gradually after a painful stimulus.

We can summarize this section by saying that each modality of sensation has its own type of receptor and each type of receptor its own adequate stimulus. The central nervous system recognizes the quality of sensation by recognizing which receptors are active.

Let us now turn to the way receptors encode information about the strength and duration of stimuli. Before getting to this, however, we should distinguish between two broad categories of receptor which use two different types of code.

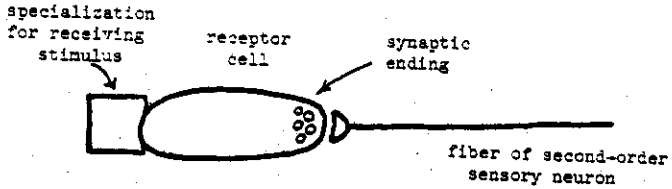
Long and short sensory receptors and the electrical signals which they use.

All the sensory receptors we know about can be grouped into two broad classes, the long and short receptors. The long receptors in vertebrates can be diagrammed schematically in the following way:



Transduction in the long receptors occurs in a peripheral ending specialized to receive the stimulus and located in skin, muscle joints, viscera, blood vessels, etc. A long fiber, connected to a cell body in a sensory ganglion, courses centrally and synapses on one or more neurons within the brain or spinal cord. The fiber connecting the peripheral ending to the cells in

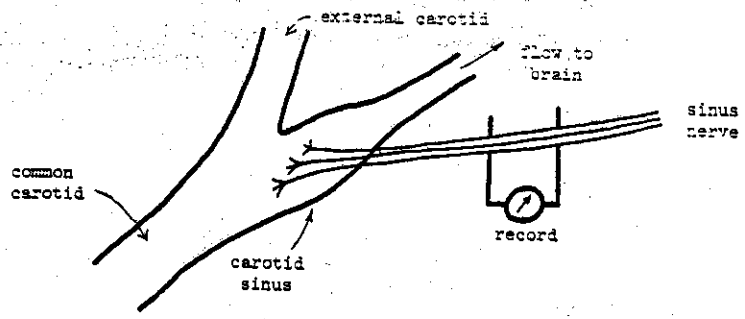
the CNS is much too long to be spanned effectively by local potentials, and thus in these receptors the action potential mechanism must be used to bridge the gap. Naturally all the receptors which Adrian examined fell into this category. The olfactory receptors in several cold blooded vertebrates have recently been shown to display action potentials, placing them in the category of "long receptors" as well. The short receptors can be diagrammed:



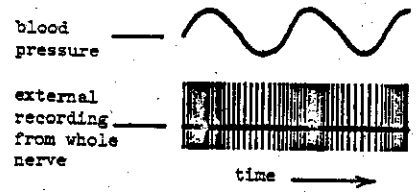
In short receptors transduction occurs at one end of the cell in a region specialized for receiving the stimulus. Although this is drawn as a "black box" above it often consists of one or more modified ciliary processes. At the opposite end of the cell is a chemical synapse which relays the sensory message to the long process of one or more second-order sensory cells. Short receptors are usually not longer than a few hundred microns, and here we would expect that local potentials could serve to relay signals over the length of the cell. In fact, in the short receptors only the local potential is used and action potentials are not present. The short receptors in vertebrates are the rods and cones in the retina, as well as the auditory and vestibular hair cells and the taste receptors.

Coding of the dimensions of the stimulus by action potentials in long receptors.

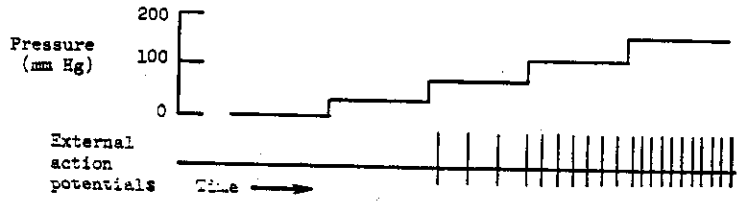
Having said that long receptors use only the action potential to transmit information over long distances, we can now turn to the question of how such a stereotyped signal encodes the strength and duration of the stimulus. Our first clear understanding of this again came from the work by Adrian, but to illustrate the points let us consider the way sensory coding occurs in the baroreceptors in the carotid sinus. These receptors were first examined by Bronk and Stella in 1935, and many of you have already heard about them in the cardiovascular portion of the physiology course. Anatomically the carotid sinus lies near the bifurcation of the common carotid artery into the external and internal carotids, and it consists of a small dilation near the origin of the internal carotid. The sinus is innervated by a population of nerve fibers whose endings branch in the walls of the vessel and which continuously monitor the critical blood pressure responsible for perfusing the brain.



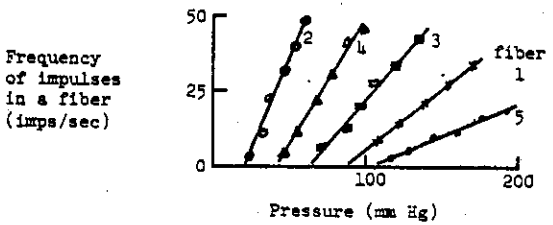
If an electrical recording is made from the nerve to the carotid sinus with external electrodes, as diagrammed above, while the pressure is recorded simultaneously in the carotid artery, one sees a massed burst of action potentials occurring at high frequency during every systolic rise in pressure and at lower frequency during every diastolic fall:



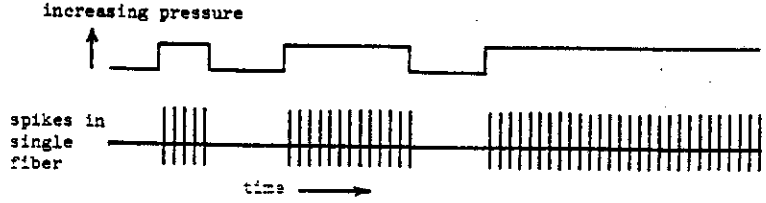
From this, it is clear that the frequency of action potentials in the nerve (total number of spikes per unit time) rises with a rise in pressure and falls with a drop in pressure. Does this reflect changes in the number of fibers active or the frequency of signalling in individual fibers, or both? This can be answered by splitting the nerve into filaments containing only a single active fiber and recording the action potentials in the single unit while the pressure changes. It is convenient to perfuse the carotid sinus to establish constant pressures of selected magnitudes. What one sees in such an experiment is shown below:



When the pressure in the artery exceeds a certain threshold value, action potentials begin to occur in a relatively constant rhythm. Their frequency rises in direct proportion to the amount by which the threshold pressure is exceeded; thus a low pressure gives a slow discharge whereas a higher pressure gives a discharge at higher frequency. We can conclude that one mechanism for coding the degree of the pressure is the frequency of impulses in individual fibers. On repeating the experiment on a number of different fibers, we find that they all behave qualitatively the same way, but some fibers begin to respond at lower pressures than others. By plotting the collected results from a number of such experiments, we might obtain results like those shown below:



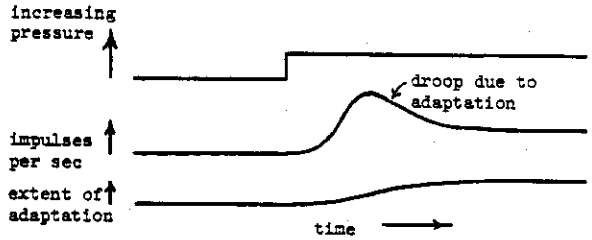
We can conclude from this sort of plot than when the population of fibers is intact, a low pressure may bring into play only the fiber with the lowest threshold, whereas as the pressure rises more and more single elements will become active. Thus, the number of active fibers as well as the frequency of impulses in the individuals codes the strength of the stimulus. (A stronger stimulus is said to recruit nerve fibers into activity.) Does the medullary center in which the compensatory reflexes are organized care about both kinds of information? Undoubtedly it does and integrates the action potentials occurring in all the afferents from both carotid sinuses in organizing the appropriate motor adjustments. Given that the stimulus strength is coded in the frequency of action potentials in single fibers as well as in the number of fibers active, how is the second dimension of the stimulus, its duration, coded? We can see this in a different experiment in which we record from a fiber while giving a series of steps of pressure of different duration. What we see is:



The duration of the pressure rise is mirrored in the duration of the train of impulses. We can look at the length of the train to infer how long the

change in pressure lasted, and of course the brain does the same thing in working out the time course of the blood pressure. It is elegant that the discharge of a number of fibers gives quite an accurate replica of the detailed form of a pulse wave, including its dichrotic notch.

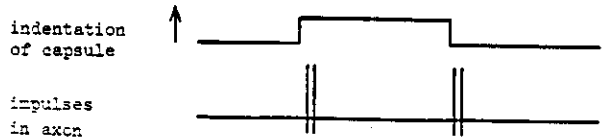
There is a very important detail of the coding of the duration of the stimulus which can be seen if we give a strong step of pressure and plot the frequency of impulses in a single receptor as a function of time after the step.



We see that after an initial peak in the impulse frequency there is a decline down to a lower steady level, as if the pressure had been lowered. Now of course the pressure is the same throughout the step, and in fact what happens is that the receptor slowly becomes less sensitive than it was initially. The delayed reduction in sensitivity, which produces the slow drop in the frequency of impulses, is called adaptation and is a common feature of the behavior of nearly all receptors. Here, because the reduction in sensitivity is slow in onset and limited in extent, the behavior is termed slow adaptation, to contrast it with the behavior of the other class of rapidly adapting receptors.

The premier example of a rapidly adapting long receptor is the Pacinian corpuscle afferent. These fibers terminate peripherally in an onion-like capsule consisting of a series of fibrous lamellae:

The adequate stimulus for these receptors is a deformation of the capsule. Impulses occur, however, only when an indentation is given to or removed from the capsule, with nothing happening during a maintained steady indentation:

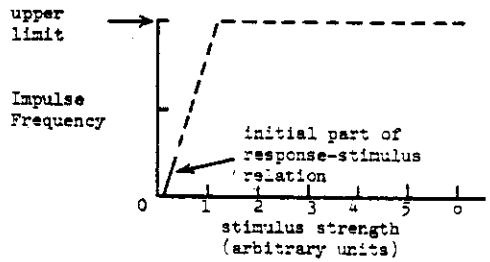


Here the desensitizing process which tends to stop impulses is very strong and comes on rapidly, thus the name rapid adaptation. What is the significance of the two forms of adaptation?

Adaptation modifies the sensory message.

In rapidly adapting receptors like the Pacinian corpuscle, excitation occurs only when a stimulus is first delivered or removed, and no impulses are present during a steady stimulus. This implies that only changes are effective in producing messages, and in fact it can be shown that the frequency of discharge increases proportionally with the rate of change of the stimulus. One would thus expect that an indentation in the form of a sine wave would be a very effective stimulus, and indeed this is correct. A Pacinian corpuscle is thus really more of a vibration receptor than a pressure receptor, although many textbooks call it a pressure receptor. To summarize, we can say that rapidly adapting receptors signal the rate of change of the stimulus and ignore information about steady states.

Why should adaptation be present in the slowly-adapting receptors which are concerned with steady states? In these receptors, adaptation represents an elegant solution to the following difficult problem. A given receptor has a limited range of frequencies of impulses which can be used for coding the strength of the stimulus (what would determine the upper limit of frequency, and what approximately would it be?) The receptor is apparently very interested in being able to respond to weak stimuli applied from rest and thus the initial part of the curve relating frequency of impulses to stimulus strength is very steep:

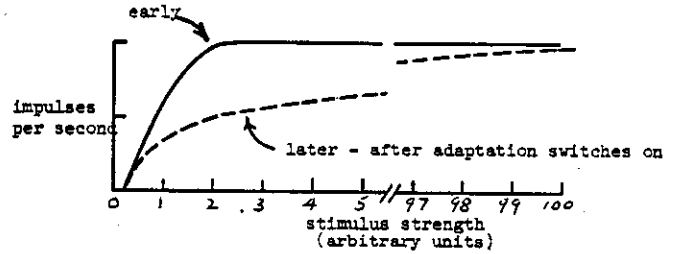


Defining the sensitivity of the receptor, S, as the slope of this curve

$S = \frac{\Delta f}{\Delta I}$ where Δf is the increment in impulse frequency produced by an increase ΔI in the strength of the stimulus

we can say that the initial sensitivity of the receptor is high. Suppose that the sensitivity remained constant for stronger stimuli. The curve relating response to stimulus would continue to rise linearly and stimuli only a bit stronger than the weak ones we have been considering would drive the cell all the way to its upper limit of response. This of course has the consequence that the range of stimulus intensity which can be handled by the response of the cell is quite limited.

Wouldn't it be useful if the cell were able to give some information about the strength of stimuli very much stronger than those just provoking a modest discharge? But granted that it might be useful, how could the cell achieve this? One of the main means the receptor uses for acheiving this is to reduce its sensitivity for strong stimuli, by the process we called adaptation earlier. That is, with a delay, the relation between response and stimulus strength shifts from being approximately linear to a slope we can represent:



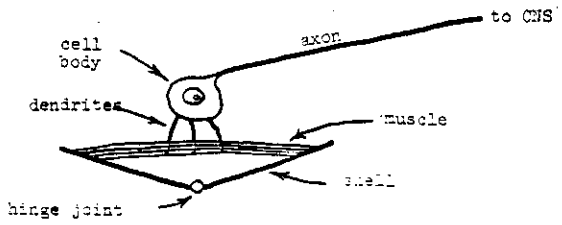
Adaptation has very little effect on the sensitivity to weak stimuli but progressively reduces the sensitivity as the stimulus strength increases. This allows some information about the strength of even very strong stimuli to be encoded. The very non-linear relation between the response of a receptor and the strength of the stimulus implies that our ability to perceive increments in the strength of the stimulus should decrease as the background level of the stimulus increases. Exactly this phenomenon has been widely observed in psychophysical experiments on many different sensory modalities, and it has been expressed in the so-called Weber-Fechner Law which says that the smallest perceivable change in the stimulus varies in direct proportion to the background level of the stimulus:

$\Delta I = kI$, where ΔI is the smallest perceivable change, I is the background level, and k is a constant.

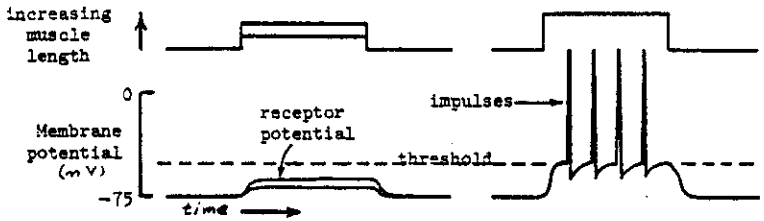
We will now examine the mechanism of sensory transduction and adaptation in long receptors.

Impulses are initiated in long receptors by a local depolarization.

The clearest experiments on transduction in long receptors were done by Kuffler and Eyzguirre. They recorded intracellularly from stretch receptor neurons in crustacea. These cells have a series of dendrites which extend into connective tissue in bundles of muscle in the animal's tail:



and they monitor the length of the muscle in a way analogous to the muscle spindle receptors you heard about in the introductory lecture. The large cell body near the dendrites is an experimenter's dream, allowing one or more intracellular electrodes to be put into it with relative ease. Two types of electrical signal are seen with an intracellular electrode when the muscle in stretched, as shown below:



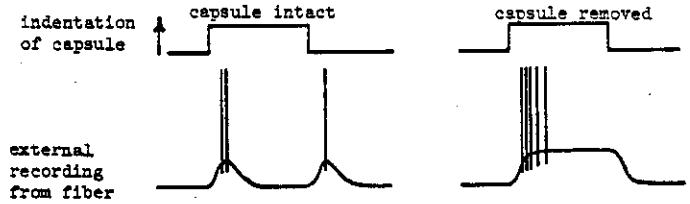
Weak stretches evoke graded depolarizations, receptor or generator potentials, with an amplitude varying roughly in proportion to the stimulus strength and a duration which mirrors the duration of the stretch. Stronger stretches, which cause the graded depolarization to exceed threshold, generate impulses at a frequency proportional to the amount by which the slow depolarization exceeds the threshold value. The slow depolarization which triggers the impulses is generated in the dendrite endings, by deformation of the membranes. Although the complete mechanism is not yet understood, it is clear that the immediate cause of the depolarization is an increase in the conductance of the dendritic membrane to ions, sodium and probably also potassium as in the end plate at the neuromuscular junction. The channels here are somewhat similar to end plate channels, inasmuch as they have an equilibrium potential near -15 mV, are not voltage sensitive, and are not blocked by tetrodotoxin. A local depolarization with similar properties has been observed in all long receptors examined. Generation of the impulses occurs at the axon hillock region of the cell, which has a lower threshold than the cell body. The main general area yet to be worked out concerns the detailed mechanism by which deformation of the dendritic membrane opens conductance channels in it. However this occurs, it is interesting that this type of transduction mechanism allows for a large amplification of the stimulus. Thus, the energy stored in concentration gradients across the cell membrane can be released by the triggering action of the stimulus, and many sodium ions can flow to the interior of the receptor when even a single channel is opened by the stimulus. This general mechanism is responsible for giving receptors their great sensitivity to weak stimuli.

Having seen these features of the transduction mechanism, we can now turn to the mechanisms responsible for producing adaptation of the sensory discharge.

Some mechanisms of sensory adaptation.

Adaptation of the sensory signals can result from several kinds of mechanism. In the Pacinian corpuscle which we discussed earlier, the most

[illegible] potential of a corpuscle afferent [illegible] spike discharge:



If, however, the capsule is carefully dissected off the ending, and it is then deformed, a maintained generator potential occurs, as shown above. The conclusion from this experiment and other analysis is that the capsule acts as a mechanical filter, transmitting to the sensory endings only changing displacements and not steady deformations produced by steady indentations. Paradoxically, even when the generator potential is maintained after the capsule is removed, the discharge of action potentials is still very brief and usually only a few impulses occur when the ending is indented.

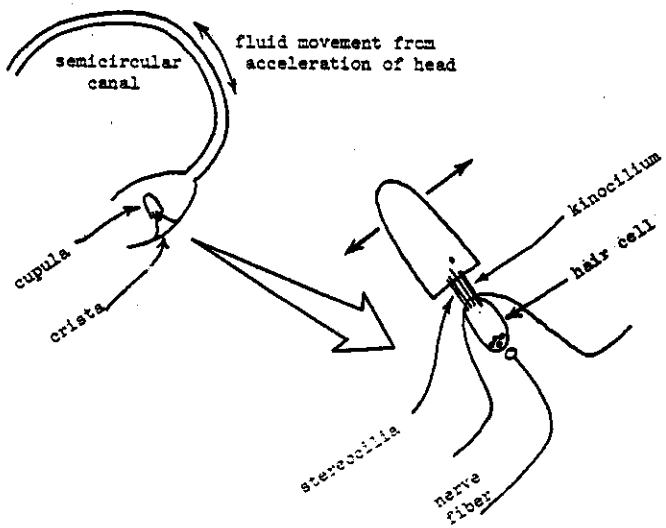
A similar "electrical" adaptation has been observed in the stretch receptor neurons of crustacea. Some of these cells give impulses which adapt rapidly during a steady stretch, whereas in others the discharge adapts slowly. In both types of cell the generator potential during a steady stretch is well maintained, and so the different behaviors are not due to mechanical factors as in the Pacinian corpuscle nor to some part of the mechanism by which channels are opened in the dendrites. The clue to the mechanism of rapid adaptation is that when a rapidly adapting receptor is depolarized by injecting a constant current through an intracellular electrode the impulses rapidly fizzle out; in a slowly-adapting cell the impulses continue as long as the current is passed. This difference can be explained by assuming that the kinetics of sodium inactivation in the voltage-sensitive channels of the two cells differ. It is as if in the slowly-adapting cell inactivation is completely removed after each impulse, allowing another to follow, while in the rapidly-adapting cell a maintained depolarization builds up a larger and larger steady inactivation which soon prevents initiation of further impulses. The slow adaptation observed in the generator potential of both types of crustacean stretch receptor is mainly due to the visco-elastic properties of the muscle fibers in which the dendrites are imbedded.

Other mechanisms operate to produce adaptation in other sorts of sensory receptor. You will hear more about one of these in a lecture on the retina.

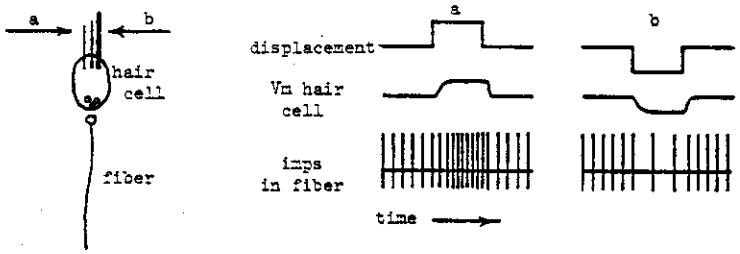
Coding and transduction in short receptors: local potentials only, generated by graded conductance changes.

In short receptors the action potential mechanism has been dispensed with and local potential changes relay information about the stimulus from the site at which the stimulus is received to the synaptic ending at the opposite end of the cell, usually not more than 100-200 microns away. It is rather surprising at first to hear that in some short receptors the adequate stimulus generates a graded hyperpolarization, in others a graded depolarization, and finally in still others a graded depolarization or hyperpolarization. One would anticipate from the lectures on synaptic transmission that a depolarizing local potential could serve as a perfectly fine presynaptic signal. Thus, whenever the presynaptic terminal depolarizes, by electronic spread of the generator potential from the site of application of the stimulus to the presynaptic terminal, chemical transmitter would be released at a higher rate and this could depolarize or hyperpolarize the second-order sensory cell(s), handing the message on. Could a hyperpolarizing receptor potential regulate transmission of signals to the second-order cells? Of course it could, if, without the stimulus, the presynaptic terminal were releasing transmitter at a high resting frequency. The hyperpolarization would then lower the rate of release, and a second-order cell could sense this, responding by a depolarization or hyperpolarization depending on the post-synaptic action of the transmitter. This is the way in which vertebrate rods and cones, which hyperpolarize to light, are thought to generate post-synaptic responses in the second-order cells.

To make these ideas more concrete, let us consider the operation of the ciliary receptors in the semi-circular canals of the inner ear. These hair cell receptors are located in the ampullae at the ends of the semi-circular canals. The hairs (cilia) projecting out of the end of each receptor are embedded in a gelatinous cupula which acts as a door swung to and fro when the endolymphatic fluid inside the canal undergoes volume displacements from movements of the head.



There is a morphological polarization of a hair cell, in that the single large kinocilium is always located to one side of the stereocilia (see above), and all the hair cells within a crista face in the same direction. Intracellular recordings from these hair cells show that they are depolarized when the hairs are bent in the direction of the kinocilium and hyperpolarized when the hair is bent in the opposite direction. The depolarization is presumably produced by opening of some of the sodium channels which are closed with the hairs in the resting position, and the hyperpolarization by closing of some of the sodium channels which are open with the hairs in the resting position. The second-order vestibular nerve fibers which the hair cells contact show a resting discharge of impulses with the hairs in the neutral position. Depolarization of a hair cell increases the frequency of firing of the vestibular nerve fiber, and hyperpolarization slows it. Can you work out how this would occur?



The arrangement here provides a "push-pull" mechanism which allows small changes in either direction to be encoded in the neural message flowing to the brain.

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