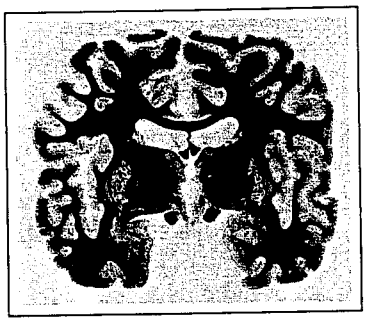
FUNDAMENTAL NEUROANATOMY



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Anatomical drawings by Carol Donner

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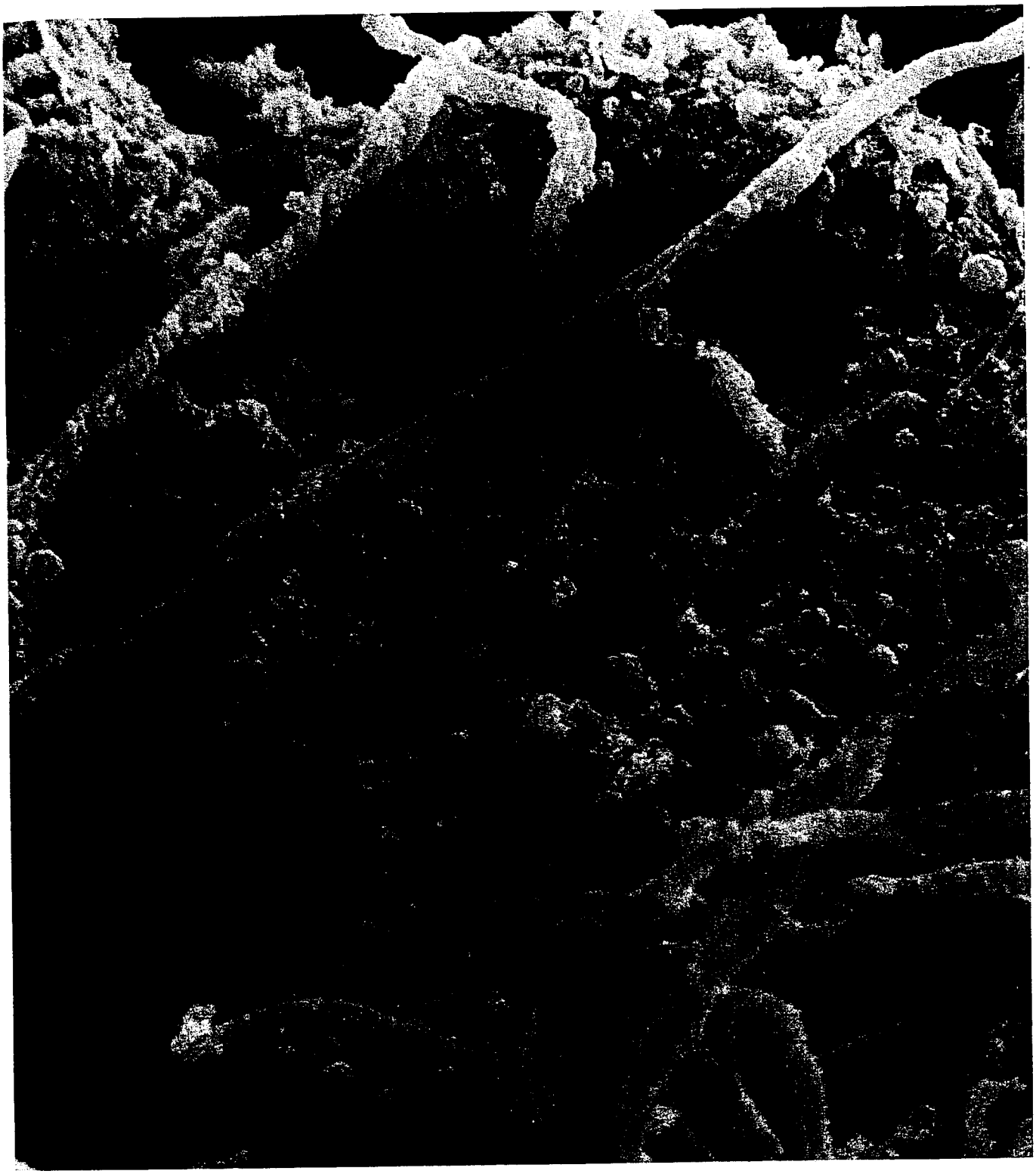


Part I

PRELIMINARIES

Facing page: **Surface of a nerve cell** occupies much of this scanning electron micrograph, which shows a nerve cell, or neuron, from the brain of a cat. (In particular, the neuron is from the part of the brainstem designated the nucleus reticularis magnocellularis.) Toward the bottom is the neuron's cell body; it is some 60 micrometers across, which makes it large as neurons go. Toward the top are the neuronal protrusions called dendrites; one dendritic trunk curves behind the other. Arrayed on both the cell body and its dendritic protrusions are a multitude of rounded swellings. They range from .5 to two micrometers in diameter. They are synaptic terminals: the sites at which the cell gets chemical signals from the axons, or nerve fibers, emitted by other neurons. A given neuron may have thousands of such connections. Indeed, neurons are embedded in neuropil, a feltwork of axons and dendrites. Here much of the felt has been stripped away. Still, a number of axons course across the field of view. The micrograph was made by Linda Paul, Itzhak Fried, Peter Duong, and Arnold B. Scheibel of the School of Medicine of the University of California at Los Angeles.

Chapter One

Early Phylogeny; The Great Intermediate Net

This book is an introduction to the structure of the brain and the spinal cord, with special reference to the brain and the spinal cord of mammals, notably man. It is, first and foremost, a medical text: it describes anatomy that medical students are called on to master. But we hope it does more than that. We hope a student of physiology, or chemistry, or psychology, or computer science and artificial intelligence—in fact we hope that anyone seeking familiarity with the tissues inside the skull and at the center of the vertebral column will find guidance in these pages. Thus we assume no special knowledge on the part of the reader; we start from scratch. At times we venture into neurophysiology, into neurochemistry, into neuroembryology, and into neurology, and there again we start from scratch. To that extent the book is an introduction not to neuroanatomy alone but to the neurosciences. The book, it must be said, is far from encyclopedic. Most notably, it slights the molecular basis of neural activity and the intricate local patterns in which nerve cells are organized. Instead it makes broad sweeps through the brain and the spinal cord, and even on that scale it offers examples, not catalogs. But then, we want to treat the nervous system conceptually, not as a mass of detail. Accordingly, the book is unorthodox: it presents the brain and the spinal cord first as a network of communications established by the fibers that nerve cells emit, and only then as a three-dimensional structure of complex architecture. The first part of the book—this part—is a set of preliminaries. It discusses the evolutionary advent of the nervous system, the nature of the nerve cell and of the cells that support its activity, the chief anatomical divisions of the brain, and the techniques that enable investigators to trace the connections a nerve

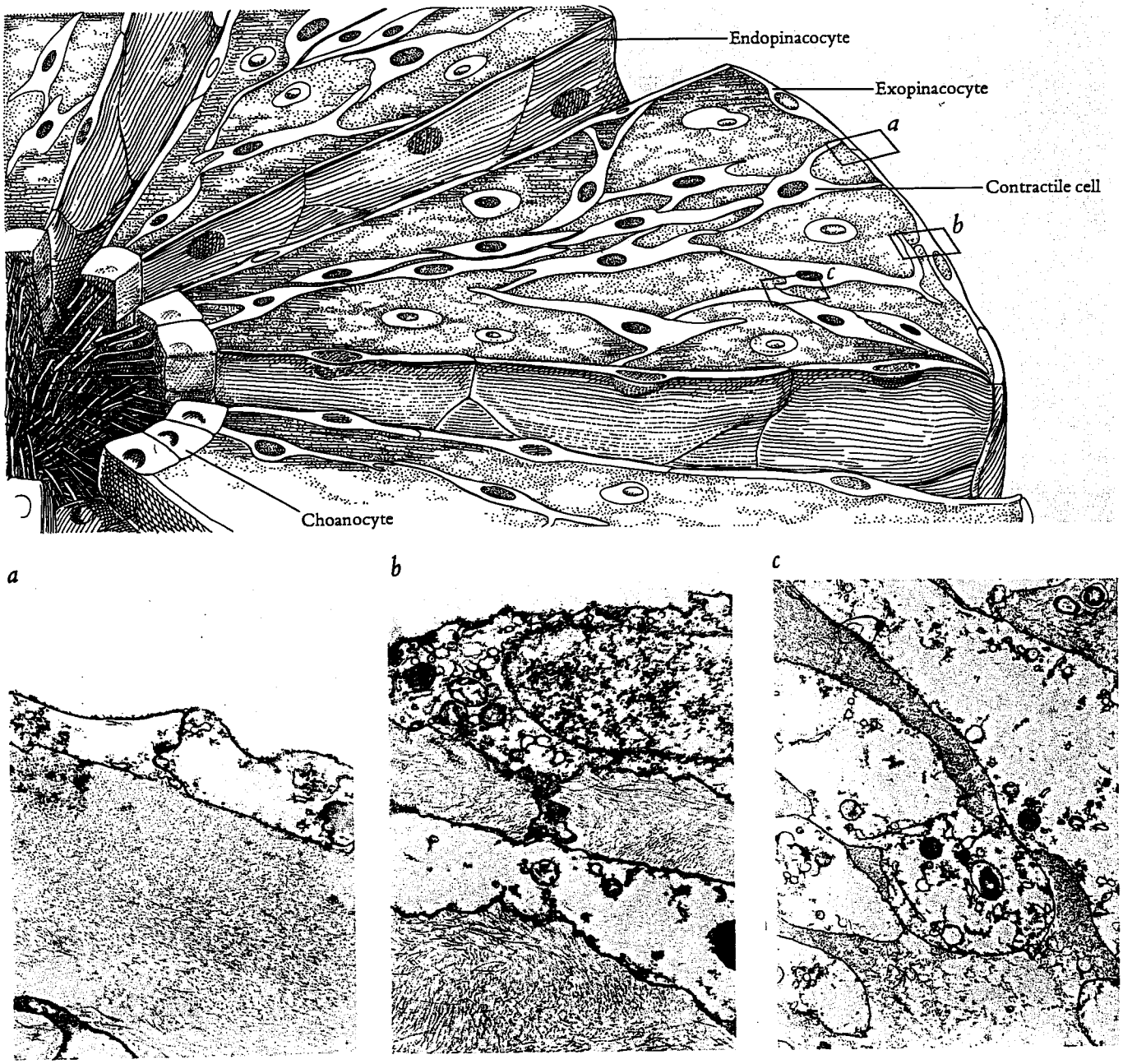


Figure 1: **Intercellular contacts** in the sponge may help to organize the filtering of nutrients from sea water; conceivably, then, they amount to a nervous system. In the gross anatomy of the sponge (*top*) no such system is explicit. The surface consists of flattened cells called exopinacocytes and others called endopinacocytes; they overlie networks of contractile connective-tissue cells, which occupy a matrix of large molecules, specifically glycoproteins and collagen. Still other cells called choanocytes beat water with their flagella. Electron microscopy reveals the connections among the cells. The surface cells make contacts among themselves (*a*); the surface cells make contacts with the underlying contractile cells (*b*); the contractile cells make contacts among themselves (*c*). The micrographs were made by Max Pavans de Ceccatty of the Université Claude Bernard in Lyon; they show tissue from the thick-walled marine sponge (the common bath sponge) *Hippospongia* at an enlargement of approximately 9,000 diameters.

cell makes with other nerve cells. The second part of the book is an overview of the mammalian central nervous system in which the brain and the spinal cord are presented topologically and the basic connectedness — a broad-scale mammalian wiring diagram, if you will — is constructed. The third part superimposes on this topology some actual neuroanatomy.

Interneuronal Communication

When did nerve cells first appear in the course of evolution? A number of biologists have been trying to answer that question — one that earlier workers were hopeful they had settled. As a result, much of the certainty about the answer has evaporated, not that the early ideas are false, but rather, as a recent investigator has written, that they are not sufficiently true. The

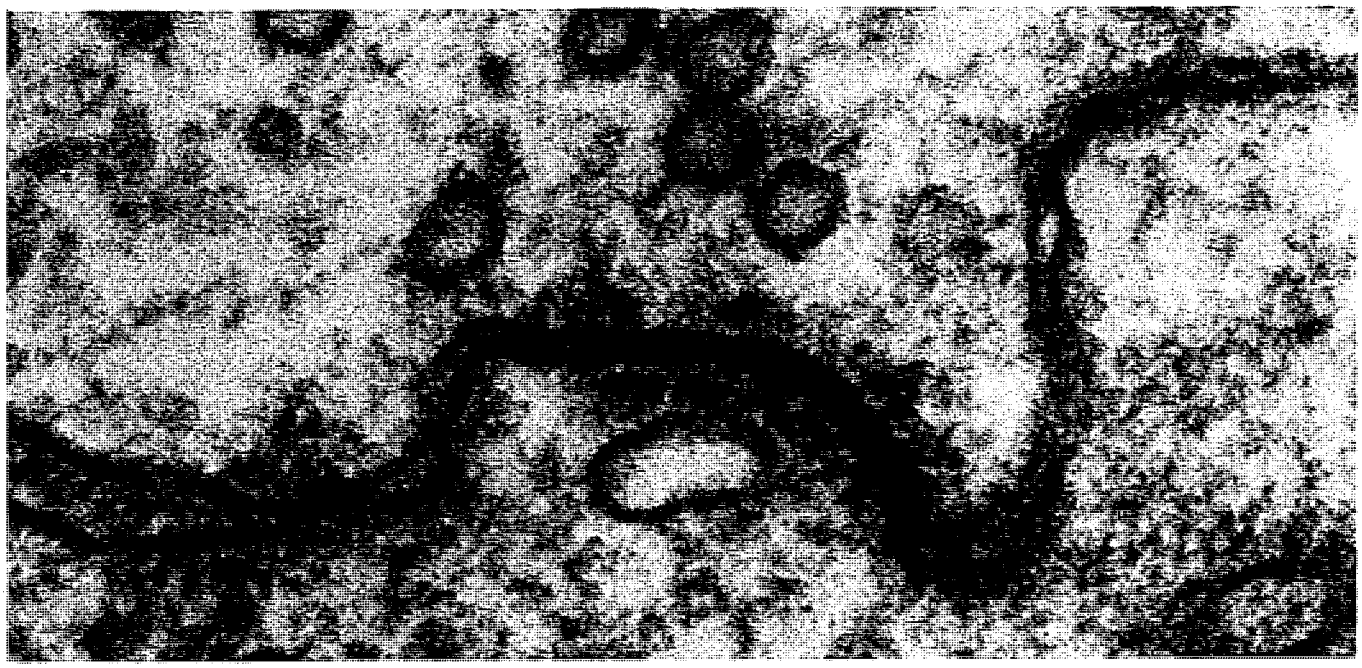


Figure 2: **Gap junction** in the nervous system of a vertebrate resembles, if only superficially, the intercellular contacts found in the sponge. In this example the junction links two neurons in the ciliary ganglion behind the eyeball of a chicken; thus it links two of the neurons that govern the contraction of the pupil of the eye. In general gap junctions enable ions and small molecules to pass from one cell to another through a lattice of channels spanning the distance between the surface membrane of the cells. Along the length of the junction that distance is only two nanometers (2×10^{-9} meter). The neuronal membrane itself is about seven nanometers thick. The electron micrograph was made by Thomas S. Reese and Milton W. Brightman of the National Institutes of Health.

problem is that nerve cells seem to have sneaked into phylogeny: they are much like other cells, with attributes all cells share. For one thing, all cells are irritable: almost any stimulus—mechanical prodding, heat or cold, electricity—can trigger a local change in the membrane forming the envelope of the cell, so that the membrane's permeability to various ions is altered. In this way, currents of ions are made to flow through the membrane; thus the concentration of ions on each side of the membrane changes, and with it the voltage difference (the bioelectric potential) across the membrane. In the second place, all cells are conductive: a local alteration in permeability can advance along the membrane, so that an altered bioelectric potential spreads over the surface of the cell. Although nerve cells, or neurons, have developed these attributes notably well—neurons are exquisitely irritable and exquisitely conductive—the universality of their most characteristic properties makes investigations into neural phylogeny extremely problematic.

Consider the sponge. It is thought to be the most primitive multicellular organism alive on the earth today. The sponge has no organized system of cells specialized for communication: it has no nervous system. Indeed, it hardly seems to have biological systems at all. Yet the sponge has several modes of contractility that give it a certain amount of responsiveness to its environment. When a sponge is touched, the surface of the colony may exhibit local, rhythmic pulsations. The local oscula (the openings through which the sponge expels water) may contract. The colony may curl up.

How is all that possible without a nervous system? Beginning with C. F. A. Pantin at Cambridge University and continuing with Max Pavans de Ceccatty at the Université Claude Bernard in Lyon, a school of investigators has examined the fine structure of sponges, in part by electron microscopy (Figure 1). The effort establishes that the surface of a sponge consists of cells that form plates resembling flagstones. One might take them to be analogous to a vertebrate's epidermis. Under the flattened cells are other, spindle-shaped cells. The spindle-shaped cells are contractile, like a vertebrate's muscle tissue. It is difficult to be certain that the images recorded by the electron microscope accurately represent the structure of a living animal. In fact, it is difficult to place untroubled faith in any histological technique. After all, the tissue under investigation must be killed and then subjected to chemical treatment before its microscopic structure can be observed. Still, the surface cells in a sponge appear to have processes (filamentous extensions of the cell body) that descend to contractile cells and touch them, it seems, at specialized regions: membrane-to-membrane appositions with little or no space intervening. It also appears that the contractile cells communicate among themselves at similar specialized regions. In the vertebrate nervous system, certain membrane appositions look very much the same (Figure 2). They are called gap junctions, and indeed, in spite of appearances, some substances applied by investigators (notably the enzyme horseradish peroxidase) can work their way between the two facing membranes. The gap junctions in the vertebrate are known to be sites of electrotonic transmission, a form of intercellular communication in which the bioelectric potential across one neuron's mem-

brane is brought to bear on the membrane of the next by the passage of currents of ions from the one cell to the other through channels spanning the junction. (Gap junctions are typically two nanometers, or 2×10^{-7} centimeter, wide.) It could readily be proposed that the membrane appositions among the cells of a sponge serve a similar function.

Even if not, there is a second (though less likely) possibility. The electron microscope shows that both the surface cells and the contractile cells of the sponge include saclike organelles. Each is some 140 nanometers (1.4×10^{-5} centimeter) in diameter. In the cells of a vertebrate such sacs, or vesicles, are common. For example, they serve as secretory vesicles: they sequester a substance the cells have produced—say, the saliva synthesized by the cells of a salivary gland. In neurons they sequester neurotransmitter: a substance the neuron releases that mediates a chemical form of intercellular communication. The most typical arrangement is shown in Figure 3. There the vesicles in a neuron are shown to cluster at strategic sites where the membrane of the cell, viewed at low magnification, appears to be thicker and denser than elsewhere. Actually the membrane is unexceptional; high-power electron microscopy establishes that the density is a membrane undercoating. Evidently the density marks an "active zone" where vesicles can attach themselves to the membrane and come open at the point of attachment, releasing their chemical contents into extracellular space. The sequence agrees with the physiological finding, made in the 1950's, that neurotransmitter is released in quantal "squirts." After such a release, the transmitter molecules promptly make contact with the specialized receptor molecules that stud a neighboring vertebrate neuron's membrane (or the membrane of a muscle cell). Indeed, a density along a second, postsynaptic neuron's membrane (or a complicated furrowing in a muscle cell's membrane) is often nearby. The interaction of the transmitter and its receptors can open channels through the postsynaptic membrane, permitting ionic currents to flow and leading, therefore, to bioelectric activity on the part of the postsynaptic cell. All things considered, it appears that some structural bases for both electrical and chemical communication in a vertebrate neuron's style are teasingly present even in a sponge. Nevertheless, a sponge has no neurons—or else all its cells are neurons.

The One-Neuron Nervous System

Among the investigators who inquired into the phylogeny of the nervous system well before the most recent efforts, George Parker of Yale University is prominent. He published his findings in 1919. Parker was seeking the primeval reflex arc. Its putative descendants had been identified in the vertebrate: they are pathways composed of one or more neurons through which the excitation caused by a sensory stimulus to some part of the body can be conducted to muscle tissue and thus can make muscle contract. In Parker's time, reflex arcs were often taken to be the simplest pattern by which nature

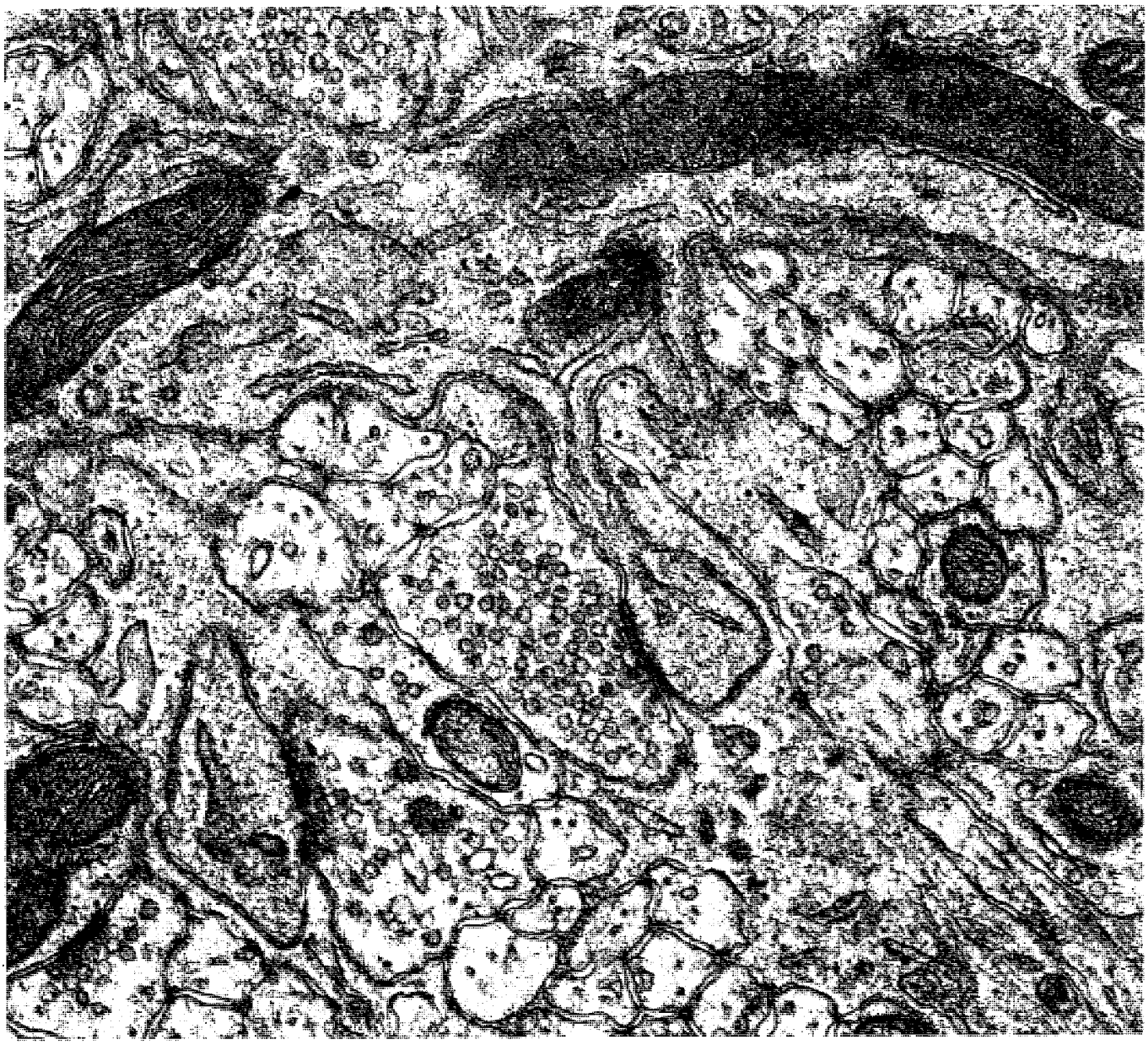


Figure 3: **Synapse** is the characteristic substrate for interneuronal communication in vertebrates and higher invertebrates; it is a site where the electrical activity of a neuron brings on the release of a neurotransmitter: a chemical messenger substance. This synapse is fairly typical. A dendrite crosses the top of the field of view: the two long, dark gray structures inside it are mitochondria cut lengthwise. At the middle of the field the dendrite emits a downward branch called a dendritic spine. The left side of the spine makes contact with an axon, cut in transverse section. The axon is the presynaptic part of the synapse: the part that releases transmitter. It is filled with round synaptic vesicles (chambers that store the transmitter). The dendrite, across a cleft of some 20 nanometers, is the postsynaptic part of the synapse. There the binding of transmitter molecules to receptor molecules induces electrical activity on the part of the postsynaptic cell. The synaptic membrane appears to be darker, thicker, and more distinct than cell membrane elsewhere. The distinctness is due to a density in the underlying cytoplasm, which forms a membrane undercoating. The electron micrograph was made by Sanford L. Palay of the Harvard Medical School. It shows tissue from the cerebellar cortex of a rat at a magnification of 54,000 diameters.

organized cells into a nervous system; hence the nervous system was thought to have originated when some organism first came to have a cell, or a chain of cells, to mediate between environmental stimuli and the organism's responsive movements. The evolution of the nervous system would then demand reflex arcs in increasing number and complexity.

Parker's search for the primeval arc was made possible by a staining technique the Italian physician Camillo Golgi had reported in the early 1870's. According to one account (that of a talkative laboratory assistant), Golgi had thought to stain the meninges: the membranous tissues that surround and cushion the central nervous system. He had not been successful at first. But when he passed a block of neural tissue that included meninges through a sequence of treatments, exposing it first to potassium dichromate and then to silver nitrate, a number of the cells in the block were rendered a deep brown approaching black (Figure 4). They were pervaded by silver chromate, which suffused even the filamentous extensions given off by the blackened cell bodies. Here, then, were neurons in silhouette, with all their processes revealed. The meninges took up no stain. It later developed that Golgi's black reaction (*reazione nera*) impregnates cells other than neurons: it stains supportive cells in the central nervous system and epithelial and muscle cells in the periphery of the body. The technique has, however, this remarkable characteristic: it picks out, from every hundred neurons in a given block of tissue, only some zero to five. That is the only reason the stain has value. If all the neurons in the nervous system were to accept the treatment equally, a slice of neural tissue would simply be blackened overall. Mysterious as the Golgi technique remains (there is still no sure explanation of its fickle selectivity), it was the greatest single blessing to befall the early studies of the nervous system's structure.

George Parker employed the Golgi stain on the tissues of many primitive multicellular organisms. He, too, saw neurons — or, at least, among the cells that form the epithelial layer in the tentacles surrounding the mouth of certain sea anemones, he saw an occasional cell that stood out in black (Figure 5*a*). At the base of each such cell Parker could see the beginning of a filament that ramified into end branches as it approached a muscle fiber. He could not be certain the two made contact, but he assumed they communicated. Surely he was correct; his findings can now be viewed as a somewhat artistic version of more recent discoveries. Still, the circuitry is simple: the entire line of conduction consists of a single cell. It is a one-neuron nervous system, and what it will do in response to a stimulus is as predictable as a doorbell. What is plain about more advanced nervous systems is that the behavior they make possible is predictable least of all.

Obviously something in phylogeny must intervene in the doorbell mechanism. Accordingly, Parker examined the action of Golgi's stain on somewhat more complex organisms. In certain jellyfishes he found an array of neurons in the epithelial layer similar to the one he had found before. Under the epithelium, however, he now found further neurons composing a widely distributed plexus (Figure 5*b*). The circuitry therefore gains some sophistica-

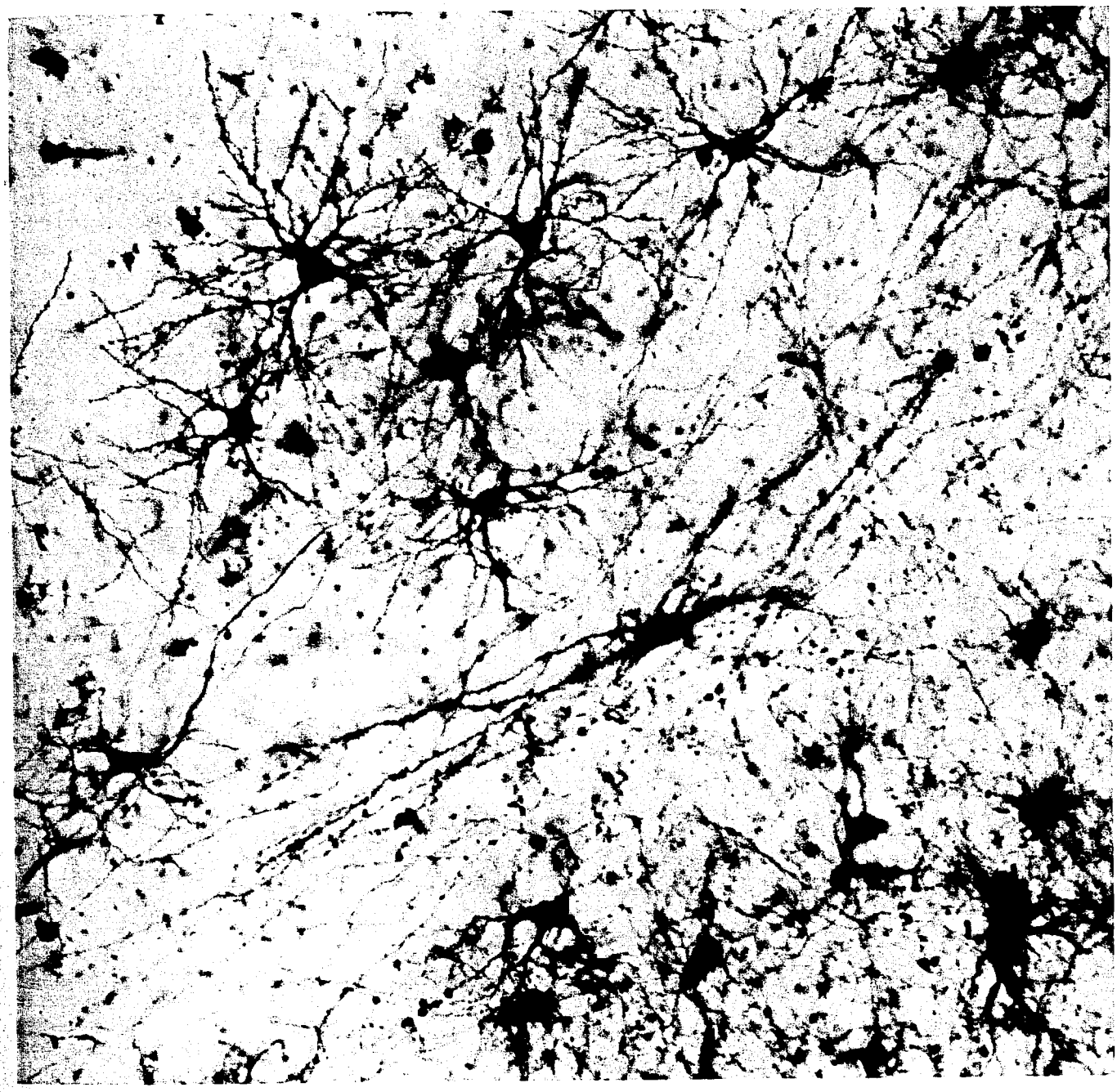


Figure 4: **Golgi technique** for staining brain tissue inaugurated the modern era of neuroanatomical investigation. Discovered by the Italian physician Camillo Golgi in the early 1870's, it blackens a sample of neurons, selecting, it seems, at random. The rest of the tissue it renders transparent. The blackened neurons are seen emitting filamentous extensions, namely axons and dendrites; the technique reveals their distribution. Before the advent of the Golgi technique investigators seeking to study neuronal extensions could do little more than attempt to work them free of their prison of neuropil. The tissue is from the brain of a cat, in particular the thalamus. The field of view straddles the border between two thalamic cell groups, the lateral anterior nucleus (*upper left*) and the ventral anterior nucleus (*lower right*). The preparation was made by Enrique Ramón-Moliner of the University of Sherbrooke in Quebec.

tion: neurons in the epithelial layer make contact with a subepithelial net, and the cells of the net make contact in turn with contractile tissue in the depths of the animal. The arrangement, for the first time in evolution (as it is known from Parker's research), requires functional contact between a neuron and a neuron. In an indisputable nervous system such contact is called a synapse. The word, which was coined in 1897 by the founder of modern neurophysiology, the English physiologist Charles Sherrington, is a contraction from the Greek *syn,* meaning together, and *haptein,* meaning to clasp. A synapse is a clasping together of cells. The crucial fact about synaptic contact is that the neurons do not fuse—a truth that was established and forcefully defended by Santiago Ramón y Cajal, a Spanish contemporary of Sherrington and the founder of modern neuroanatomy. It came as the essential ingredient of the "neuron doctrine," in which the neuron is affirmed to be the anatomical, histological, embryological, and functional unit of the nervous system. In a

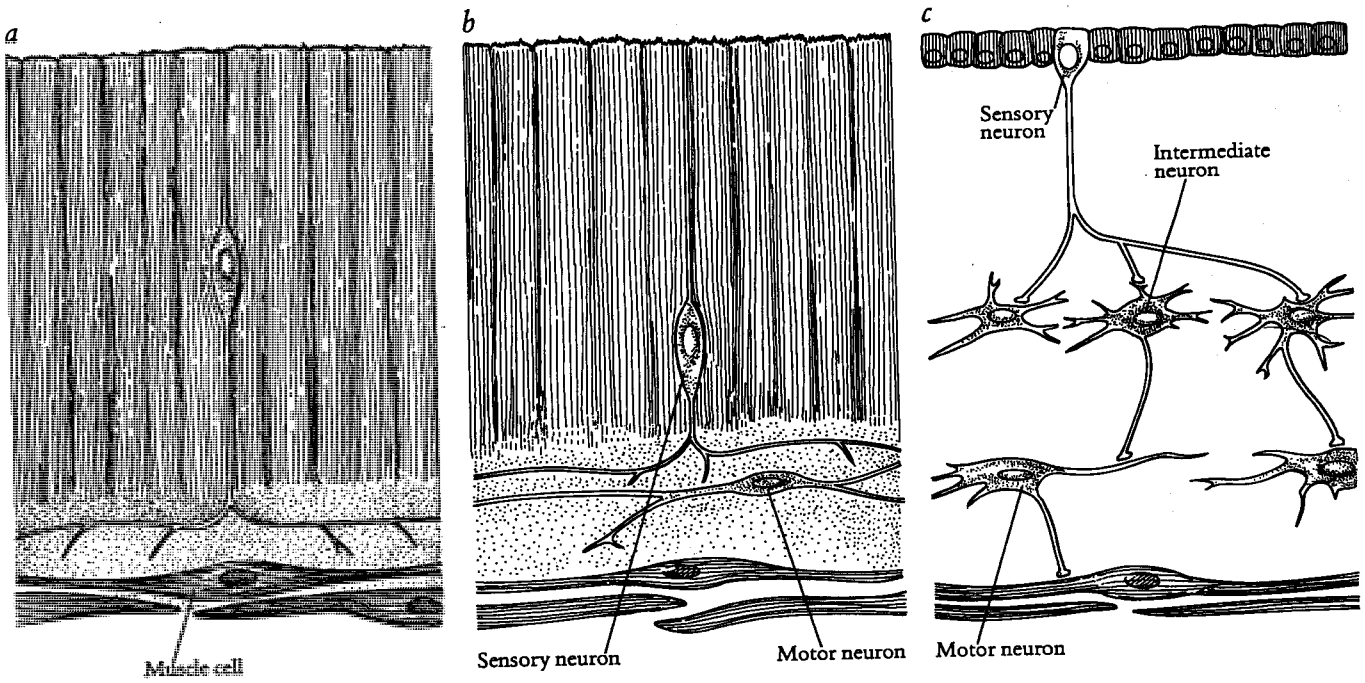


Figure 5: **Nervous system arose** by three stages of evolution, in the view of George Parker of Yale University, who studied Golgi-stained tissue from a progression of multicellular organisms and published his findings in 1919. In certain sea anemones he found a one-neuron nervous system (*a*). That is, the chains of conduction from sensory stimuli to motor responses are established by single cells. In certain jellyfishes he found a two-neuron nervous system (*b*). In it sensory neurons make contact with motor neurons, which in turn can cause muscle cells to contract. Finally, in certain jellyfishes and mollusks he found a three-neuron nervous system (*c*). There motor neurons are isolated from sensory neurons by a network of intermediate neurons.

word it is an individual, and all functional contacts between neurons are really confrontations of membrane with membrane, across a gap now called a synaptic cleft. The cleft is commonly 15 to 25 nanometers wide for a humoral synapse (one at which neurotransmitter is released), or about 10 times the width of an electrotonic, or gap-junction, synapse.

To summarize: Certain jellyfishes boast a two-neuron nervous system, in which sensory neurons (in these simple creatures the nerve cells in the epithelial surface layer of the body, in contact with the organism's ambient environment) communicate with motor neurons (nerve cells that make contact with effector cells, in this case contractile cells, and thus in essence muscle fibers). Does the arrangement remain predictable? Perhaps not. Imagine that the motor neurons communicate with one another, so that the input to any one of them includes not only messages from the ambient environment conveyed by sensory neurons but also messages from neighboring motor neurons. Imagine further that some messages are excitatory and make the motor neuron more likely to generate and transmit its own bioelectric activity, whereas others are inhibitory. Under these conditions there is a riddle to solve: predicting what a neuron will do in response to its inputs seems to be a matter of algebraically summing the excitatory and inhibitory messages that converge on it.

Then comes a third advance. It, too, is found in primitive marine organisms, such as certain jellyfishes and mollusks. In a way it is the final advance, because the nervous system of these jellyfishes and mollusks and the nervous system of man both consist in essence of only three classes of neurons. In mollusks as in man, most of the sensory neurons no longer communicate directly with motor neurons. Between the two a barrier of neurons has developed that have connections not only with motor neurons but also with one another (Figure 5*c*).

To be sure, this third and final step may already have been taken by all organisms that have subepithelial neurons. In the foregoing account of a two-neuron nervous system, all such cells were assumed to be motor neurons: cells that innervate effector tissues. In reality, only some of the many subepithelial cells may make those effector connections. The rest may be positioned in such a way that they get input from sensory neurons in the epithelium but can communicate only with others of their kind or with true motor neurons, not with effector tissue. Neither sensory nor motor, they are placed as go-betweens in the paths of sensory-to-motor conduction. In short, here, too, are intermediate neurons — the final step, as it were. Although a three-neuron organization is difficult to identify in a diffuse neuronal net, it is abundantly evident elsewhere, because in animals that are more highly developed than a jellyfish and whose bodies have become polarized so as to have a leading end (that is, a head), a tail end, and bilateral symmetry, the subepithelial neurons are concentrated into either sequences of ganglia (nests of neurons encapsulated by connective tissue) or a single, unsegmented central nervous system. The important point is the advent, shadowy though it is, of the great intermediate net: a barrier of intermediate neurons that interposes itself between sensory neurons and motor neurons quite early in evolution.

The Neuron; Some Numbers

A stain representing a kind of counterpart to a Golgi preparation was developed in Munich in the 1880's, a time when investigators of the nervous system were mostly neurologists and psychiatrists driven by the hope that if the structure of the brain were known, its workings and disorders would soon be understood. Franz Nissl, a 24-year-old student later to become a distinguished clinical psychiatrist, recognized the need for sharper definition of neurons in slices of brain tissue. He devised a fitting method, one that revealed intraneuronal detail none of the earlier methods could show. He arrived at his procedure (Figures 7 and 17) in two steps: first by specifying alcohol as the fixative for brain tissue, and second by staining the neurons in the fixed tissue with magenta red, a dyestuff he later replaced, with steadily increasing success, by a curious mixture of methylene blue and soap (he specified shredded Venetian soap) and finally by aniline dyes, specifically thionine or toluidine blue. A century later, the need for Nissl's method persists. The method, however, can now be applied to tissue fixed in aldehydes (formaldehyde, glutaraldehyde, or both), and a variety of alkaline dyes can be used.

Axon and Dendrites

Figure 7 displays two Nissl-stained motor neurons from a human brain. The dye was cresyl violet. The appearance of the cells is characteristic of what the Nissl stain does. The cell bodies are distinct, and so are the beginnings of their processes: their filamentous extensions. But all of the latter soon disappear

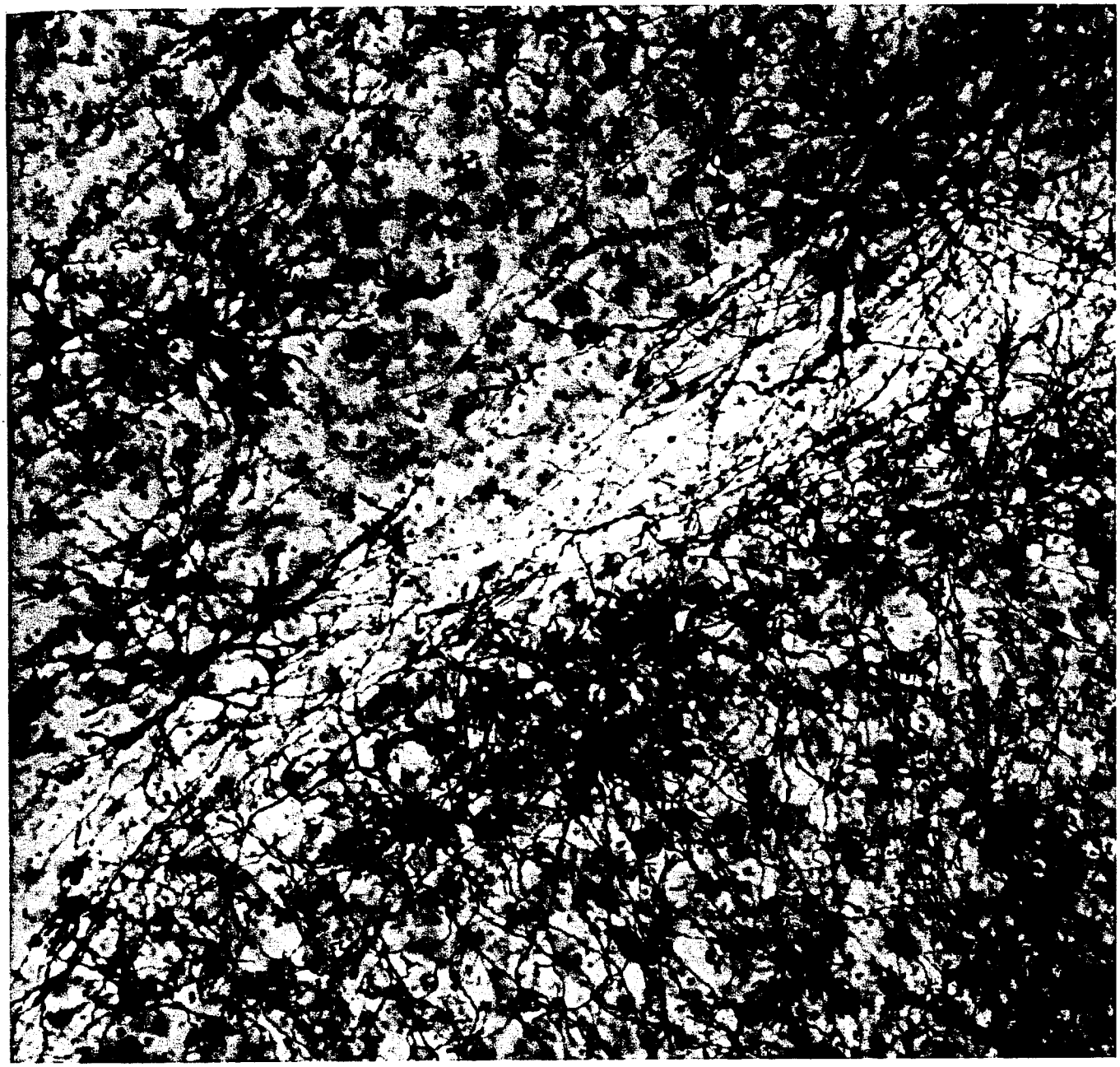


Figure 6: **Nissl technique** is a counterpart to the Golgi technique; discovered by the psychiatrist Franz Nissl in Munich sometime in the 1880's, it equably stains all neurons. In particular, a Nissl-stain dyestuff such as cresyl violet binds to Nissl substance: aggregations of ribosomes, the intracellular machines that make proteins. Ribosomes are notably abundant in neurons. In the illustration the tissue is counterstained: the Nissl technique and the Golgi technique have both been applied to the slice of brain tissue adjoining the slice shown in Figure 4. Again the staining was done by Enrique Ramón-Moliner. Golgi-stained neurons show up in black; Nissl-stained neurons are vaguer gray bodies. The narrow cell-poor zone between the lateral anterior nucleus and the ventral anterior nucleus runs diagonally across the field of view. The preparation establishes that the Golgi stain marks no more than some five percent of the neurons in a given sample of neural tissue. The Nissl technique finds its greatest utility as a means of surveying tissue architecture.

from sight, in what Santiago Ramón y Cajal once called "the dismal fog." Still, the Nissl stain makes possible some valuable observations. Note that both of the neurons in Figure 7 are filled with patchlike, dark-staining masses. The masses sometimes resemble stripes; thus they inspired the name tigroid substance. They are now called Nissl substance or Nissl bodies. According to current understanding, they consist largely of stacks of flattened caverns known as endoplasmic reticulum, on whose membranous walls are mounted ribosomes, the machines that assemble amino acids into proteins by

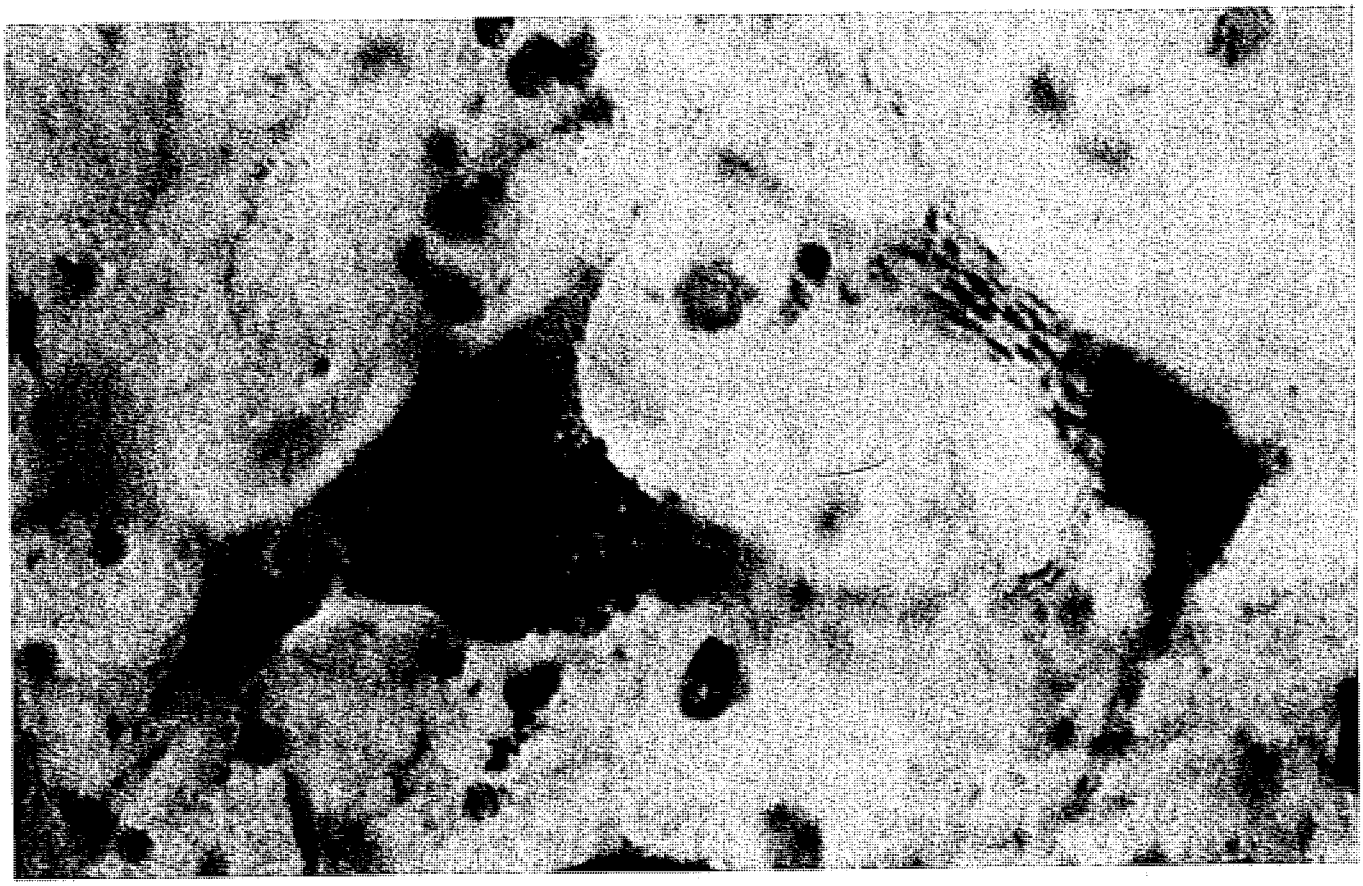


Figure 7: **Two Nissl-stained neurons** from a human brain demonstrate the most reliable distinction between axons and dendrites, namely that ribosomes are lacking in axons. In general, a neuron has only one axon. It is a smooth, cylindrical fiber with which the cell sends signals to other cells. Here an axon emerges from the top of the cell body at the left; its emergence, the axon hillock, is a conical region whose even gray tone in this micrograph shows that it harbors no Nissl substance. In contrast, a typical neuron has many dendrites. They are gnarled, irregular filaments with which the cell gathers signals. Here two dendrites emerge from the cell body at the left; four or five emerge from the cell body at the right. The dendrites are filled, like their parent cell bodies, with dark-staining masses of Nissl substance. The cells have additional dendrites, which are out of the plane of the section. The cells are motor neurons from the oculomotor nucleus; their axons join the oculomotor nerve to extend from the brain to a muscle that rotates the eye.

means of the coded instructions borne by strands of ribonucleic acid (RNA) that leave the nucleus of the cell. (The Nissl-stain dyestuffs combine with acid groups such as those in RNA. As it happens, ribosomes themselves are two-thirds RNA.) Ribosomes mounted on endoplasmic reticulum are present in every cell, but only in neurons do they aggregate so impressively.

An examination of Figure 7 will show that elongated islands of Nissl substance enter two of the neuronal extensions exposed at the plane of the cut through the cell at the left of the figure but fail to enter the third. By that criterion two varieties of neuronal process are distinguished. The first has Nissl substance at the beginning of its length; a typical neuron incorporates several such extensions. The second lacks Nissl substance. Given a Nissl preparation there is no more to be said. Examine, however, the drawings of neurons in Figure 8. The drawings are based on Golgi preparations. Here the processes are revealed in their full extent. Most of them — the ones whose beginnings are filled with Nissl bodies — turn out to be short; those as long as two millimeters can rightly be thought of as giants. These processes, distinguished most reliably (though not infallibly) by the Nissl substance in them, are known as dendrites, the diminutive of the Greek word *dendron,* meaning tree. The name is apt. The Golgi technique reveals that dendrites tend to arborize: they bifurcate repeatedly into ever thinner branches. Sometimes the result is a dense, shrublike tangle of ramifications. In other cases the density of arborization is less extreme. Even in the smallest details of a dendrite's overall shape, the treelike appearance persists, because dendrites are often gnarled: they have excrescences on their surface, like galls on the trunk of an oak. The most pronounced of the excrescences are called dendritic spines.

The remaining process emitted by the neuron at the left in Figure 7 — the one free of Nissl substance — is an axon. The name is the Greek word for axis. Nearly every neuron has only one such process, in contrast to a multiplicity of dendrites. Figure 7 shows the beginning of the axon to be a conical region whose absence of Nissl substance gives it a glassy appearance. This region is called the axon hillock. The axon itself is smooth and cylindrical throughout its length: the axon has no excrescences. In contrast to dendrites, axons can reach impressive lengths: as long as a meter in the human nervous system. Surely that helps to explain the pervasiveness of ribosomal aggregates — Nissl bodies — in neurons. Consider a neuronal cell body 100 micrometers (a tenth of a millimeter) in diameter. It is among the very largest in the human central nervous system; even a cell body half that size is quite large as neurons go. Let the cell body emit an axon tens of centimeters long. The axon is several thousand times longer than the cell body from which it arises. Yet since the axon lacks ribosomes, its need for various molecules must be met by the parent cell body. The axonal membrane, for example, must call chronically for maintenance, like a long bridge that must constantly be repainted. It is known that if an axon is severed from the parent cell body, it will die. It also is known that molecules synthesized by ribosomes in the cell body flow toward the ends of the axon. Some of these molecules (including enzymes soluble in cytoplasm) move no more than a few millimeters per day. Other

molecules, or rather molecular assemblages (for the most part assemblages of protein and lipid that amount to prefabricated parcels of cell membrane), move a hundred times faster. Axons (and dendrites, for that matter) contain filamentous proteins. The thinner filaments are threads no more than 10 nanometers in diameter; they tend to be roughly coaxial with the axon (or the dendrite) in which they are arrayed. They make crosslinks with one another, thus forming a tensile internal skeleton. Perhaps they support the axon. The thicker filaments are microtubules. They, too, are longitudinal, but are as much as 30 nanometers across. Their disruption by drugs such as colchicine inhibits the swift form of transport. It is thought that the fast-moving material travels on the surface of the tubules rather than inside them.

The arborization of an axon is often limited to a short end stretch far from the parent cell body, where the process ramifies into what is called a terminal arbor. This pattern, however, is far from universal. Sometimes an axon ends in a single tip, without having ramified at all. Sometimes it gives off side branches fairly close to the parent cell body, and sometimes it gives off branches throughout its length. All such side branches, whose end stretches may likewise vary from single tips to prodigious terminal arbors, are known as axon collaterals. They have a tendency to leave the main trunk of the axon

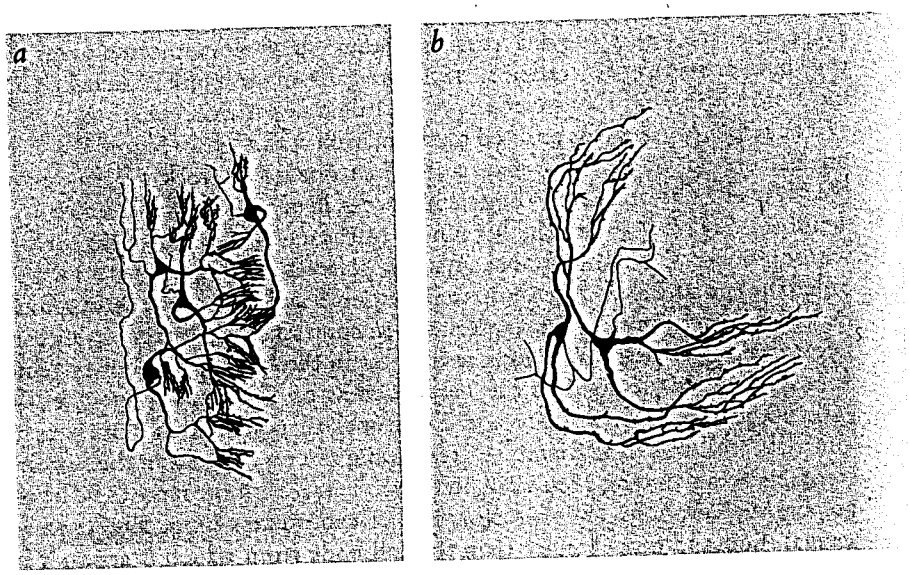
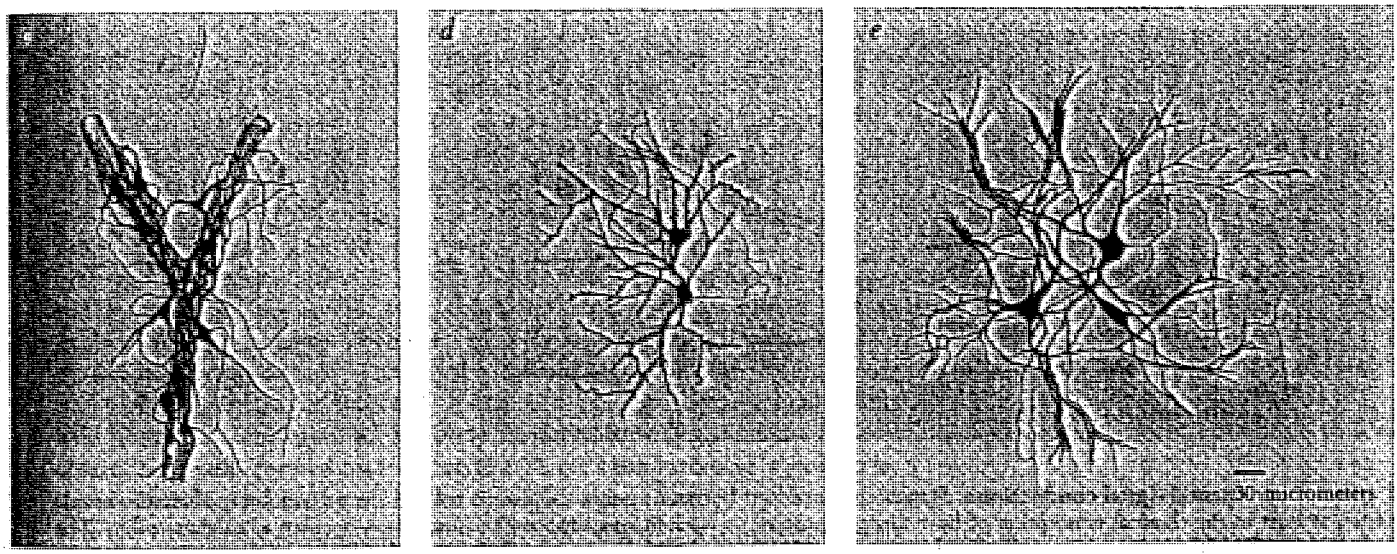


Figure 8: **Variety among neurons** is suggested by these camera-lucida drawings of neuronal constellations, all from the brainstem of the cat. At one site, the nucleus reticularis lateralis (*a*), the dendrites are short and bushy, and end in signal-gathering "pools." The axons, rather thinner than the dendrites, run upward. At a second site, near the hypoglossal nucleus (*b*), the dendrites are long and gently curved, with rather few branchings. The axons again are thinner, and tend toward the right. At a third site, the raphe nuclei (*c*), the dendrites form dense plexuses, in this case on the surface of a bifurcating blood vessel. The

at a right angle, and at each division point the diameter of the main trunk diminishes. Axon bifurcations are too infrequent, however, to produce the appearance of rapid tapering, which is typical of dendrites. The endings of an axon are commonly marked by terminal swellings. Ever since Cajal described them, the swellings have been called *boutons terminaux,* or terminal buds (Cajal is most frequently quoted from the French translation, *Histologie du Système Nerveux,* of the volumes he wrote in Spanish on the structure of the nervous system). Under the electron microscope, the boutons are nearly always found to contain synaptic vesicles, the organelles that store the neurotransmitter with which the cell communicates with other neurons or with effector cells in the periphery of the body.

Signal Transmission

A note on neuron physiology: According to the classical conception the path of electrical activity in a neuron leads from its dendrites to its axon, with the neuron's cell body (in the great majority of cases) interposed between the two. The first part of this path — the spread of an excitatory or an inhibitory signal



axons turn downward. At a fourth site, the nucleus reticularis gigantocellularis of a newborn cat (*d*), the dendrites project in all directions and are covered with dendritic spines. The axons tend to bifurcate. When a cat is five months old (*e*), the dendrites of the neurons in the nucleus reticularis gigantocellularis are lacking most of their spines. Moreover, the dendrites tend by then to run in small, dense packets. The cells are now impressively large: their cell bodies can measure more than 30 micrometers across. The drawings were made by Arnold Scheibel at U.C.L.A.

along the membrane investing the dendrites and the cell body—is decremental. That is to say, the altered bioelectric potential diminishes in intensity as it spreads along the membrane (Figure 9). Ultimately the altered potential arrives at the axon hillock, where it contributes to the algebraic sum of the signals converging on the axon. Thus the shape of the neuron and the positions of synapses on it make the cell unique in the way it integrates data. Now begins the second part of the conduction path. Electron microscopy shows that the cell membrane just beyond the axon hillock takes on a granular undercoating throughout a short length of the axon known as the initial axon segment. Doubtless this special structure has something to do with a special kind of bioelectric activity: the initial axon segment is the site at which, if the arriving excitation is sufficiently greater than the arriving inhibition, a spike of bioelectric voltage called an impulse or action potential (Figure 10) is generated. The impulse invariably has the same electrical rise and fall and magnitude; it exists or it does not, and so it is described as all-or-none. If it is generated, it travels without decrement along the axonal membrane, because this type of bioelectric signal is self-renewing. On arriving at the axon terminals, it initiates the release of neurotransmitter, in most cases at a recognizable synapse, with specialized receptors on the membrane of a second neuron at the other side of a cleft some 20 nanometers wide. In the classical conception

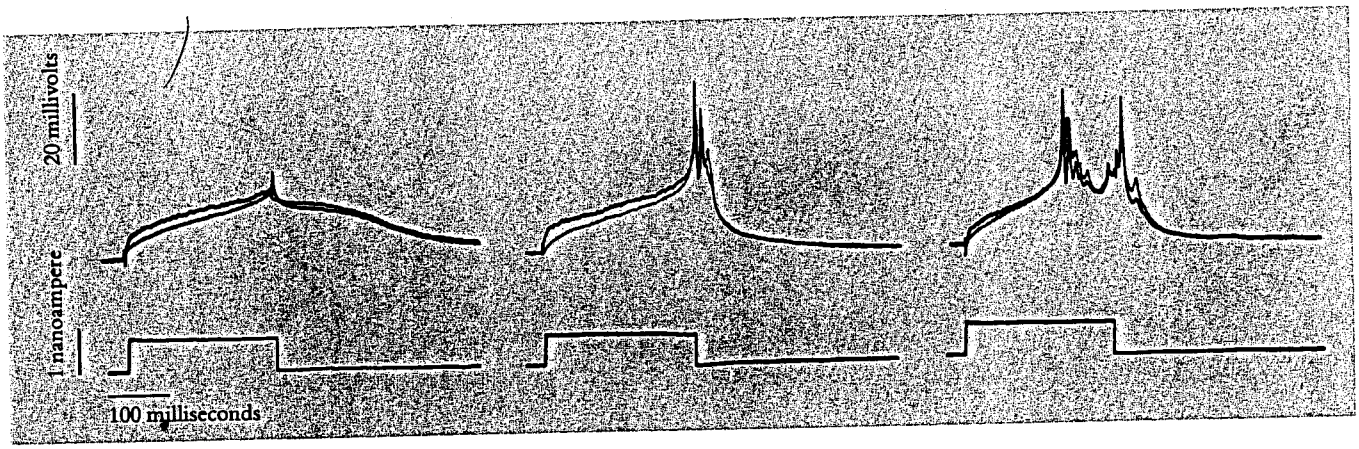


Figure 9: **Decremental signal conduction** is characteristic of dendrites, at least in the classical conception of how neurons process information. The electrical recordings displayed in this illustration were made with two microelectrodes, one positioned in the cell body of a neuron (a Purkinje cell) in the cerebellum of a rat, the other positioned some distance up a dendrite of the cell. In each of three experiments the first electrode injected square-shaped pulses of current (*gray*); it also measured the resulting dome-shaped change in the cell body's voltage (*black*). The change was conducted along the dendrite (in a direction antidromic to the physiological direction) and so had a lower height in the second electrode's record (*color*). At some point, the altered voltage opened membrane channels for calcium ions, enabling the dendrite to generate a "calcium spike." The spike (or set of spikes) protrudes above the dome. The spike was conducted back to the cell body; thus its measured intensity is lower in the latter. In essence, the recordings are a double demonstration of the decremental ("passive") conduction of signals by a dendrite. In contrast, the calcium spikes demonstrate the dendrite's "active" electrical properties, which defy the classical understanding of dendrites as purely passive. The experiments were done by Rodolfo R. Llinás and his colleagues at the New York University School of Medicine.

of interneuronal communication, the synaptic contact occurs on one of the second neuron's dendrites (dendritic spines are sites of predilection for synapses) or on the membrane of the postsynaptic cell body. In either case, the receipt of neurotransmitter initiates electrical activity in the postsynaptic cell—activity that will spread decrementally toward the second cell's axon hillock.

This, then, is the classical conception: in Cajal's phrase the nervous system is dynamically polarized, so that axons send signals to dendrites (or to a postsynaptic cell body). Neurons, however, are far more varied than that. Axons can transmit signals to axons. Dendrites can transmit signals to dendrites. Most surprisingly—in that it constitutes not a short circuit but a reversal of "dynamic polarization"—dendrites can transmit signals to axons. In sum, every possible mode of transmission between neuronal processes now seems to have a place in the organization of the brain and the spinal cord.

Axons signaling axons. The discovery of such a synapse is often achieved by inference. It can happen, for example, when an investigator examining an electron micrograph observes that a serial set of synapses is established by the terminals of three neuronal processes. The first and the second contain synaptic vesicles; the third does not. The investigator decides that the first and the second are the terminals of axons; hence the axoaxonal synapse. The problem is that synaptic vesicles do not invariably specify an axon. Dendrites, too, can be presynaptic. Other cases are less equivocal. For example, an axon may synapse on what is clearly an initial axon segment (Figure 11). Thus the path of signal conduction excludes much of the postsynaptic cell, namely its dendrites and cell body. What might this shortcut accomplish? The morphology of such a synapse is often of the type that tends to be correlated with inhibition. The zone of synaptic contact is rather small, the density at the membranes is not pronounced, and the synaptic vesicles in the presynaptic terminal are flattened, not round. Moreover, the insertion of a microelectrode into the postsynaptic cell often shows that the cell undergoes intervals of powerful inhibition. It seems a reasonable surmise that an axoaxonal synapse is always inhibitory, and in fact, neural circuits are known in which an axoaxonal synapse vetoes the output of the postsynaptic cell: it transmits an inhibition so potent that the postsynaptic axon is temporarily rendered incapable of conducting the impulses generated by the postsynaptic cell itself. One does well to be cautious, however. In a number of invertebrate species, conduction paths formed by giant neurons have been intensively studied by neurophysiologists. In the squid, for example, a path formed by three giant neurons enables the animal to propel itself from danger by jetting water out of its funnel. Each of the synapses in such a path is apparently axoaxonal, and some of the synapses are known to be chemical, not electrotonic. Yet some of the chemical synapses are demonstrably excitatory: the arrival of an action potential at the presynaptic terminal leads to the generation of an action potential in the axon next in the sequence.

Dendrites signaling dendrites. Such contacts have been encountered in many parts of the brain. One notable place is the olfactory bulb (Figure 12). An-

other is the retina. Indeed, it has been maintained that the retina processes visual data by dendrodendritic synapses almost exclusively. The retinal neurons called horizontal cells furnish, in any case, an extreme example of a neuron's reliance on dendrodendritic synapses. The horizontal cell has an axon, or at least one of its numerous processes is notably thinner (one micrometer) and notably longer (several hundred micrometers) than the others the neuron emits. The axon branches extensively as it nears the end of its course, so that the cell has two dense bushes of dendritelike ramifications, one near the cell body, the other some distance away. Each gets signals from retinal photoreceptors (the rods and cones of the eye), and each transmits signals to the dendrites of bipolar cells, the retinal neurons next in line to process visual data. Thus each bush dispatches its output through dendrodendritic synapses. The axon has no role in the transmission of visual data: it carries no bioelectric activity from one bush to the other. It appears, therefore, to be no more than a metabolic pipe enabling a single neuronal cell body to sustain two integrative apparatuses: in effect two independent microcomputers. In a dendrodendritic synapse the path of conduction excludes a presynaptic axon, and with it the all-or-none site of the presynaptic cell. This suggests that no all-or-none action potential is involved in the signal conduction. Instead, the varying electric activity — the so-called graded potential — conventionally associated with a dendrite might give impetus for the release of neurotransmitter. Presumably the release is quantal: a given synaptic vesicle contains a specific amount of transmitter, and the number of vesicles tapped for their content at a presynaptic dendritic terminal might vary with the strength of the arriving bioelectric activity. On the other hand, cases are emerging in which patches of dendritic membrane prove capable of generating spikelike signals.

Dendrites signaling axons. The best instance discovered so far is in the substantia gelatinosa, a district of the spinal cord first described in the 18th century by the Italian anatomist Luigi Rolando. Examining freshly cut spinal cord, Rolando saw that a part of the face of the cut was notably gelatinous: notably lacking in opacity. The Nissl stain would later show that the substantia gelatinosa is composed of small and very small neurons: the smallest in the spinal cord. Still later, the microelectrode would show that some of the neurons — the larger among them; the smallest ones cannot yet be probed — respond more or less selectively to nociceptive stimuli affecting the periphery of the body. They respond, in other words, not to touch or to moderate variations in temperature but to extremes of mechanical irritation and temperature that threaten harm to the body's tissues and indeed are perceived as painful. Then the electron microscope revealed the dendroaxonal contacts. They seem, as a rule, to be paired with the reciprocal connection: an "orthodox" axodendritic synapse. Perhaps they prolong the transmission of a signal (a nociceptive signal?) by feeding excitation back into the presynaptic terminal. Perhaps they damp the transmission by feeding back inhibition. Perhaps they perform a complex mixture of both.

One should not despair; the doctrine of dynamic polarization must apply

broad-scale. After all, a given region of the brain or the spinal cord plainly has inputs and outputs. They travel on long nerve fibers that indisputably are axons, and they are coded as trains of action potentials, which are self-renewing signals. But then, only a self-renewing signal could travel more than a few millimeters along a neuronal process; a signal of smaller magnitude would be decrementally conducted, and so would die away. Inside a given region things are not that simple. A given site on a given neuron can prove to be presynaptic, postsynaptic, or both. It can prove, moreover, to be active (capable of generating spikelike bioelectric signals) or passive (capable only of conducting signals decrementally). Under these circumstances the best strat-

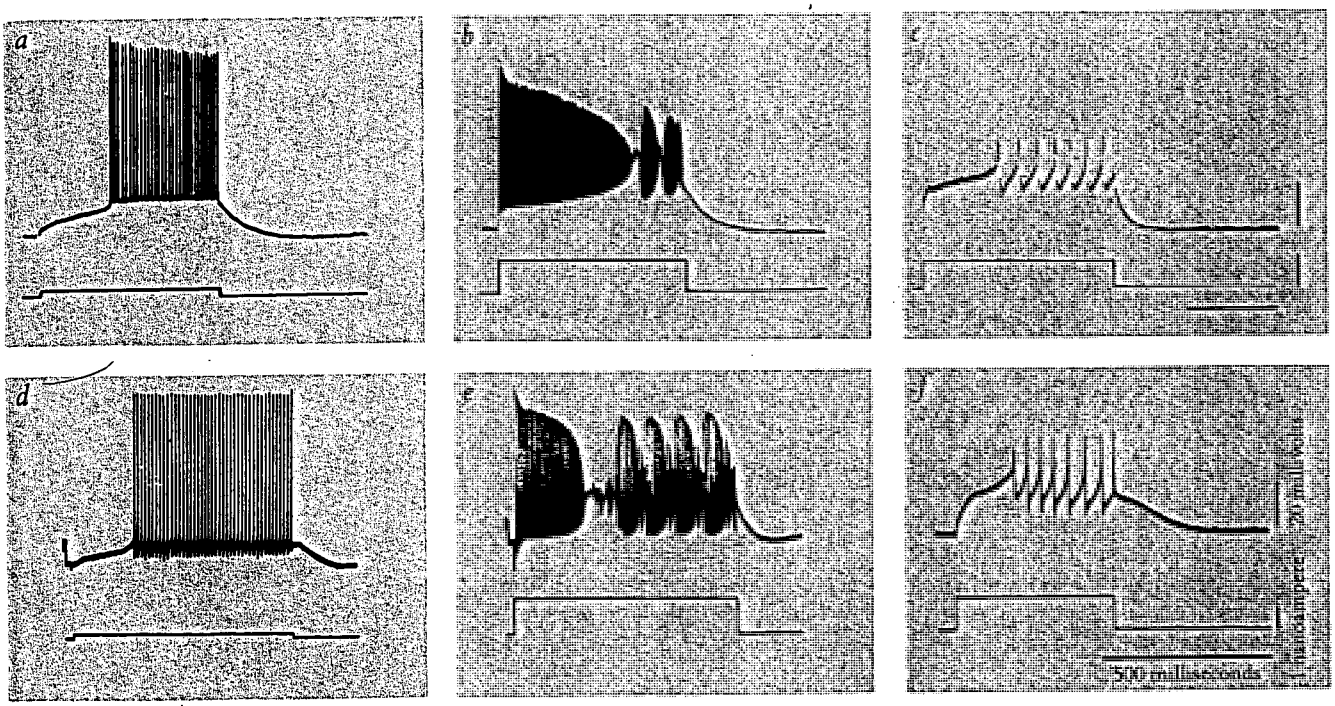


Figure 10: **Self-renewing signal conduction** is characteristic of axons, which are called on to carry signals over distances as great as several tens of centimeters. In the experiment documented at the top of this illustration the axon of a Purkinje cell from the cerebellum of a reptile (a turtle) was induced to "fire" (*a*) by the injection of electric current (*bottom trace*) into the neuron's cell body. The result was a volley of action potentials (*top trace*): a series of self-renewing, spikelike changes in the voltage across the axonal membrane. Greater current (*b*) yielded a pattern of oscillating voltage, recorded in the cell body. The administration of the blowfish poison tetrodotoxin (*c*), which eliminates action potentials by blocking sodium conductance across the neuronal membrane, suggested the source of the oscillation: the action potentials were being modulated by calcium spikes generated in the dendrites of the cell. The experiment was done by Jorn Hounsgaard at the New York University School of Medicine. Across the bottom of the illustration a similar set of recordings was made in a Purkinje cell from a mammal (a guinea pig). Current injected into the cell body again yielded action potentials (*d*); greater current yielded voltage oscillation (*e*); the administration of tetrodotoxin exposed dendritic calcium spikes (*f*). The experiment was done by Rodolfo Llinás and Mutsuyuki Sugimori at New York University. Neurons are revealing a multiplicity of conductances to sodium, potassium, and calcium ions, but across virtually all of evolution trains of action potentials based on inflows of sodium ions and outflows of potassium ions are the currency for axonal signal conduction.

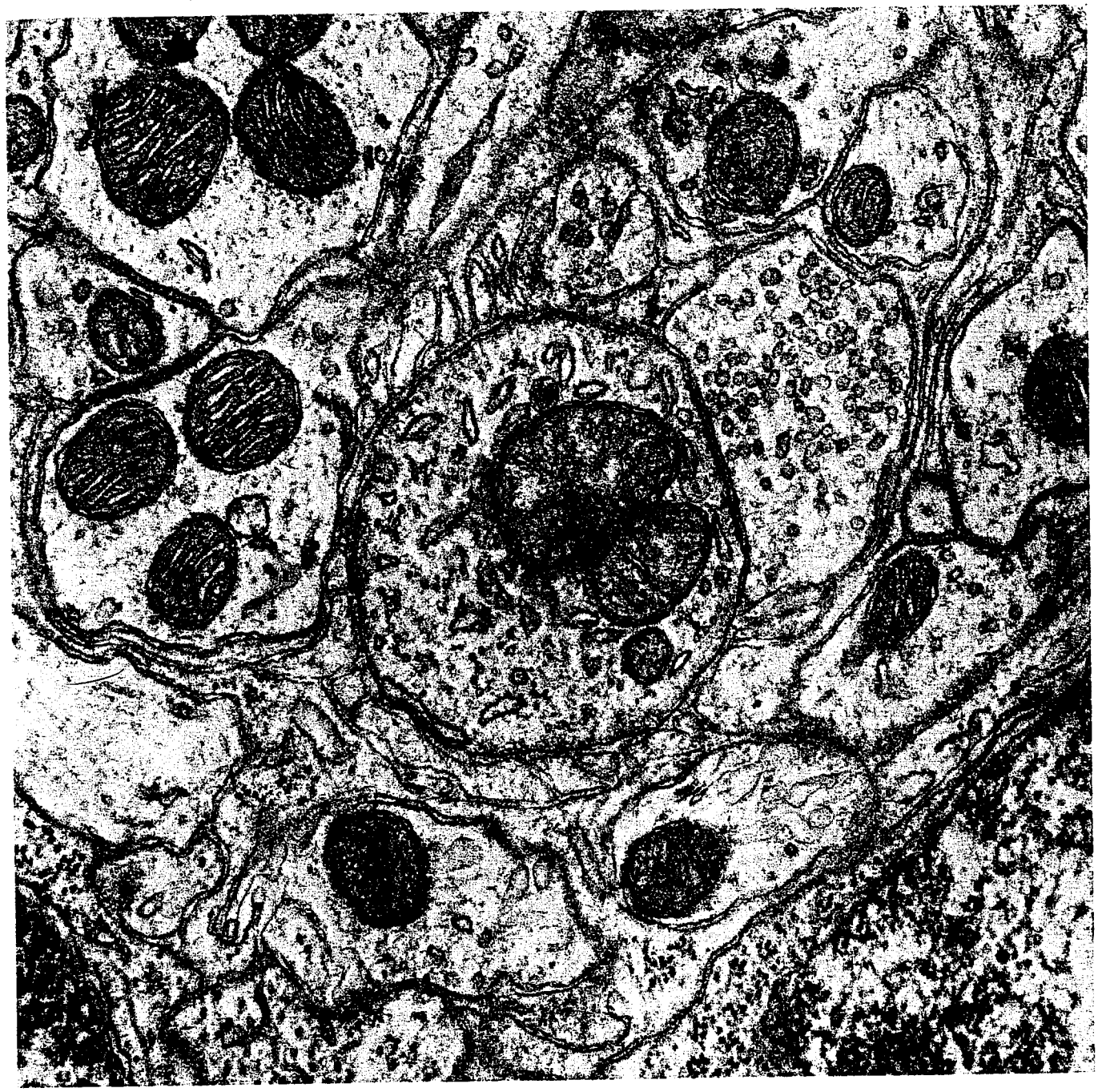
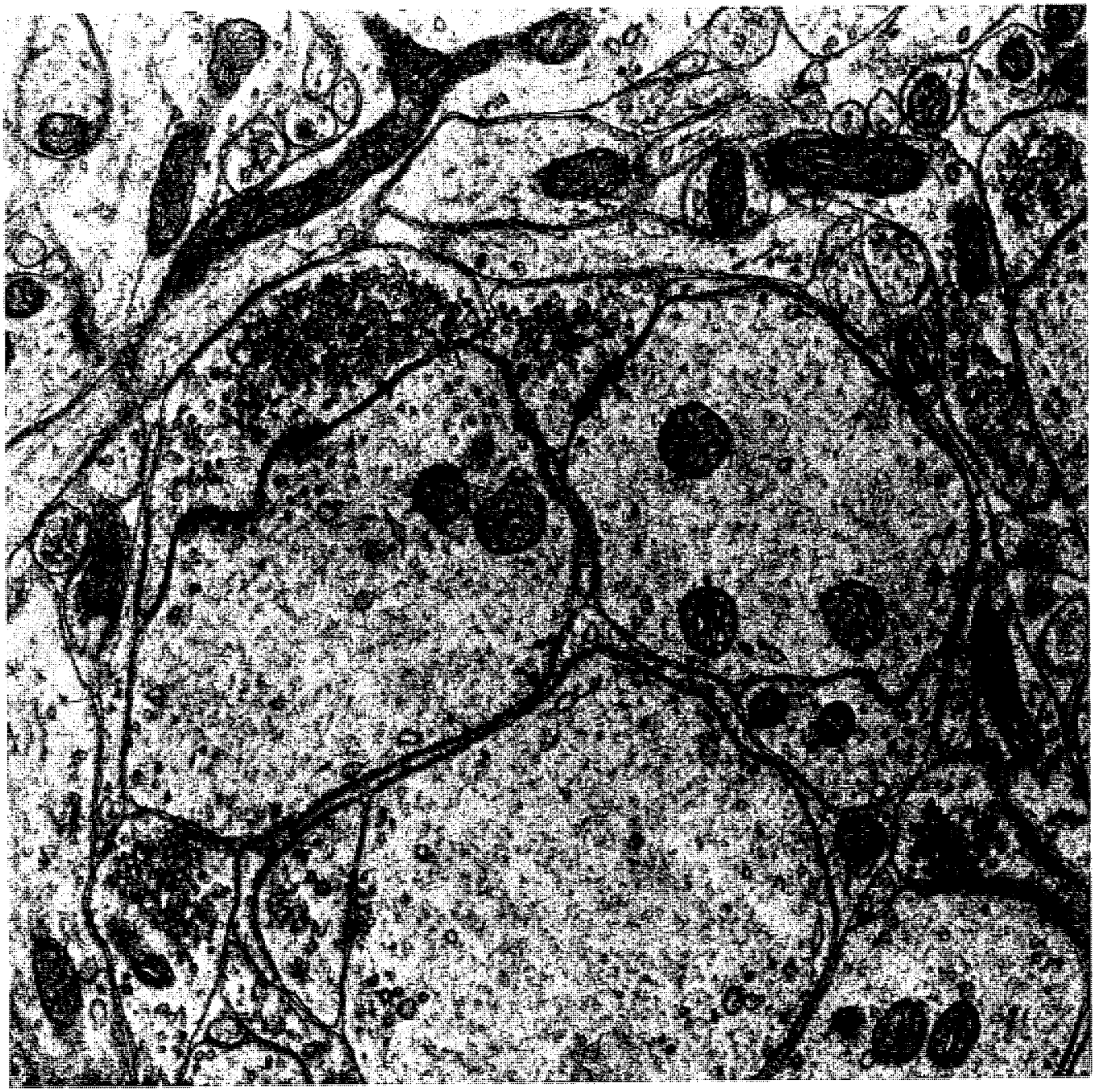


Figure 11 (left): **Axoaxonal synapse** subverts the classical concept that axons always transmit signals to dendrites or to cell bodies. The large, more or less circular region bounded by cell membrane at the center of the field of view is an axon cut in cross section. In particular, it is the initial segment of the axon of a Purkinje cell from the cerebellum of a rat. It includes an assortment of intracellular structures, ranging from mitochondria to neurofilaments and occasional ribosomes. Its microtubules are in groups, a characteristic of the initial axon segment. At the right the axon is abutted by an axon terminal: that of a cerebellar basket cell. The latter is filled with synaptic vesicles. The vesicles are flattened, which betokens an inhibitory synapse. Cross sections through other basket-cell axons



occupy much of the rest of the field. The micrograph was made by Sanford Palay at Harvard at a magnification of 52,000 diameters.

Figure 12 (right): **Dendrodendritic synapses** further subvert the classical concept of interneuronal communication. The micrograph shows tissue from the olfactory bulb of a rat at an enlargement of 27,000 diameters. Three large dendrites cut in cross section dominate the field of view. The one at the upper left establishes reciprocal synapses with a smaller dendrite above it. That is, the cells trade information. The smaller dendrite is packed with synaptic vesicles. The micrograph was made by Thomas Reese and Milton Brightman at the National Institutes of Health.

egy may be simply to identify a neuronal process as an axon or a dendrite by morphological criteria, including, for example, the pattern of branching or the presence or absence of Nissl substance. The functional properties of neural processes could then be studied independently. What proportion of synapses might turn out to be "orthodox" axodendritic synapses? It is of little help to know. In the retina the synapses are often "unorthodox." Yet one cannot conceive of the retina as exotic. It is certainly not inefficient. Presumably the retinal circuitry reflects an extreme need for miniaturization. In the cerebral cortex the synaptic arrangements are far more "conventional." The majority of synapses are probably axodendritic, and most of the rest are probably axosomatic (they convey signals from an axon to a postsynaptic cell body). Yet the cerebral cortex appears to serve the newest functions of the brain. In the human brain it is the neural substrate for language. The extreme variety of neuronal microcircuits suggests that evolution is open-minded—that a design is workable if it advances the brain's computational abilities.

One final heterodoxy. Axons have been discovered that fail to release neurotransmitter at recognizable synapses. For example, axons containing

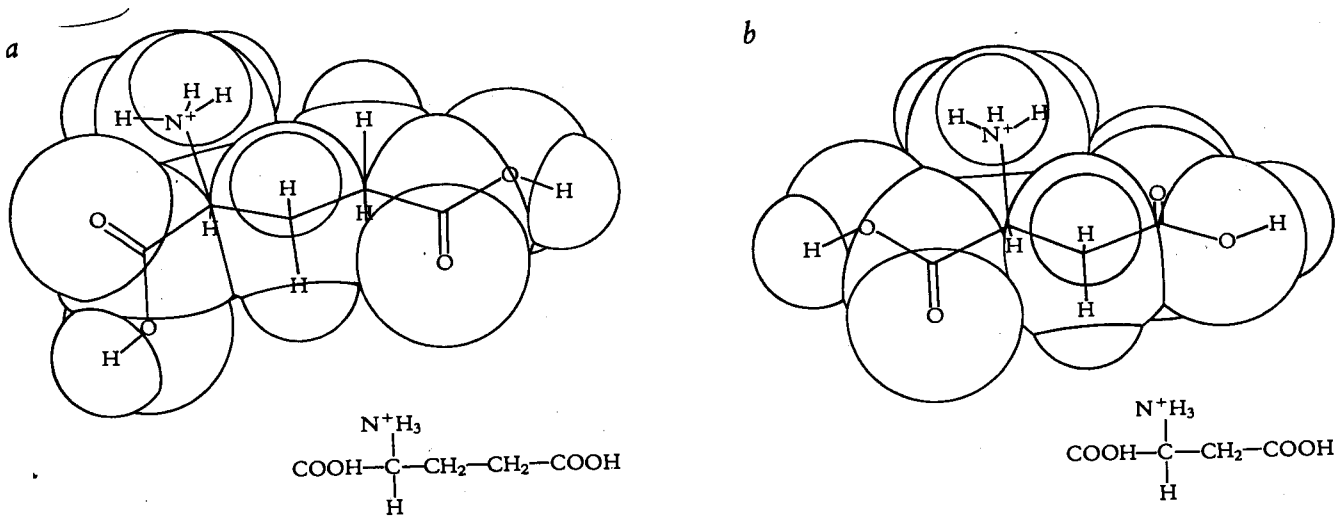
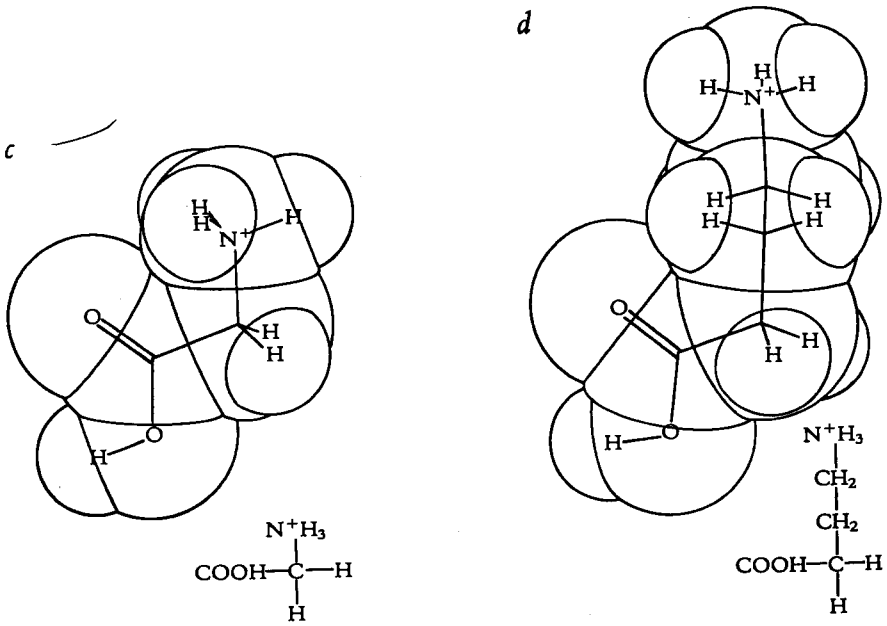


Figure 13: **Amino acid neurotransmitters,** or more precisely the amino acids now thought to serve neurons as chemical messenger substances, are four in number. Glutamate (*a*), aspartate (*b*), and glycine (*c*) are constituents of protein. Gamma-aminobutyric acid, or GABA (*d*), results when a carboxyl group (COOH) is removed from glutamate. The four are displayed as models generated by a computer from X-ray diffraction data that specify the positions of the atoms in a crystal of the substance. In essence, each model depicts an

the neurotransmitter serotonin have been found to enter the cerebral cortex of the rat. The axons have swellings, or varicosities, that contain synaptic vesicles. Yet a great number of the swellings have no presynaptic density. Moreover, the swellings tend to lie where no postsynaptic membrane is nearby. The swellings might simply release their serotonin into extracellular space. That hardly seems to be a way to send a private message. It seems more like throwing leaflets from a rooftop. On the other hand, the arrangement has the advantage that neurons throughout a volume of brain tissue could be affected by a release of neurotransmitter. Indeed, the function of such non-synaptic axon terminals might be not the transmission of discretely ordered information but the mediation of widespread changes in the functional state of the cerebral cortex. Moreover, it is possible that even a seemingly aimless arrangement (an arrangement lacking recognizable synapses) affects only certain neurons—say the ones with a particular type of receptor arrayed inconspicuously on their surface. It should be said that the motor axons entering smooth muscle tissue also fail to make recognizable synapses. In smooth muscle (the contractile tissue in the walls of hollow viscera), the



electronic shape. (Simpler stick figures are also shown.) Under physiological conditions the shapes are doubtless different. For example, at the pH characteristic of cytoplasm, glutamate and aspartate have a net negative charge owing to the loss of a hydrogen ion from each carboxyl group. The models (and the ones in the next two illustrations) were produced by David Barry with the PROPHET Computer System, operated for the National Institutes of Health by Bolt, Beranek & Newman, Inc., in Cambridge, Massachusetts.

endings of axons are scattered, perhaps at strategic sites in the matrix of muscle fibers, and when a given smooth-muscle fiber is made to contract by the receipt of neurotransmitter, its response can be communicated to neighboring muscle fibers through gap junctions among the fibers. In contrast, the muscle fibers composing striated muscle tissue each make private contact with an axon at the elaborate membrane specialization known as a motor end plate or neuromuscular junction. It should also be said that neurons may turn out to have many modes of communication that bypass synapses altogether. After all, cellular mechanisms for expelling molecules and cellular mechanisms for absorbing them are ubiquitous. Perhaps such transfers produce slow metabolic modulations in their target cells.

Neurotransmitters; Neuropeptides

A note on neuron chemistry: The chemical substances that best typify neurons are neurotransmitters: the substances neurons release to signal other cells. Their identity can be elusive. Ideally the neuropharmacologist seeks to establish that the stimulation of a particular group of neurons causes them to release from their presynaptic terminals a particular chemical that affects particular postsynaptic cells in the way observed in nature. In the periphery of

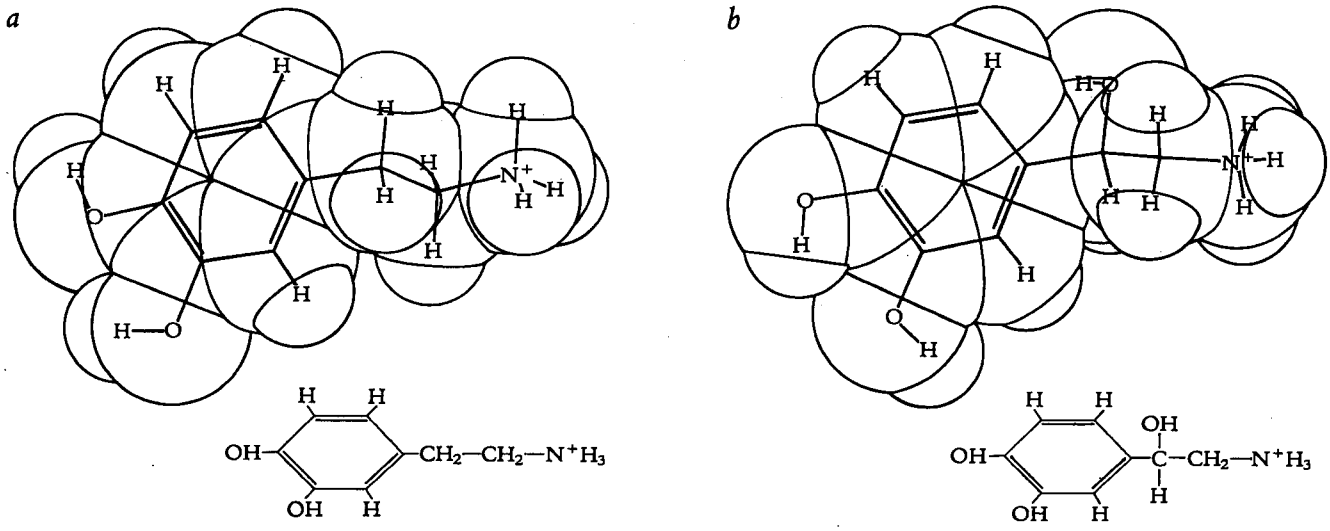
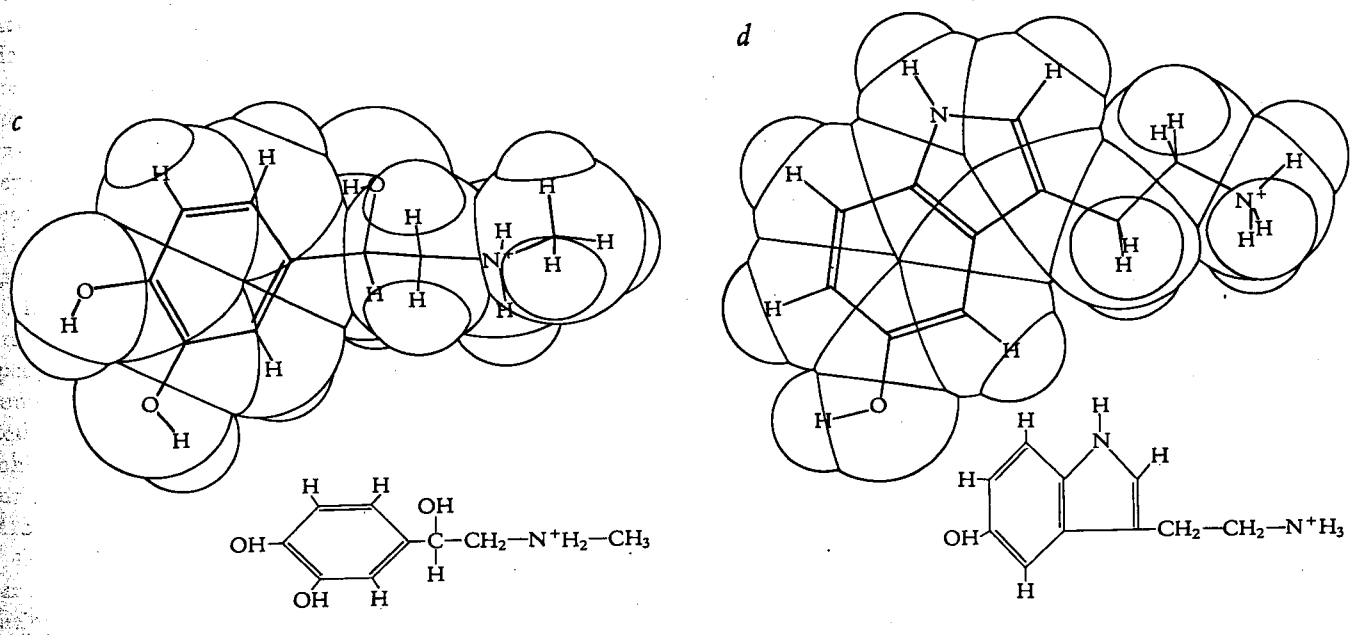


Figure 14: **Monoamine neurotransmitters** (that is, the ones well established today as being neurotransmitters) are four in number. They all result when enzymes in a neuron edit an amino acid. Dopamine (*a*), norepinephrine (*b*), and epinephrine (*c*) emerge in a

the body the project is feasible; in fact a crucial success was reported in 1921. Otto Loewi, a German pharmacologist, perfused the heart of a frog; the heart continued to beat. A length of the vagus nerve remained attached to the organ. He stimulated the nerve electrically; the beating slowed. He collected the perfusate and applied it to the heart of a second frog. It, too, slowed its beating. The perfusate eventually proved to contain acetylcholine; the vagus nerve had released it. In the brain and the spinal cord the project has never fully succeeded. Presynaptic terminals are measured in micrometers (thousandths of a millimeter); the presynaptic membrane has a surface area of no more than perhaps two square micrometers; the terminals lie in a welter of neural circuitry; the arrival of a presynaptic action potential induces each terminal to tap perhaps a few hundred synaptic vesicles, each containing no more than a few tens of thousands of transmitter molecules.

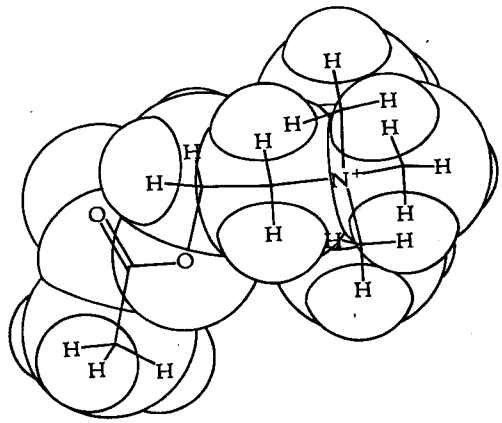
One seeks, therefore, to establish circumstantial evidence. If neurons must synthesize the putative neurotransmitter instead of simply capturing it from the extracellular environment, they ought to include the machinery, say the enzymes, that work the synthesis. The putative neurotransmitter ought to be present in presynaptic terminals. Electrical stimulation ought to bring on its release. The application of the putative neurotransmitter to postsynaptic neurons (say by bathing tissue slices in it) ought to duplicate the effect of natural neural events: for example, a transmitter might change the permeabil-



series of chemical steps from the amino acid tyrosine. Each is a catecholamine: a monoamine that incorporates a six-carbon ring. Serotonin (*d*) derives from the amino acid tryptophan. It is an indoleamine: it incorporates a six-carbon ring and a five-atom ring.

ity of the postsynaptic membrane to certain types of ion. There ought to be a mechanism by which the putative neurotransmitter is inactivated. Presynaptic reuptake will do, or alternatively the catalysis of the substance by extracellular enzymes. Otherwise a synaptic transmission would never end. Drugs known to affect a stage in the cycle of a transmitter — its synthesis, its storage in synaptic vesicles, its release from presynaptic terminals, its interaction with postsynaptic receptors, its inactivation — ought to modify the efficacy of the transmission: the drugs should predictably be agonists or antagonists.

Nine substances found in the vertebrate brain and spinal cord are canonical neurotransmitters: it is agreed that the circumstantial evidence is comparatively conclusive. Remarkably, four of them — glutamate, aspartate, glycine, and gamma-aminobutyric acid, or GABA — are amino acids (Figure 13). Indeed, all but GABA are dietary amino acids: they are molecules found in protein. Thus they are part of an animal's diet. The synthesis of GABA requires merely the decarboxylation of glutamate; that is, the removal of a carboxyl group (COOH). Another four — dopamine, norepinephrine, epinephrine, and serotonin — are monoamines (Figure 14). That is, they are derived from amino acids by no more than minor editing: the addition of hydroxyl groups (OH), the removal of a carboxyl group, the addition of a methyl group (CH_3). Dopamine, norepinephrine, and epinephrine are de-



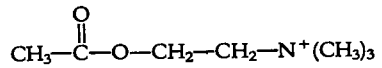
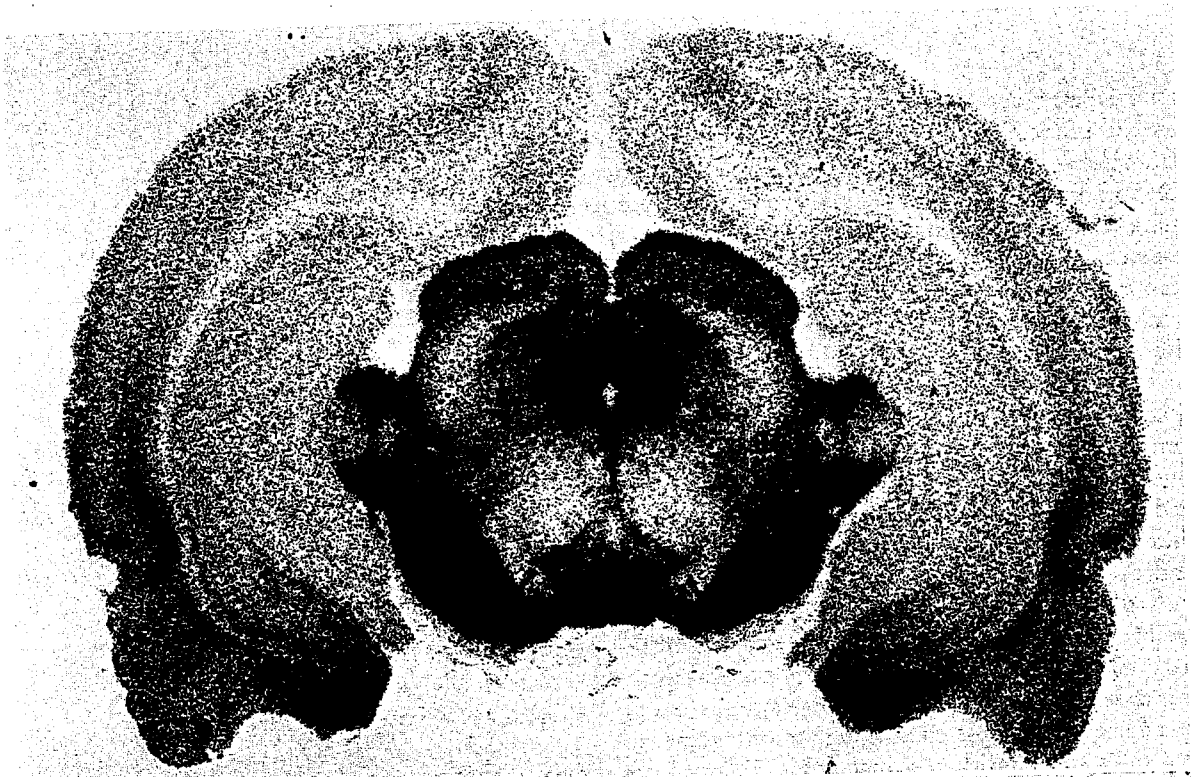


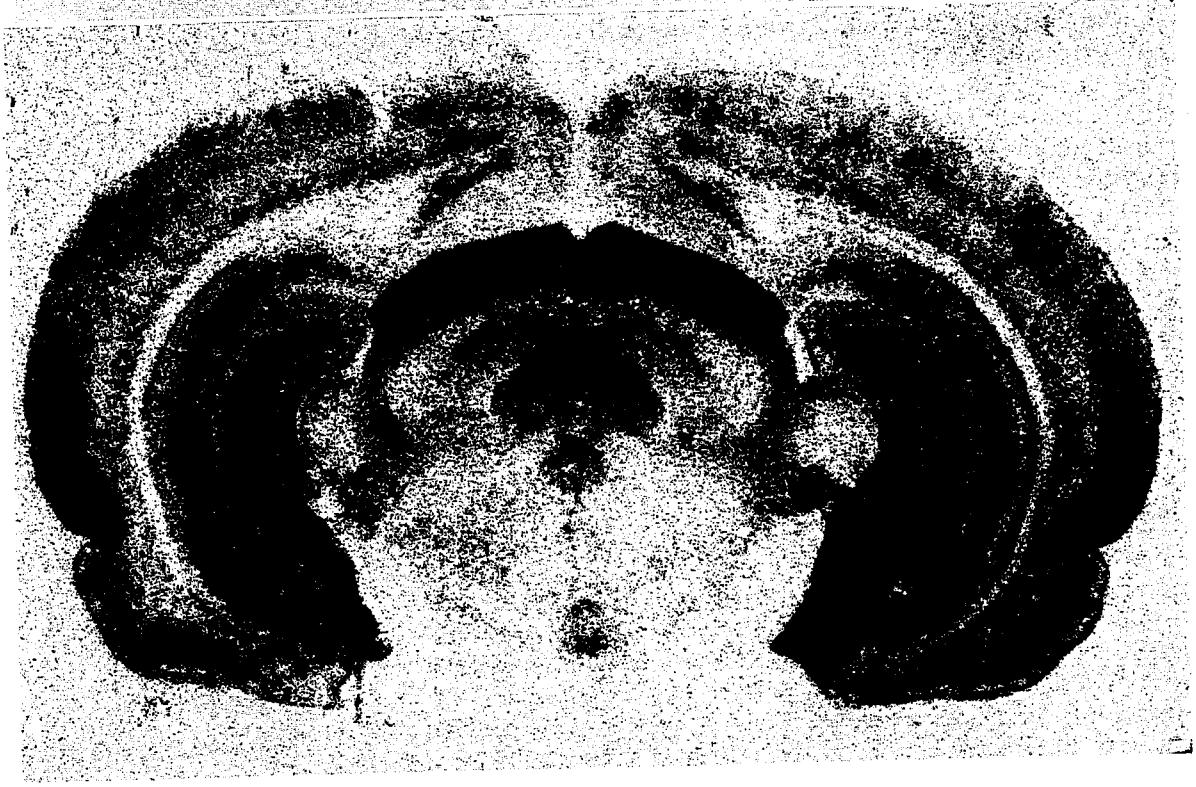
Figure 15: **Acetylcholine** is the ninth substance well established as being a neurotransmitter. Indeed, it was the first known neurotransmitter. It results from the joining of an acetyl group (CH_3CO) to choline, a constituent of lipids. Like the other eight, it is a fairly simple molecule produced in a small number of chemical steps from precursors available in what the animal eats.

rived from the dietary amino acid tyrosine; a six-carbon ring in their structure places them all in the class of molecules called catecholamines. Serotonin is derived from the dietary amino acid tryptophan; the presence of a six-carbon ring linked to a five-atom ring of carbon and nitrogen atoms marks it as an indoleamine. The ninth canonical neurotransmitter is acetylcholine (Figure 15). It results from the joining of choline to an acetyl group (CH_3CO). The choline is a problem: the brain cannot produce it. Choline, however, is a notable constituent of lipids in the diet. The liver can release it or make it de novo. Thus the pattern is unbroken: all nine canonical neurotransmitters are simple molecules made (or liberated) in a small number of chemical steps by enzymes acting on substrates readily available in what the animal eats. This suits them well for high-volume use and rapid replenishment. In that regard it may be relevant that the very simplest canonical neurotransmitters, the amino acid ones, seem to account for synaptic transmission from the majority of synapses in the central nervous system. A crude assay of brain tissue suggests this is the case. The amino acid neurotransmitters occur in concentrations of micromoles (10^{-6} mole) per gram of tissue. That corresponds to 10^{18} molecules per gram. The catecholamine neurotransmitters occur in nanomoles (10^{-9} mole) per gram. The simplest assumption consonant with the disparity is that the brain has a thousand synapses employing an amino acid transmitter for every synapse employing a monoamine.

In the 1930's the English pharmacologist Henry Dale proposed that a given neuron might employ the same neurotransmitter at all of its synapses. At the time acetylcholine and norepinephrine were known to be transmitters. Hence if one or the other could be identified at any one of a neuron's presynaptic terminals, the substance could be trusted to be the transmitter at other terminals less accessible to the investigator. This is not to say that the action of a neuron must always be the same at each of the neuron's synapses. It depends on the interaction between the transmitter and its postsynaptic receptor. The nervous system of the sea snail *Aplysia* offers a spectacular example. In the abdominal ganglion of *Aplysia,* workers including Eric R. Kandel and his colleagues at Columbia University can repeatedly identify more than 50 large neurons. Such identifications are one of the reasons why invertebrates are valued by neuroscientists. No one examining a more advanced species has much hope of finding large neurons that make precisely the same connections in animal after animal. Among the identified neurons the one designated *L*10 contacts the ones designated *L*2, *L*3, *L*4, *L*6, and *R*15. It inhibits all but the last. That one it excites. Acetylcholine applied to the surface of *L*2, *L*3, *L*4, and *L*6 proves to be inhibitory. Applied to the surface of *R*15 it proves to be the opposite.

Dale's principle is also being updated by recent discoveries suggesting that certain peptides (short amino acid chains) serve as intercellular messengers in the brain (Figure 16). The peptides tend to be found throughout the central nervous system. Receptors that bind them tend to be similarly widespread. In one place or another the concentration of any one of them may far exceed its background level. Elsewhere it may be notably lacking. The level is low: the





concentration of a peptide in the brain is typically measured in tens or hundreds of picomoles (10^{-12} mole) per gram. Moreover, a close look at the brain often places the peptide inside only a scattering of neurons; thus it is hard to ascertain whether the electrical stimulation of the neurons containing the peptide causes it to be released. On the other hand, the application of the peptide to slices of brain tissue affects the activity of many neurons. Three such "neuropeptides" — substance *P*, vasoactive intestinal peptide, and cholecystokinin octapeptide — showed up first in the mammalian gut. Then they showed up in the brain: they are now found, for example, in neurons in the cerebral cortex. Conversely, the peptide somatostatin first showed up in the brain: it was purified in minute quantity from literally tons of brain samples on the basis of its ability to suppress the secretion of growth hormone in the anterior lobe of the pituitary gland. Somatostatin now turns out to be present almost throughout the nervous system, from the cerebral cortex to peripheral ganglia. In addition it is found in cells that line the intestine and in certain cells in the pancreas. The peptides known as endorphins showed up both in the brain and in a gland. Specifically, neurons in the brain and cells in the anterior lobe of the pituitary proved to synthesize a protein, pro-opiomelanocortin, that incorporates several messenger peptides, including beta-endorphin, a putative neurotransmitter, and corticotropin, a hormone. (Released from the anterior lobe, it stimulates the cortex of the adrenal gland.) Neurons at a number of sites in the brain have receptors that bind opiates such as morphine, and some — not all — of the sites are known to participate in the perception of pain. The endorphins, occurring naturally in the brain, can bind to these receptors. So can the enkephalins, a pair of pentapeptides. The

Figure 16: **Substance *P* and its receptors** exemplify recent discoveries that a number of peptides (short amino acid chains) are active in the brain, perhaps as neurotransmitters. They are collectively called neuropeptides. Substance *P* is a neuropeptide consisting of 10 amino acids. Like several other neuropeptides it was found first in the gut and then in the brain: in neuronal cell bodies, axons, and axon terminals. Neurons with substance-*P* receptors on their surface have also been encountered. In these low-power micrographs, which show transverse slices of the brain of a rat, the distribution of substance *P* (*top*) was demonstrated with an immunological technique. An antibody to substance *P* was labeled with the radioactive isotope iodine 125 and applied to brain slices. The slices were then coated with photographic emulsion, in which the radioactivity laid down a visible pattern by transforming silver halide into black metallic grains. The micrograph shows the emulsion, not the underlying tissue. The distribution of substance-*P* receptors (*bottom*) was demonstrated in a similar way by labeling substance *P* with iodine 125 and applying it to tissue slices, so that it, and its label, could bind to receptors there. Curiously, the broad-scale distribution of substance *P* appears to correlate poorly with that of substance-*P* receptors. For example, the substantia nigra, at the base of each slice about two centimeters to each side of the midline, is rich in substance *P* but lacking in receptors. Perhaps a class of receptors with low affinity for substance *P* escaped detection. The distribution of substance *P* was determined by Stafford McLean of the National Institutes of Mental Health; the distribution of substance-*P* receptors was determined, again at the N.I.M.H., by Richard B. Rothman, Miles Herkenham, and Candace B. Pert.

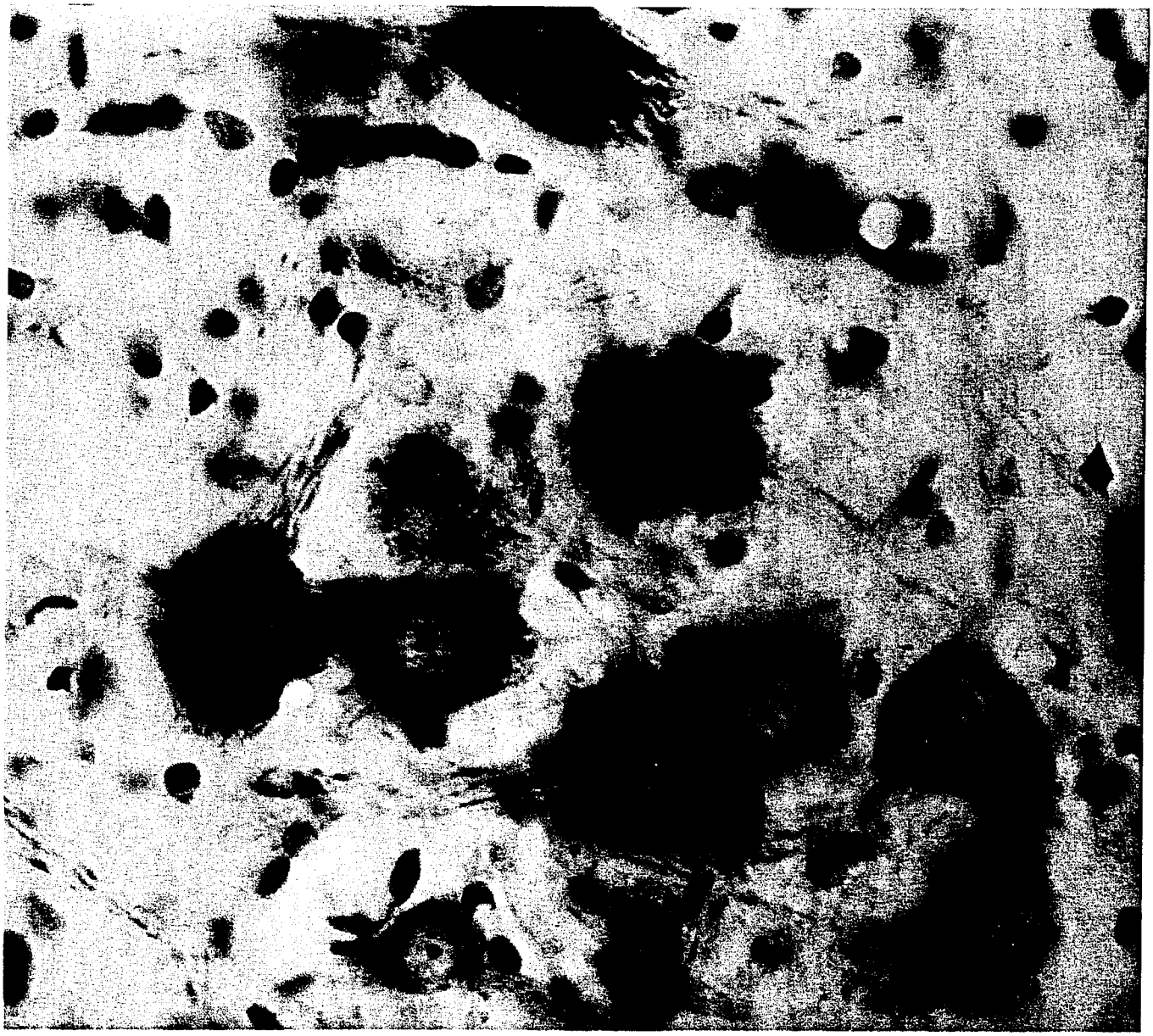


Figure 17: **"The dismal fog"** is the expression the Spanish neuroanatomist Santiago Ramón y Cajal employed to signify his dismay at the sight of neuronal processes vanishing as they emerge from their parent cell bodies in tissue stained for cell bodies. Here, in Nissl-stained tissue from the brain of a rat (in particular the motor nucleus of the trigeminal nerve), dendrites emerge from several neuronal cell bodies. The dendrites are invaded by elongated strands of Nissl substance, which enable their early trajectories to be traced. Then, however, the dendrites disappear. Even so, the dismal fog is far from featureless. It is populated by the nuclei of nonneuronal cells called glia. The largest, lightest-staining spots are the nuclei of glial cells called astrocytes; darker spots are the nuclei of glial cells called oligodendroglia; the darkest, angular spots are the nuclei of glial cells called microglia. To give examples: some closely spaced glial nuclei form an eyebrow-shaped crescent at the upper left. Three round, rather dark-staining bodies form the middle of the crescent; they are the nuclei of oligodendroglia. The larger, lighter-staining body abutting the leftmost of the three is the nucleus of an astrocyte; the very dark pellet just past the right end of the crescent is the nucleus of a microglial cell. The small white circle at the upper right is a blood vessel. It is lined by two dark crescents: the nuclei of vascular endothelial cells.

enkephalins are beginning to turn up in longer peptides, including one called dynorphin.

The updating of Dale's principle suggested by the study of neuropeptides comes about because the peptides are sometimes found in neurons already known to employ one of the canonical neurotransmitters. Some cells include both vasoactive intestinal peptide and acetylcholine. Others include cholecystokinin and dopamine. Still others include substance *P* and serotonin. A neuron may therefore release a mixture of chemical messengers; their actions doubtless differ. In particular, the canonical neurotransmitters open ion-conductance channels in the postsynaptic membrane. Thus they mediate rather directly between bioelectric activity on the part of the presynaptic cell and bioelectric activity on the part of the postsynaptic cell. The monoamine neurotransmitters have a further mode of action: they seem to alter the metabolic state of the postsynaptic cell. Neuropeptides, too, do both. In addition some of them, including the endorphins, are implicated in a curious mode of action. Here the arriving neuropeptide opens no postsynaptic channels and initiates no metabolic changes. And yet somehow it impedes the ability of the postsynaptic cell to respond to the arrival of canonical neurotransmitters, excitatory and inhibitory alike. To use a term favored by Floyd E. Bloom of the Scripps Clinic in California, it temporarily "disenables" the postsynaptic cell. The prospect arises that a mixture of messengers crossing a synaptic cleft and arriving at a postsynaptic membrane entails a complex course of events. The prospect is further complicated by the knowledge that neurons quite typically get input from a variety of brain structures, and that alone implies a variety of chemical messengers. In the cerebellum, for instance, the neurons called Purkinje cells are known to have receptors for norepinephrine and receptors for GABA. Other cerebellar neurons (ones that influence Purkinje cells) are known to have receptors for GABA and receptors for serotonin. Does the finding of a peptide in the intestine and in the brain seem like a humbling joke? It may simply reflect the usefulness of certain molecules as messenger substances. Evolution has employed them in many places. Does the number of substances—now in the dozens—suspected to be neurotransmitters seem like an excess? It may simply reflect the variety and the subtlety inherent in intercellular communication.

Glial Cells

Now let us peer into the dismal fog that surrounds a Nissl-stained neuron (Figure 17). It is far from featureless; one sees dark spots within it. For the most part they are the nuclei of glial cells, or glue cells in literal translation: cells that provide support to neurons throughout the central nervous system. Glial cells are thought to be 10 times more numerous than neurons. Yet the cell body in which each glial-cell nucleus lies embedded is invisible in Nissl preparations because it contains too few ribosomes to bind much of the Nissl dyestuff.

The largest but lightest-staining spots in the dismal fog are called open-faced nuclei; each belongs to a cell body of irregular shape that may look like a stylized star in the histological preparations that reveal it because its jagged sides sometimes taper to points (Figure 18). In addition (and perhaps more characteristically), the cell has numerous processes that radiate outward like a starburst. For either reason, the name astrocyte (from the Greek *astron*, or star) is descriptive. Astrocytes form the greater part of the matrix or scaffolding in which neurons are embedded. The astrocytes near the surface of the central nervous system have an additional function. They send processes to the surface, where the aggregation of their end feet forms a limiting membrane: a glial capsule that constitutes the outer wall of the brain and the spinal cord. The surface of the capsule is just under the pia mater, the innermost of the meningeal coverings of the central nervous system. In fact the glial capsule and the inner layer of the pia mater fuse: they form what is called the pial-glial membrane. The membrane is ubiquitous: its covering of the central nervous system is complete and unbroken. Indeed, wherever a blood vessel seems to invade the central nervous system, it never truly does so, because the pial-glial membrane funnels in around it. In this way, blood vessels are kept from making physical contact with neurons.

A second type of glial cell is characterized in Nissl preparations by a far more darkly staining nucleus, a round one in which the nucleic acids tend to aggregate more compactly. It is the oligodendroglial cell (Figure 19). The name means the glue cell with few processes. It does in fact have fewer than an astrocyte. Still, it has dozens; the electron microscope reveals them. Oligodendroglia coat axons in the brain and the spinal cord with an investment of lipid and protein called the myelin sheath (Figure 20). In particular, the membrane that forms the surface of an oligodendroglial cell compacts on itself to make as many as 40 veil-like extrusions, and each extrusion—in essence a doubled cell membrane—wraps around an axon. As the axon grows thicker in the developing central nervous system, more wraps are added. Thus the thicker the axon (the thickest ones in a mammal are from 12 to 14 micrometers in diameter, myelin included), the thicker its myelin sheath. There is, on the other hand, a cutoff diameter below which axons get no sheath. The cutoff seems to lie at about half a micrometer. When the myelination of an axon is complete, the axon is enveloped by lipoprotein except at the axon hillock, the initial axon segment, and the axon terminals. Moreover, the axon lacks myelin at a series of short gaps throughout its length where the sheath provided by one oligodendroglia ends and that of another begins. The gaps are known as nodes of Ranvier. They are the only places beyond the initial axon segment where ions can enter or leave a myelinated axon. The conduction of an impulse in a myelinated axon must therefore be discontinuous: it cannot be construed as simply the onrushing of an altered bioelectric potential. It can occur only because each node of Ranvier, like the initial axon segment, is an all-or-none site, complete with a granular undercoating, at which the impulse is regenerated. The impulse jumps from node to node, and in consequence its conduction is far faster than is possible in a process lacking

myelin. The impulse is said to undergo saltatory conduction, meaning "moving by leaps." Small wonder that long-distance neural communication lines —those spanning centimeters rather than millimeters—almost always are established in vertebrate animals by myelinated axons.

The last variety of glial cell in the central nervous system is the microglia. Its nucleus is rarely round or even ovoid; it often is angular. It is the darkest among the glial nuclei that can be seen in the dismal fog. Microglial cells seem not to originate inside the embryonic tissues that become the central nervous system. Instead they are thought to arrive in the company of ingrowing blood vessels. In any event, microglia are the only cells in the central nervous system that can become militant. That is to say, they respond to a

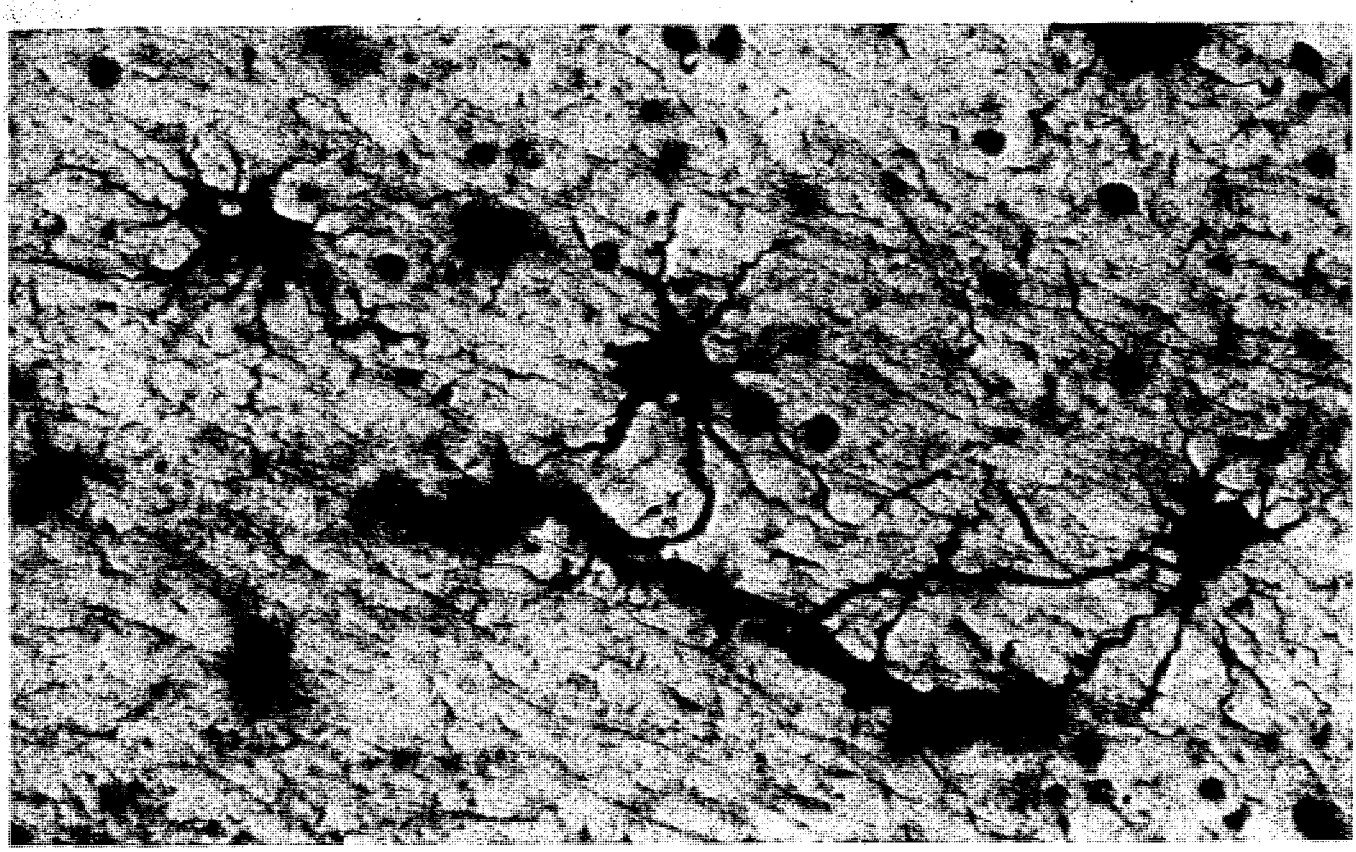


Figure 18: **Astrocytes** are the largest glial cells; their angular cell bodies emit a wealth of processes, which radiate outward like a starburst. In this micrograph, tissue from the axonal "cable basement" under the cerebral cortex of a human brain has been stained by the Cajal gold sublimate method, which is specific for astrocytes. Several astrocytes can be seen. Two of them extend their processes toward a small blood vessel crossing the bottom center of the image. There the end feet of the astrocytic processes contribute to a membranous glial capsule that keeps the vessel from making contact with neural tissue. Astrocytes serve, it seems, to establish neural compartments. Indeed, the end feet of astrocytic processes close off the surface of the brain and spinal cord.

pathological process by changing into phagocytes: cells that ingest microbes and the products of tissue breakdown.

How Many Neurons?

Since the cell bodies of glial cells are invisible in Nissl preparations, the Nissl technique can do no more than display the distribution of neuronal cell bodies

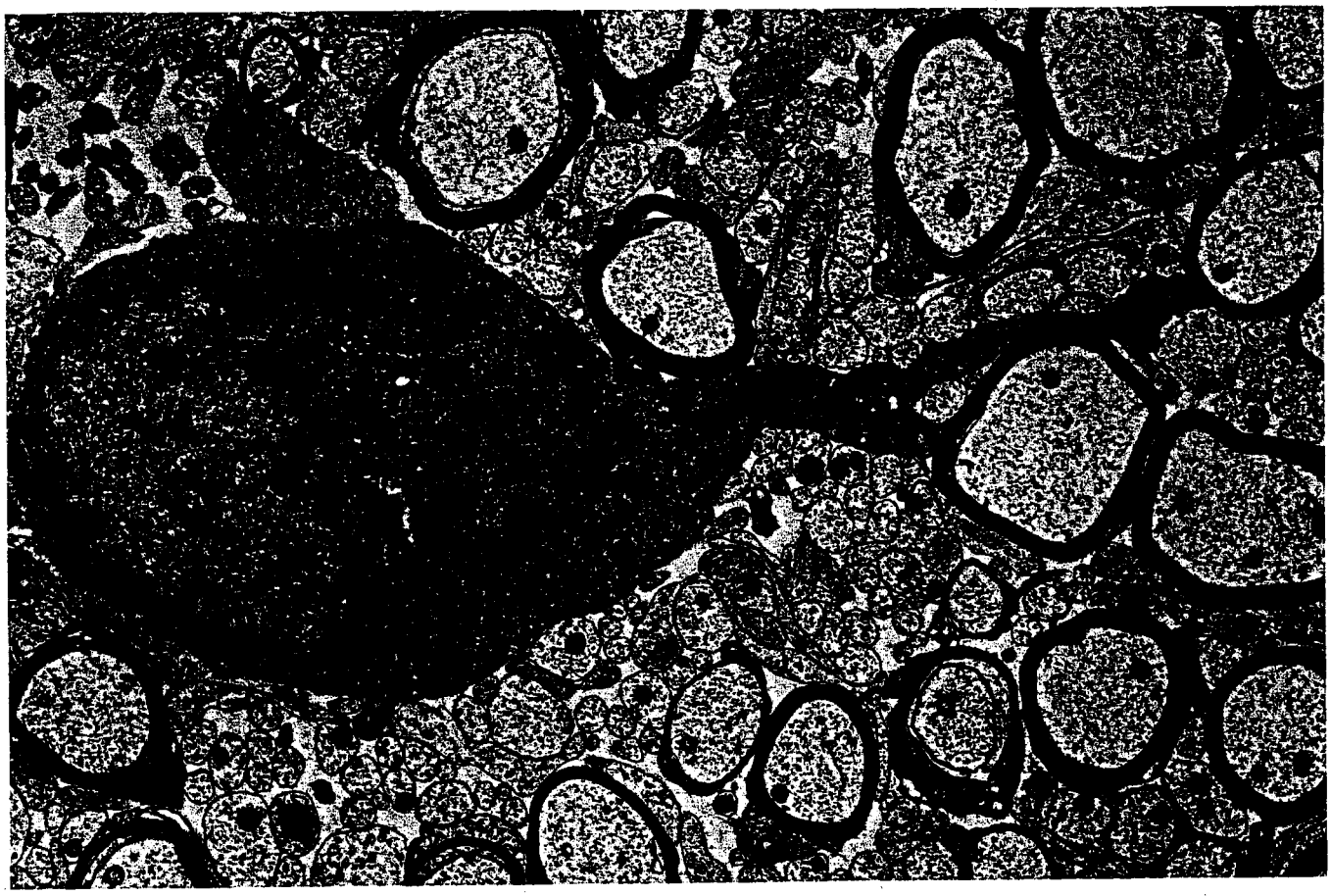
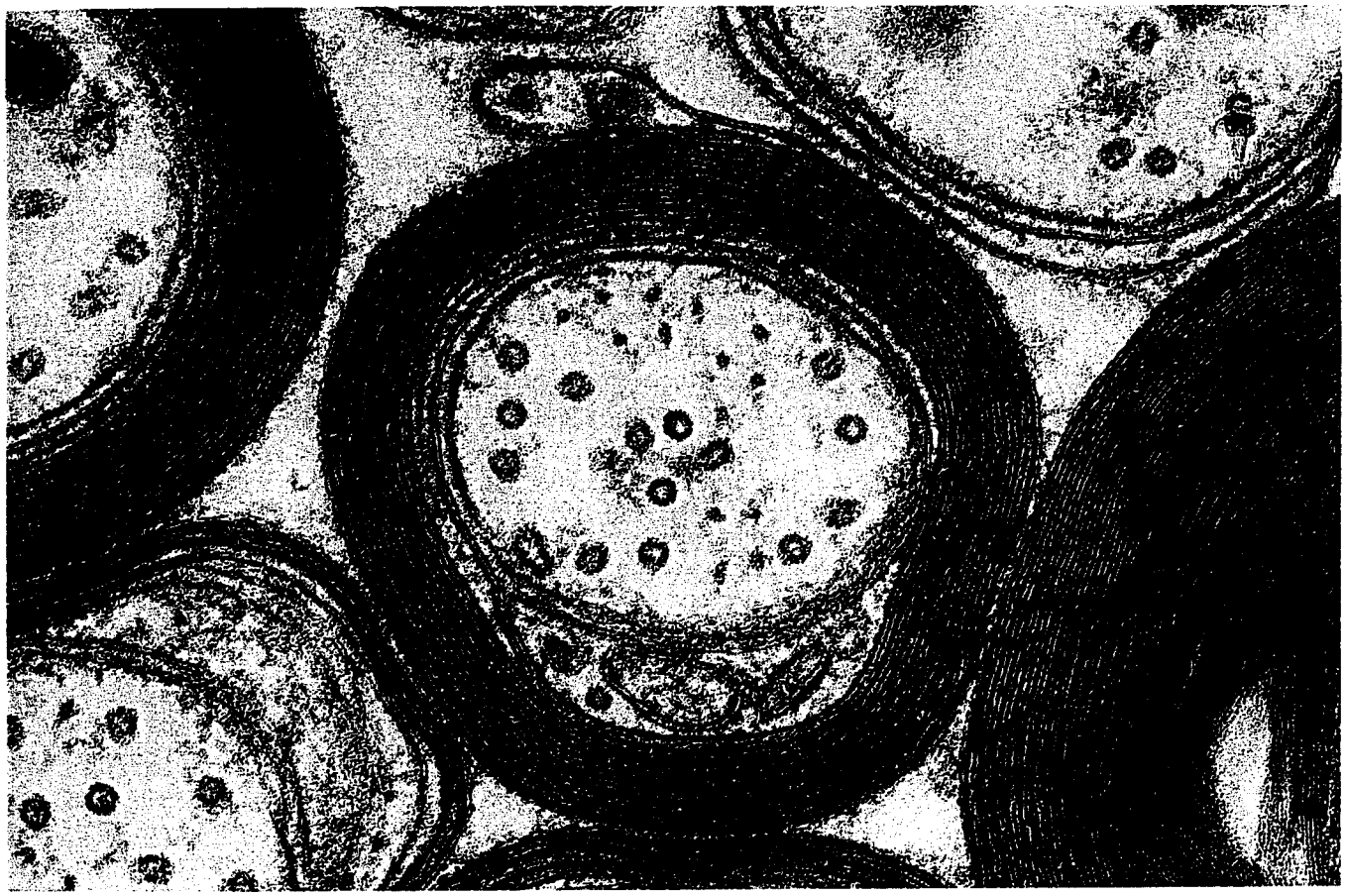


Figure 19 (left): **Oligodendroglia** invest the axons of neurons in the brain and spinal cord with a sheath of the fatty insulation called myelin. This electron micrograph was made in the spinal cord of a newborn cat at a magnification of 12,200 diameters. The cell body of an oligodendroglial cell is at the left. Its nucleus, which fills almost half the cell body, is at the extreme left. Toward the right the cell emits an extension, or process, that contacts two axons, cut in cross section. An oligodendroglial cell emits many such extensions and so may contact 40 axons or more, providing each with a sheath that covers part of the length of the axon. Oligodendroglia along the rest of the length of the axon contribute the other sheath segments. In this image a multitude of smaller axons fills much of the rest of the field; some would have increased in size and received a myelin sheath as the animal grew. The micrograph (and the one shown in Figure

in a section of tissue. But by that shortcoming, or rather by that virtue, it provides a survey of the cytoarchitecture of the brain and the spinal cord, and most basically, it permits a simple count of neurons per unit volume. How many neurons occupy the human central nervous system? True sensory neurons lie not in the central nervous system but in ganglia that flank the brain and the spinal cord; hence the answer must be an accounting of intermediate neurons and motor neurons. It often used to be said that the answer is 10^{10}. It is an attractive number, easy to remember and easy to state. Yet there



20) was made by Cedric S. Raine of the Albert Einstein College of Medicine of Yeshiva University.

Figure 20 (right): **Close view of myelin** shows details of how oligodendroglia make the myelin sheath. An axon is at the center of the field. On top of it is an oligodendroglial process, cut, like the axon, in cross section. Toward its right-hand side the process compacts on itself to form a double layer of cell membrane; then the double layer wraps repeatedly around the axon. In this instance the wrapping is clockwise. At the end of the innermost wrap the process expands again, producing a final cytoplasm-filled chamber. The axon itself includes a number of microtubules. The electron micrograph displays tissue from the spinal cord of a dog at a magnification of 163,000 diameters.

are classes of neurons so small and so densely crowded together that it is difficult or even impossible to judge their number. One such class is the granule cell. There are so many granule cells in just one part of the human brain, the cerebellum, that the estimate of 10^{10} neurons in the entire central nervous system becomes quite suspect. The total could easily be an order of magnitude higher—perhaps two orders of magnitude.

Assume, then, that the total is 10^{12}. How many are motor neurons? An estimate of the axons leaving the central nervous system to animate muscle fibers implies an answer of two or three million, which is disconcertingly few, because only through motor neurons can the workings of the nervous system find expression in bodily movements. The figure suggests that motor neurons are at a premium, and hence that a great number of influences must converge on them; it suggests, in other words, that a typical motor neuron must receive synapses from a multitude of axons emitted by a multitude of neurons in the great intermediate net. The facts agree. A typical motor neuron has perhaps 10,000 *boutons terminaux* on its surface. About 8,000 are on its dendrites and 2,000 are on its cell body. This is not to say that 10,000 intermediate neurons impinge on the motor neuron; the intermediate neurons tend to make multiple synaptic contacts when they communicate with a cell. Even so, being one among very few, the average motor neuron must be heavily impinged on; a neuron count of 10^{12} in the central nervous system implies as many as half a million neurons of the great intermediate net for every one motor neuron. Charles Sherrington had good reason to refer to the motor neuron, and in particular its axon, as the nervous system's "final common path."

One last conclusion remains to be drawn from the numbers we have cited: With the exception of a mere few million motor neurons, the entire human brain and spinal cord are a great intermediate net. And when the great intermediate net comes to include 99.9997 percent of all the neurons in the central nervous system, the term loses much of its meaning: it comes to represent the very complexity one must face when one tries to comprehend the nervous system. The term remains useful only as a reminder that most of the brain's neurons are, strictly speaking, neither sensory nor motor. Strictly speaking, they are intercalated between the true sensory side of the organization and the true motor side. They are the components of a computational network.

Anatomical Divisions

The brain and the spinal cord of every vertebrate animal first appear in the embryo as no more than a tube formed by an epithelium only one cell thick. The forward part of the tube becomes enclosed inside the cranium. Long before that, however, it shows a series of three bulbous swellings called the primary brain vesicles (Figure 21). From back to front they are the rhombencephalon, or hindbrain; the mesencephalon, or midbrain; and the prosencephalon, or forebrain. In each case, the Greek name derives from the suffix *-encephalon,* meaning "within the head." Of the three primary vesicles the forebrain is the most productive in higher vertebrates, in terms of both further subdivision and further differentiation. The major event in its ontogenesis is the formation of a chamber on its left and right side. These become the cerebral hemispheres, also called the telencephalon or endbrain, which in lower vertebrates such as fishes are of modest size but in higher forms are enormous. Between the hemispheres lies the unpaired central part of the forebrain, from which the hemispheres diverge. It is called the diencephalon, which literally means "between-brain." Concurrent with these developments, the prosencephalon in a slightly more ventral position grows a further pair of lateral diverticula.* They are the optic vesicles. Even sightless animals

*The word lateral amounts to a navigational aid. There are several others. Throughout this book they have the following definitions. The word median (from the Latin *medium,* or middle) signifies a position at the midplane of the central nervous system, and hence at the midline of a slice through the nervous system. The word medial signifies a position toward the midplane. Thus a structure is medial only with reference to some other structure. The

have them, but in animals that can see they elongate toward the surface of the head. Ultimately they become the two retinas, connected to the base of the forebrain by their stalks, the optic nerves. Lastly, the ventral wall of the primary prosencephalon develops an unpaired midline diverticulum that differentiates to form the posterior lobe, or neurohypophysis, of the pituitary, or hypophyseal, complex.

Caudal Divisions

Figure 22 suggests the outcome of all these events; it is a schematic diagram that holds, by and large, for all mammals, and it shows the mammalian central nervous system broken up into several divisions. Many of its boundaries are more conceptual than biological, because every neural structure is continuous with its neighbors. Still, dividing lines, even arbitrary ones, are welcome. At the left of the figure is the first and most caudal subdivision of the central nervous system, the spinal cord, drawn with extreme foreshortening. Then, at no certain level—the transition is not abrupt—one's attention is transferred to the fully formed rhombencephalon, the caudalmost part of the brain. We shall often call it the hindbrain even though that straightforward English name is seldom used; the preferred name for the division, like the preferred names for all the great subdivisions of the brain, is the Greek one. "Rhombencephalon," then, was coined by affixing the prefix *rhomb-* to the ubiquitous stem *-encephalon;* "rhomb" refers to a progressive widening of the hindbrain, which reaches a maximum width at about the middle of its length, and tapers above and below. The organization of this brain division is remarkably consistent throughout the vertebrate orders. Its caudal half—the part immediately continuous with the spinal cord—is called the myelencephalon, from the Greek *myelon,* or marrow. Alternatively it is called the medulla oblongata, Latin for "the extended marrow." Both names have the same basis. Early anatomists described the spinal cord as the medulla spinalis, the spinal marrow. After all, the spinal cord is soft, whitish tissue and it is encased in bone: the bone of the vertebral column. The caudal half of the rhombencephalon was named by extension of this logic.

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word lateral (from the Latin *latus,* or width) signifies a position away from the midplane. Again the term is relative. The word rostral (from the Latin *rostrum,* or snout) means "toward the end of the organism nearest the nose." Anterior is a synonym. The word caudal (from the Latin *cauda,* or tail) means "toward the tail end of the organism." Posterior is a synonym. The word dorsal (from the Latin *dorsum,* or back) means "toward the back of the organism." The word ventral (from the Latin *venter,* or belly) means "toward the front." In giving these definitions we are suppressing a complication that becomes important in the comparative anatomy of the brain. Basically, some animals walk on four legs, so that the spinal cord trails out horizontally behind the brain, whereas others walk on two legs, so that the spinal cord descends vertically under the brain, and this difference bedevils all attempts to establish a nomenclature that is totally unambiguous.

The rostral half of the rhombencephalon is called the metencephalon. Perhaps the name derives from *meta* and signifies "that which follows the myelencephalon." In any case, the metencephalon can be further divided. Its ventral part is the pons district, so named because it bulges outward there to form the pons Varolii, the bridge of Varolio. (Costanzo Varolio was an Italian anatomist of the 16th century.) Its dorsal part is an appendage called the cerebellum, the "little brain." In fact the size of the appendage varies greatly in different species: some fishes have an impressive cerebellum whereas most amphibians have a tiny one. The ancestry of the cerebellum suggests it was initially an auxiliary to the vestibular system but that other sensory systems later claimed its attention, too. Finally almost all the senses came to do so. Yet this cataloging of cerebellar input begs the more important question: How do the efferent, or outgoing, connections of the structure serve the needs of a living animal? One line of evidence is immediately available. When a person with cerebellar pathology is asked to bring his finger to the tip of his nose, his effort is decomposed: he jerks about, and as the finger approaches its target the jerkiness grows worse. This motor impairment is called ataxia—literally, loss of taxis, or order, in bodily motion. It is the only form of deficit that can be found in the patient. There is no sensory loss. Evidently, then, the cerebellum is a mechanism that integrates messages from all or most of the senses, and then brings that integral to bear on the organism's movements.

Rostral to the rhombencephalon is the mesencephalon, or midbrain. The English term is often used. In a mammal the midbrain develops two dorsal pairs of protrusions that together form a region of four hills known as the lamina quadrigemina, the tectum mesencephali, or simply the tectum, meaning roof. The more caudal pair are the inferior colliculi, from *colliculus,* or little hill; they are part of the central conduction paths for hearing. The rostral pair, the superior colliculi, are part of the paths for vision. Other than the colliculi, the mesencephalon gives little outward reason for subdivision; in fact, the mesencephalon is a rather short stretch in the human brain. The dorsal midbrain of vertebrates more primitive than the mammals has only a single pair of hills. Each hill corresponds to a superior colliculus, in the sense that its function is visual, not auditory. Accordingly, the hills are called the optic lobes or optic tectum. The functional homolog to an inferior colliculus in these nonmammalian forms occupies a deeper position and raises no bulge at the surface.

Rostral to the mesencephalon is the central, unpaired division of the prosencephalon, namely the diencephalon. Its dorsal two-thirds is the thalamus (where "thalamus" derives from the Greek for chamber). The thalamus will emerge as a crucial way station, a final checkpoint that intercepts messages to the cerebral cortex from all of the senses (except, it seems, olfaction). It is tempting to call any such interruption a relay. What happens at a break in neural circuitry, however, is far more than what happens in an athletic relay, where each runner simply hands a baton to the next and the baton arrives unmodified at the end of the course. In the central nervous system, the "relay" is quite different. At each synaptic interruption in a sensory pathway

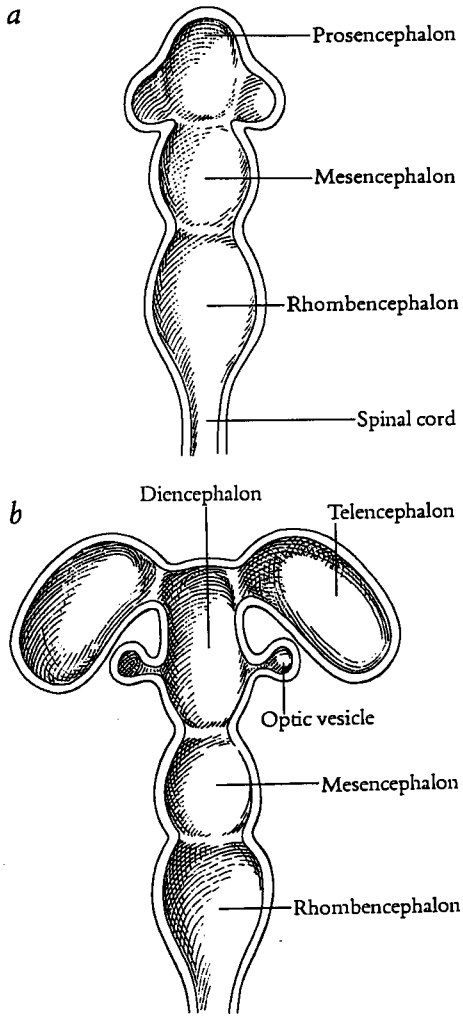


Figure 21: **Primary brain vesicles** are an early sign that the brain is taking form in the embryo of a vertebrate. They are a set of three bulbous swellings at the forward end of the neural tube: the precursor of the brain and the spinal cord. The swellings, from back to front, are the prospective rhombencephalon, or hindbrain; the prospective mesencephalon, or midbrain; and the prospective prosencephalon, or forebrain. In a human embryo the primary brain vesicles are apparent before the fifth week of gestation (*a*). Then, in the fifth week, the diencephalon, or central chamber of the forebrain, begins to develop side chambers: the telencephalon, or cerebral hemispheres (*b*).

the input is transformed: the code in which the message arrived is fundamentally changed. Presumably the data could not be "understood" at other levels; translation is needed, and the synaptic relays are better spoken of as processing stations.

Many such stations are found in the thalamus. Each is called a nucleus, a term that may be confusing. In a cytological context, the term refers, of course, to the nucleus of a cell. In neuroanatomical nomenclature, however, the term indicates a multitude of neuronal cell bodies lying in proximity to one another, and thereby forming a cluster that is more or less clearly delineated from neighboring structures in properly stained sections of the brain or the spinal cord (Figure 23). The ventral nucleus of the thalamus is a large cell

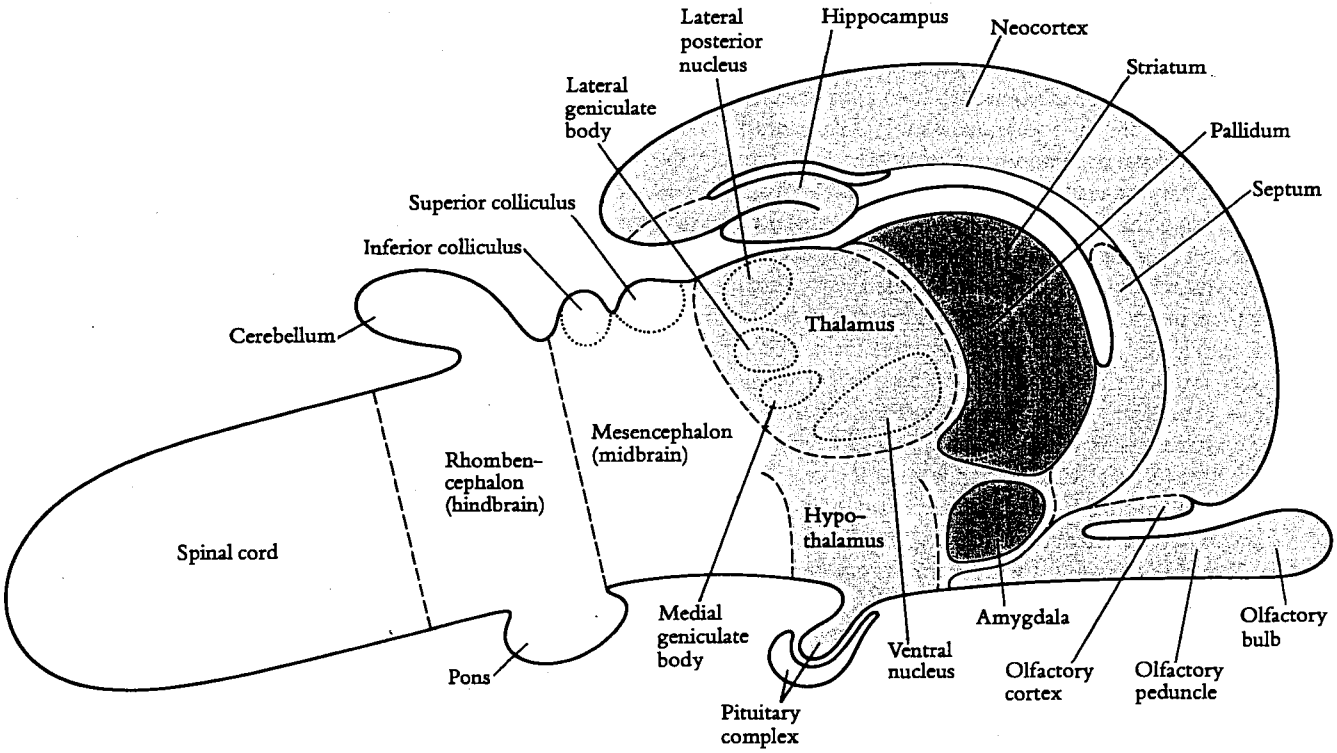


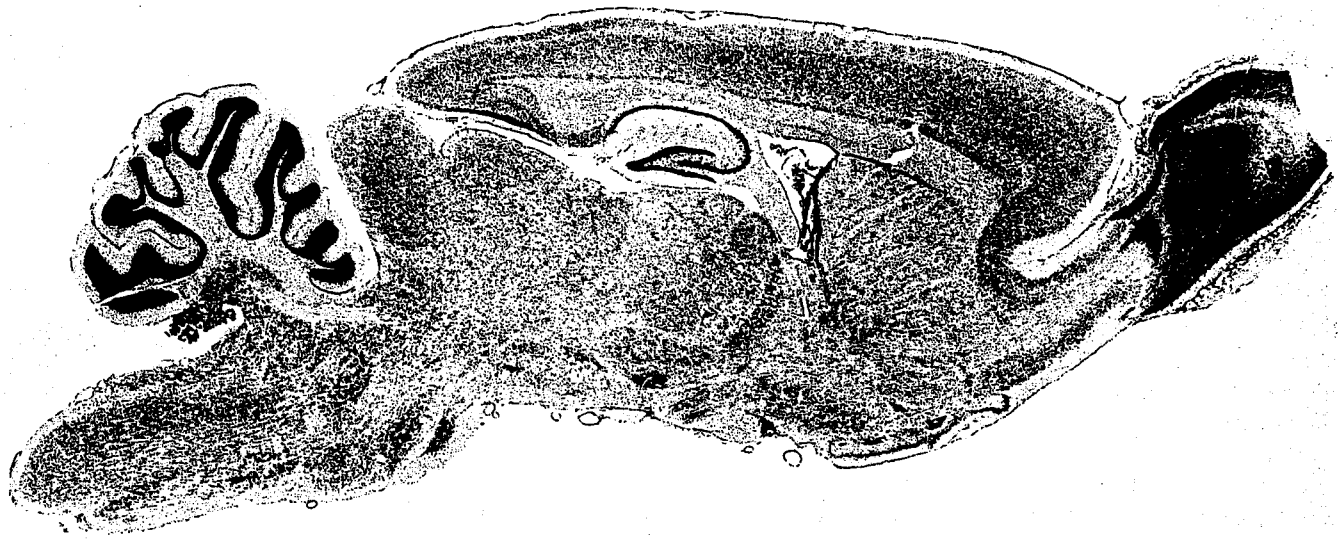
Figure 22: **Large-scale divisions** of the central nervous system arise from the neural tube. Here the mammalian brain is pictured. The rhombencephalon, or hindbrain, includes a massive protrusion, the cerebellum, and at the other side of the hindbrain an elevation called the pons. The mesencephalon, or midbrain, includes two elevations, the inferior and superior colliculi. The prosencephalon, or forebrain, is more complex. It has an outer part, the cerebral hemisphere (*color*), and an inner part, the diencephalon (*gray*). Each has further divisions. The cerebral hemisphere includes a "rind," the cerebral cortex (*light color*), which incorporates the hippocampus, the neocortex, and the olfactory fields. Beneath them (*dark color*) are stationed the amygdala and the corpus striatum. (The latter has two divisions, the striatum and the globus pallidus.) Meanwhile, the diencephalon includes the thalamus and the hypothalamus. The latter connects to the posterior lobe of the pituitary complex. The septum is best considered a diencephalic outpost. The scheme established in this illustration will serve as the basis for a sequence of diagrams throughout Part II of the book.

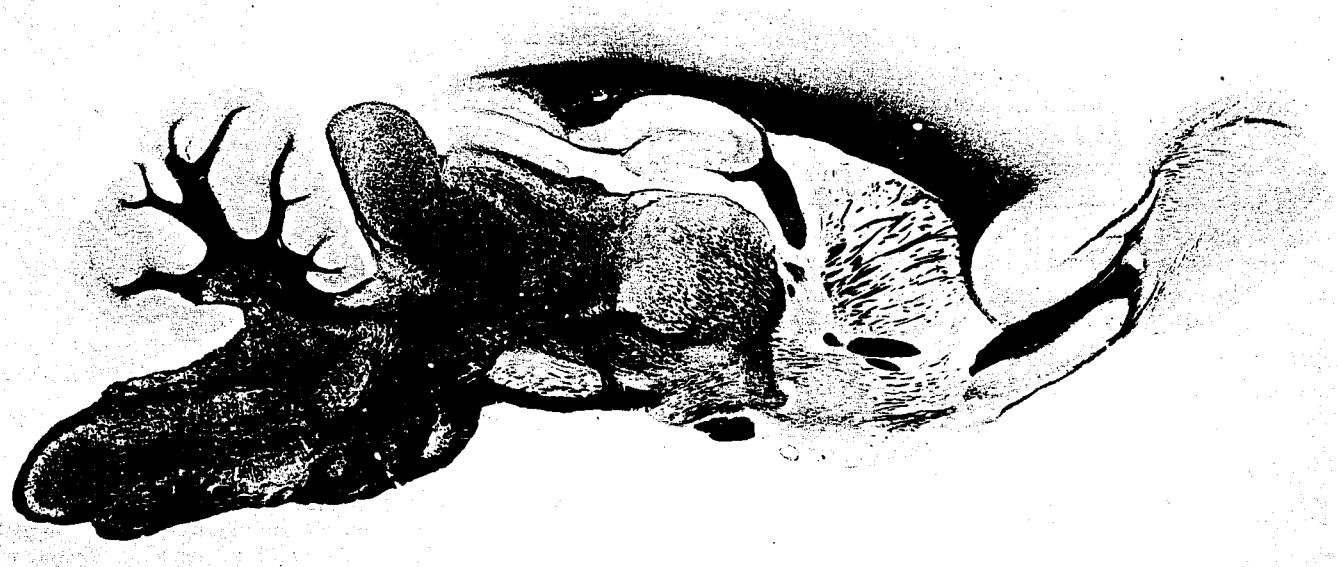
mass. Part of it serves the somatic sensory modality, specifically exteroception, comprising touch, pain, and temperature signals from the body surface, and proprioception, or perception of the body itself, comprising signals from muscles, tendons, and joint capsules and ligaments. Other thalamic nuclei serve other senses. The medial geniculate body (geniculate comes from *genu,* meaning knee) is a processing station for auditory sensation, whereas the lateral geniculate body is specialized for the processing of visual information. Figure 22 shows a further thalamic cell mass known as the nucleus lateralis posterior. This one resists any facile description; it cannot be characterized as solely a processing station for incoming sensory data. For now the best thing to say is that its position in the circuitry of the brain places it deep in the great intermediate net. The same must be acknowledged of many thalamic nuclei not shown in the illustration. In fact, the thalamic nuclei that are "merely" interposed in ascending sensory conduction paths make up only an eighth of the human thalamus.

The ventral district of the diencephalon is the hypothalamus, a part of the brain whose activity is expressed in the neural regulation of the viscera and of the endocrine glands. Like the thalamus it comprises a number of nuclei. One would estimate, however, that in the human brain its volume is no more than a tenth that of the thalamus. In the rostral direction the hypothalamus is continuous with the septum, a more or less triangular sheet of brain tissue best classified, despite its deceptive position, as a part of the diencephalon. In the ventral direction the hypothalamus becomes a narrow stalk. The stalk marks the alliance between the hypothalamus and the hypophysis, or pituitary complex. The stalk is thus a symbol of a way in which the hypothalamus governs the viscera. The stalk ends as the appendage called the posterior lobe of the pituitary. Sometimes called the neurohypophysis, it is a true part of the brain. It releases two hormones. The rest of the complex, namely the anterior lobe, or adenohypophysis (the prefix *adeno-* referring to gland), is not a part of the brain: it is an epithelial organ that develops from the roof of the embryonic oral cavity and applies itself closely to the posterior lobe, like a barnacle that clings to a piling. It releases several hormones. Regarding the name pituitary: it derives from a misconception on the part of the ancient anatomists who discovered the structure in unembalmed cadavers. The complex had decomposed, becoming a blob of mushy matter. Since it sat just above the roof of a side chamber to the nasal cavity, it was taken to be the master gland of mucus secretion. Thus it was called the *glandula pituitaria,* the mucus-producing gland; the Latin for mucus is *pitus.* The name survives in spite of the misconception.

Cerebral Hemisphere

The remaining subdivision of the forebrain is the telencephalon, or cerebrum, or cerebral hemisphere, or endbrain. In the brain of a mammal it is by far the largest part, and in many mammalian species its outer shell, the





cerebral mantle or cerebral cortex (also called the pallium), is heavily furrowed into convolutions called gyri and fissures called sulci. The degree of furrowing is not indicative of a creature's phylogenetic status. Instead it appears to depend solely on the size of the species. Many small New World monkeys—for example the marmoset, and also the squirrel monkey—have brains whose surface is almost completely smooth. The sheep, the cow, and the horse, on the other hand, all boast respectable convolutions. So does man. Thus the human brain accommodates a cerebral cortex whose surface area is about 1.5 square feet. Every large mammal has a highly convoluted cortex; the whale's convolutions are perhaps the most extreme.

A matter of definition: The name cortex is bestowed on any neural structure that combines the following attributes. First, it is a sheet of gray matter*

*The term gray matter is best defined by reference to white matter, which is its opposite. White matter denotes districts of the central nervous system consisting of myriad axons. For the most part the axons are sheathed in myelin, a glistening, fatty substance highly

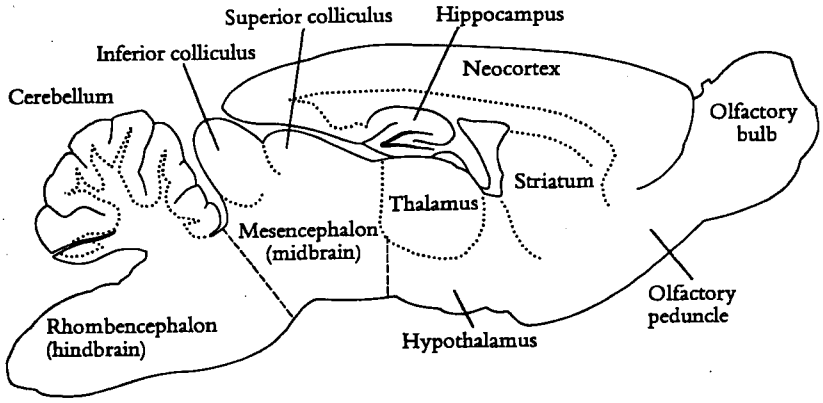


Figure 23: **Brain of a rat** looks much like the schematic mammalian brain displayed in Figure 22. Two adjoining slices are shown; they were stained by complementary techniques. In the Nissl-stained slice (*top*) each dot is a neuronal cell body; the dark-staining regions and bands are dense neuronal packings. The preparation establishes that the brainstem (the hindbrain and the midbrain) includes distinct neuronal communities, or nuclei. So does the diencephalon: the thalamus and hypothalamus. In contrast, the cerebral cortex and the cerebellar cortex have a layered cell architecture. In the Loyez-stained slice (*bottom*) only myelin is marked: the Loyez technique stains the fatty sheathing of axons. Accordingly, the dark-staining regions (including all of the brainstem) are regions dense in axons. The very darkest regions are axon bundles: the great communication channels of the central nervous system. For example, the dark band under the neocortex is in essence a neocortical cable basement. A number of smaller axon bundles traversing the corpus striatum validate the name of the structure: corpus striatum means "striped body." The map (*above*) identifies the anatomical structures visible in the stained slices.

at the surface of the brain; it is indeed the brain's rind or bark—the literal meaning of the term. Second, the neuronal cell bodies in a cortex are arranged in layers, so that under the microscope cells of one size and shape seem to occupy a common depth in the structure, and at different depths one finds different populations of cells. In a word, a cortex is laminated. Third, the outermost lamina contains axons and dendrites but few neuronal cell bodies. This zone is called the molecular or plexiform layer. Finally, and perhaps most characteristic, there is a tendency for the neurons in a cortex to have among their dendrites a long one that rises perpendicularly through the cortical laminations and into the plexiform layer, in which it ramifies. Such processes are called apical dendrites. In stained sections of the tissue they produce a palisadic appearance: a clear polarization of the dendritic field in a direction perpendicular to the surface of the brain. Only two loci wholly satisfy these conditions: the mantle of the cerebral hemisphere and the mantle of the cerebellum.

The mammalian cerebral cortex can be divided into a number of districts. It is convenient to begin at the base of the endbrain, where a structure juts forward composed entirely of cortex, though of varying cytoarchitecture. Its foremost, swollen end is the olfactory bulb; its shank is the olfactory stalk or olfactory peduncle. Only the portion under the rest of the cerebral hemisphere is the olfactory cortex proper. All three are notable for their primitive architecture: no more than three layers can be distinguished, including the plexiform layer, whereas in more advanced cerebral cortex one can distinguish six. The olfactory cortex is a common denominator in the brains of all vertebrates; it is often called paleocortex, Greek for "the old cortex." It forms the larger part of the cerebral cortex in a fish, whereas in reptiles and birds one finds a further field of cortex, also primitive in structure and appreciable in extent but more dorsal in position. Its function has proved embarrassingly difficult to determine. For that reason, it has tactfully been called general cortex.

A second portion of the mammalian cerebral cortex is of vast extent and structural complexity: in man and the other primates, it is estimated to contain no fewer than 70 percent of all the neurons in the central nervous system. This is the neocortex (Figure 24). It is the latest form of cortex to appear in evolution. We owe it to a branching: beyond the reptiles, one strain of animals elaborated on the reptilian pattern and became the birds, while another, more venturesome strain developed the neocortex as it became the mammals. From a strictly phylogenetic point of view, birds are thus the

reflective of light. Thus it is myelin that confers on white matter its eponymous white appearance; gray matter looks rather darker. Gray matter denotes districts of the central nervous system rich in neuronal cell bodies but poor in myelin-covered axons. The cell bodies are embedded in neuropil: a dense feltwork made up mostly of incoming axons and their terminal ramifications, along with the dendrites the local cells emit to meet the axons. Neither dendrites nor the thin end stretches of axonal ramifications are invested by myelin.

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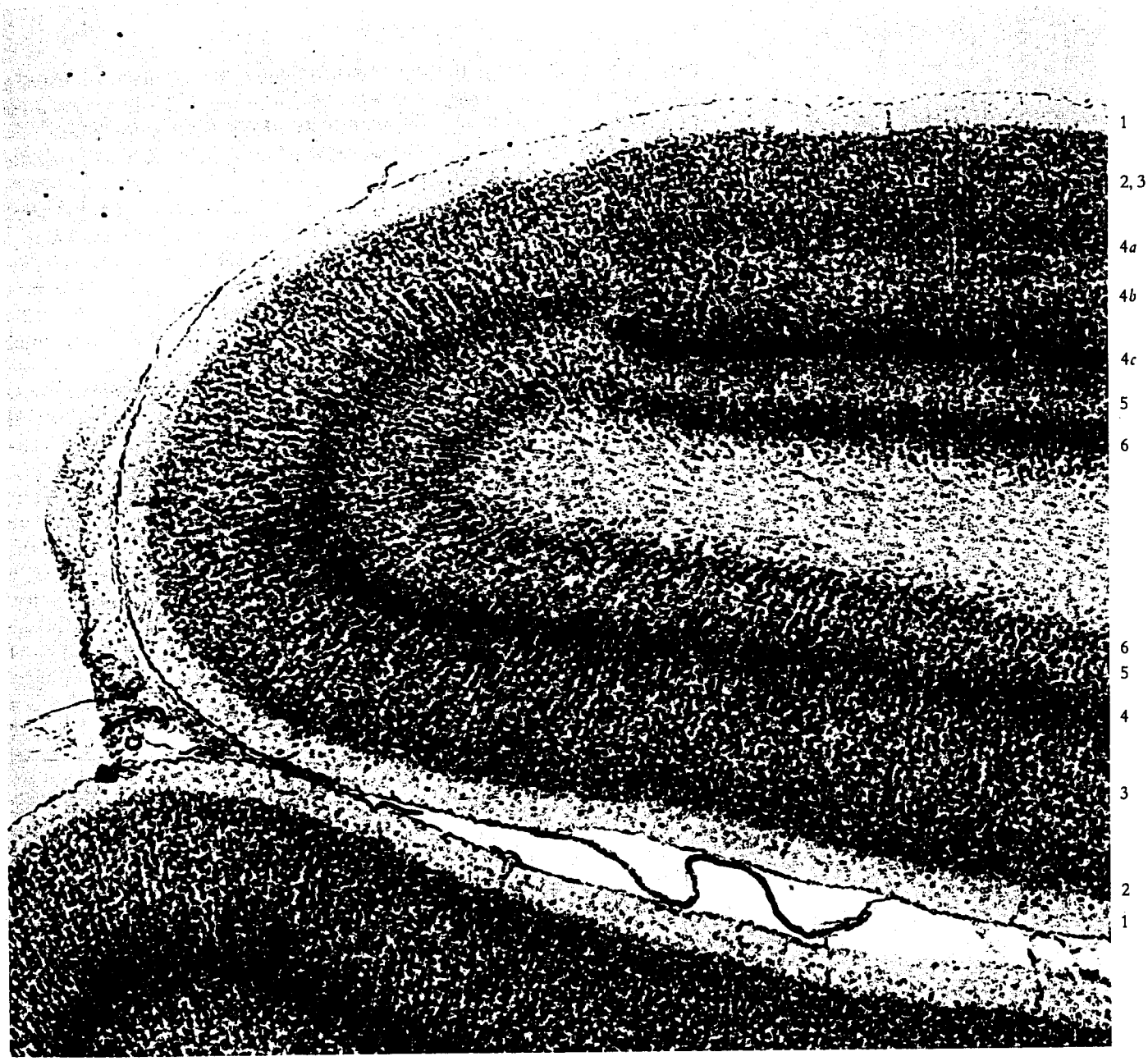

Figure 24: **Neocortex,** shown in a Nissl preparation from the brain of a macaque monkey, demonstrates three of the criteria distinguishing cortex from other neuronal organizations. The cortical tissue is at the surface of the brain; its neuronal cell bodies are organized into layers; and the outermost layer, called the plexiform layer, consists of closely packed dendrites and axons, with neuronal cell bodies more or less absent. In neocortex the plexiform layer is designated layer 1. The field of view includes a gyrus, or cerebral convolution. Most of the upper turn of the gyrus is primary visual cortex, the part of the neocortex where visual data arrive. Within its layered pattern three strata are dense in cell bodies. They are layer 6, sublayer 4*c*, and sublayer 4*a*. At the border of the primary visual cortex the sublayers abruptly coalesce, and layer 4 becomes unitary. The micrograph was made by David H. Hubel and his colleagues at the Harvard Medical School; the primary visual cortex of a human brain is shown in the micrographs in Figure 112.

logical end of the brain's traditional development. In contrast, mammals are deviants: no birds can be found in their ancestry. In one of the many radiations of mammalian evolution the primates appeared, an order in which the neocortex reaches its maximal development. We human beings are heir to all the consequences, perhaps including psychiatry.

A final district of the mammalian cerebral cortex is found at its medial edge, where the cortical sheet rolls inward and folds on itself to form a composite gyrus whose cross section is reminiscent of a rococo ornament. This remarkable structure is called the hippocampus — the sea horse — and in the human brain, it so strikingly resembles a sea horse, at least in the shape of its dorsal surface, that no other name seems conceivable. Hippocampal cortex is unique in that its neuronal cell bodies occupy only a single layer. (In olfactory cortex they occupy two layers; in neocortex they occupy five.) It is therefore called archicortex ("primitive cortex"). That name, however, is problematic. It suggests a phylogenetic sequence in which the hippocampus (and the olfactory cortex) arose before the neocortex. Yet the hippocampus cannot be placed in a scheme of evolutionary primacy; it is simply a characteristic feature of the edge of the cortical mantle. Specifically, the approach of the neocortex to its medial free edge is marked by a stepwise reduction in the number of laminations until a single cell-body layer remains. It should be said that the free edge of the cerebral mantle in reptiles and birds — that is to say, the free edge of the general cortex — also has a cytoarchitecture of only one cell-body layer. Hence the district is sometimes taken to be the homolog of the mammalian hippocampus. The extent of the homology remains a subject of debate. A true correspondence would require a similarity of function or a similarity of connections with other parts of the brain. The folding of the free edge of the cerebral mantle into a complex convolution is, in any case, peculiar to mammals.

In the depths of the mammalian cerebral hemisphere are further neuronal assemblages. One of them, the amygdaloid body, or amygdala (Greek for almond), is gray matter in which anatomists distinguish several nuclei. It lies immediately under the olfactory cortex. In fact, one of its nuclei (the cortical nucleus of the amygdala) fuses into the overlying olfactory cortex. The amygdala and the hippocampus are the main components of what is called the limbic system. It seems an odd alliance. For one thing, the hippocampus is cortex; the amygdala is not (except for the aforementioned cortical nucleus). Still, the hippocampus and the amygdala are allied (to give the most straightforward reason) by virtue of their placement in the circuitry of the brain. They stand out in the cerebral hemisphere because their axons descend most massively to the hypothalamus.

A final assemblage of neurons deep in the mammalian cerebral hemisphere is larger than the amygdala; indeed, it can impress one as being the solid core of the cerebrum. It is the corpus striatum: the striped or striated body. Clinical evidence reveals it is of crucial importance in the programming of complex bodily movements. In man, for example, extensive destruction of tissue in the corpus striatum may bring on motor automatisms. That is, it brings on com-

plex movements, far more than mere tremors or muscle twitches, that begin without the patient's volition and are beyond his will to stop. The complexity of the movements may make them resemble a purposeful act: kicking or seizing, for instance. The name corpus striatum was coined several hundred years ago, when anatomists noted that the depth of the cerebral hemisphere is occupied by a large, gray mass crossed by slender, transverse white stripes. The stripes are now recognized to be bundles of myelinated axons. The corpus striatum is nonetheless composed of two great districts that are histologically distinct. One of them is a relatively large-celled inner zone called the pallidum or globus pallidus: the pale globe. The other one, darker looking, especially in a fresh (that is, an untreated) brain, is an outer zone whose cells are smaller and more densely packed. It is known as the striatum. In many mammalian species, including man, a plate of axons called the internal capsule cleaves the striatum into two anatomical divisions: the caudate nucleus and the putamen. "Caudate" refers to the trailing end of the cell mass: *cauda* is Latin for tail. "Putamen" is Latin for husk; in botanical usage it refers to structures such as cherry stones.

Axon Tracing

The gross-anatomical distinctions made in the preceding chapter are helpful. Even so, they leave unanswered the first thing one asks about the organization of the central nervous system. What are the major pathways by which it conducts information?

Let us start with a more or less circumscribed part of the nervous system: a nucleus or a field of cortex. Fundamentally, it includes two classes of neurons. The neurons that make up one class each have a long axon with which they send signals out of the region: in neuroanatomical usage, they "project" to other regions, namely other nuclei or other fields of cortex. They are called projection neurons, or principal neurons, or (in honor of Camillo Golgi) Golgi type I cells. The neurons of the other class each have a shorter axon (or no axon, but only dendrites) and confine their connections to cells in their vicinity. They process information locally. They are called intrinsic neurons, or local-circuit neurons, or Golgi type II cells.

Let a thalamic "sensory relay nucleus" (the ventral nucleus, the medial geniculate body, or the lateral geniculate body) serve as a source of specifics. In a thalamic sensory relay nucleus, intrinsic neurons are relatively few: they amount to no more than a quarter the number of the projection neurons there. That proportion is quite unusual. In the brain overall, and especially in the brain of a primate, intrinsic neurons outnumber projection neurons by a ratio of at least three to one. (In the striatum they outnumber projection neurons by almost 20 to one.) The amount of data processing in a thalamic sensory relay nucleus must therefore be relatively modest. The transformation the nucleus performs on its incoming sensory data is nonetheless profound. Much of the

synaptic contact inside the nucleus is in synaptic glomeruli: juxtapositions of numerous axon and dendrite terminals insulated by a glial capsule about 10 micrometers in diameter, which is formed by astrocyte membranes. The analogy to an electrician's junction box is irresistible. Such capsules are found elsewhere in the brain: notably the cerebellum. Within each thalamic glomerulus, an axon that enters the thalamus bearing sensory data passes signals synaptically to the dendrites of projection neurons. In turn, the projection neurons export data from the thalamus: they project to the neocortex. The sequence seems simple enough. The simplicity, however, is disrupted by a number of complications. Inside the glomerulus the arriving sensory axon signals not only projection neurons but also the dendrites of intrinsic neurons. In turn, those dendrites make dendrodendritic synapses with the projection neurons' dendrites. Outside the glomeruli the intrinsic neurons contact projection neurons in the more orthodox fashion of axon signaling dendrite. One imagines the intrinsic neurons help to determine which synapses interrupting sensory conduction lines will be open to impulse traffic and which will temporarily be closed.

The patterns of connection among inputs, intrinsic neurons, and projection neurons are extremely varied from place to place in the central nervous system (Figure 25). Still, it remains a fact that certain axons enter a nucleus or a field of cortex, often in circumscribed bundles, and that other axons leave it, often in circumscribed bundles. These axons establish the major conduction paths in the brain and the spinal cord. Tracing them is one task of this book. In practice the tracing begins with two questions. Given a part of the brain or the spinal cord, say a nucleus or a field of cortex, where do its axons go? The answer requires that the axons of its projection neurons be traced forward—that is, in the direction in which information is conducted along the axons toward their terminals. In brief, it requires anterograde (or orthograde) axon tracing. Conversely, given a part of the brain or the spinal cord, where do the axons that reach it begin? The answer requires that the axons synapsing on its neurons be traced backward to the projection neurons from which they arise. In brief, it requires retrograde tracing. A number of methods have been developed in efforts to meet each need. In general, the earliest methods, devised a century ago, were serendipitous discoveries. They were superseded a decade or two ago by methods capitalizing on the finding that an axon transports substances not only forward to its terminals but also backward to its parent cell body. The newer methods are now being superseded by still newer ones devised to capitalize on recent discoveries in molecular biology.

Retrograde Tracing

Consider the methods of retrograde tracing. The earliest way to identify the neurons sending their axons to a given neural structure was to destroy the structure. The axon terminals in the structure would also be destroyed, and throughout the nervous system the neuronal cell bodies emitting those axons

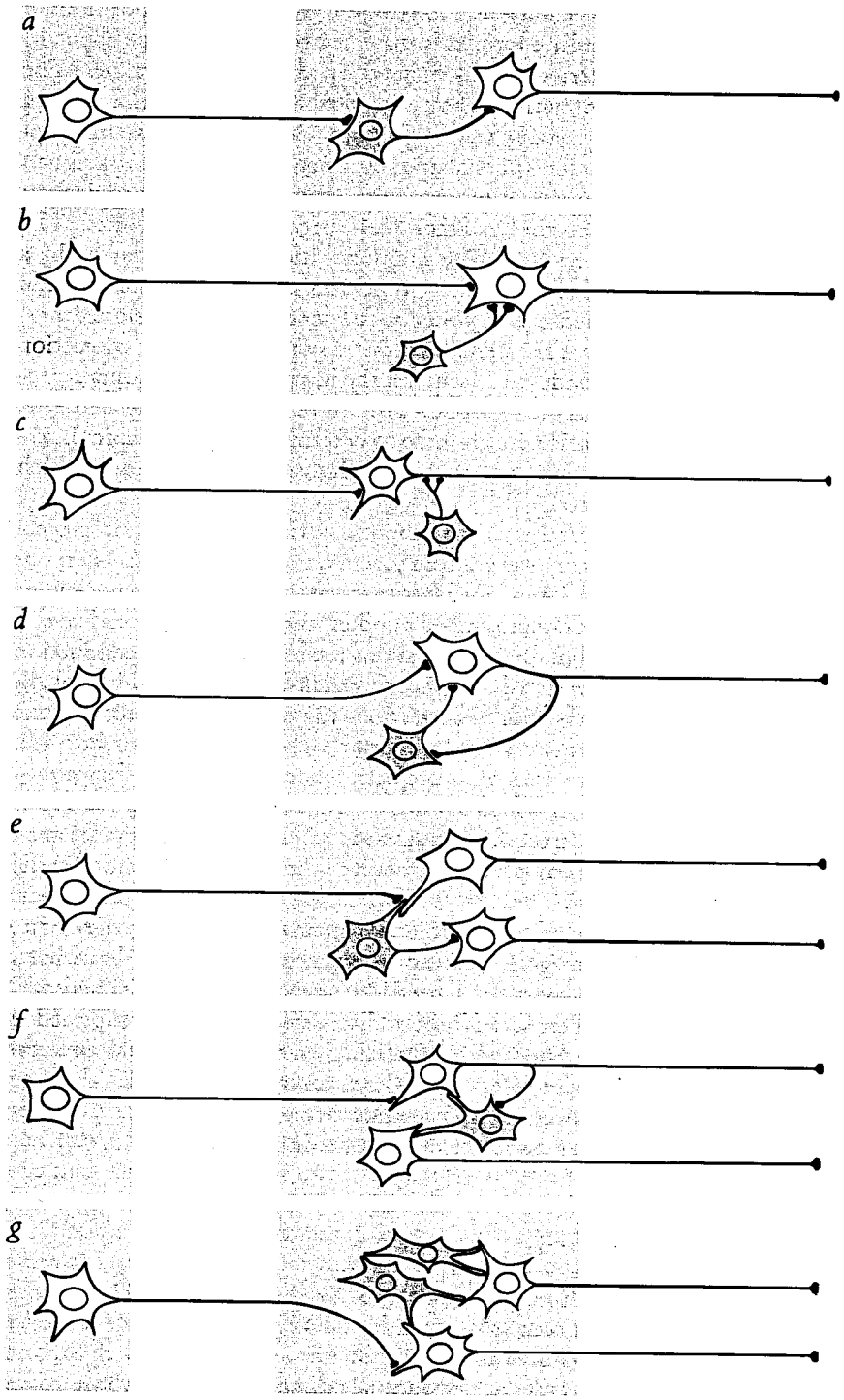

Figure 25: **Local circuits** among neurons in the central nervous system vary from place to place. This illustration gives some examples. In each a projection neuron exports signals from a nucleus at the left; it might equally well dispatch signals from cortex. Toward the center the projection-cell axon makes synaptic connections with cells in a target nucleus (or a cortical field). In *a* the axon contacts a local-circuit neuron (*color*): a nerve cell whose axon and dendrites ramify locally. In turn the local-circuit neuron contacts a projection neuron. In *b*, *c*, and *d* the local-circuit neuron receives no long-distance signals; it is a satellite of projection cells nearby. (In *d* it closes a local feedback loop.) In *e*, *f*, and *g* the local-circuit neurons act as bridges between projection neurons; they span what might be termed throughlines. (In *f* and *g* they lack an axon and transmit signals dendrodendritically.) Synaptic complexes in the brain and spinal cord can be far more complex than the ones diagrammed. The illustration was devised by Pasko Rakic of the Yale University School of Medicine.

would undergo what is called the retrograde cell reaction. Such cells take on a typical appearance, which often can be recognized when the nervous system is sectioned and examined under a microscope two to three weeks after the lesion is made. In each such cell, the nucleus swells and becomes displaced from the center of the cell body to a position near the cell membrane. Meanwhile, most of the cell's Nissl substance disappears. (The process is called chromatolysis.) The most peripheral Nissl bodies—the ones just under the surface of the cell—persist the longest. Accordingly, the cell body becomes extraordinarily pale, but it keeps a dark edging.

The technique of destroying neural tissue and searching the rest of the nervous system for chromatolytic cell bodies was introduced in the 1880's. A different and far more effective technique appeared some 90 years later. Krister Kristensson, a Swedish neuropathologist, was studying the mechanism by which motor neurons are paralyzed by tetanus toxin. He had found that toxin labeled with radioactive iodine was absorbed by the axon endings in muscle tissue and transported in a direction opposite to the direction of axoplasmic flow. That is to say, it was transported up the axons and into the parent cell bodies, where he could demonstrate its presence by the ability of the radioactivity to fog the photographic emulsion with which he coated slices of neural tissue. Such transport is not astonishing. When a nerve in the periphery of the body is cut, the motor neurons that supply the motor contingent of the nerve show the retrograde cell reaction. They are trying to regrow their axons. In many cases they succeed, because in the peripheral nervous system the myelin that invests axons is the membrane of what are called Schwann cells, and Schwann cells, unlike oligodendroglia, maintain orderly positions after the death of the axons they envelop, providing tunnels through which regrowing axons can find their way. During the months that pass while the denervated muscle group is totally paralyzed (and therefore flaccid), its governing motor neurons remain in a state of chromatolysis. Then comes the first reinnervation of the muscle, and in a short time all signs of the retrograde cell reaction disappear. How does the cell body of a motor neuron in the central nervous system learn that its axon has reestablished motor end plates on the muscle? It must receive a signal: at the reestablished end plates the axon endings must take up a substance produced by the muscle—even an extremely atrophied muscle—and this substance must somehow send sign of its presence up the axon.

Kristensson found that bovine serum albumin, a large protein, was likewise absorbed by axon terminals and transported up the axon. But his most important success with retrograde axon transport involved horseradish peroxidase. HRP is one of a class of peroxidase enzymes found in various plants, and not in horseradish alone; it might equally well have been purified from the potato. It frees an oxygen atom from hydrogen peroxide, reducing the latter to water. Its ability to cross neural barriers had previously been exploited—to study gap junctions, for instance. Now it became the basis of a retrograde tracing technique (Figure 26). The technique begins with the injection of HRP into a given structure in the nervous system. The HRP is absorbed by

the axon terminals there; then it travels up the axons toward the cell bodies that emit them. The HRP travels at a rate of 200 to 300 millimeters per day; hence the animal is sacrificed one or two days after the injection. The animal's brain is perfused with substances such as formaldehyde, glutaraldehyde, or both. The perfusate coagulates proteins, and so it fixes the tissue: it gives the tissue rigidity. The brain can thus be sectioned, typically into slices no more than 50 micrometers (a twentieth of a millimeter) thick. At such a thickness the brain of the rat, a common experimental animal, becomes a few hundred slices. Each of the slices is exposed to diaminobenzidine or, in more recent

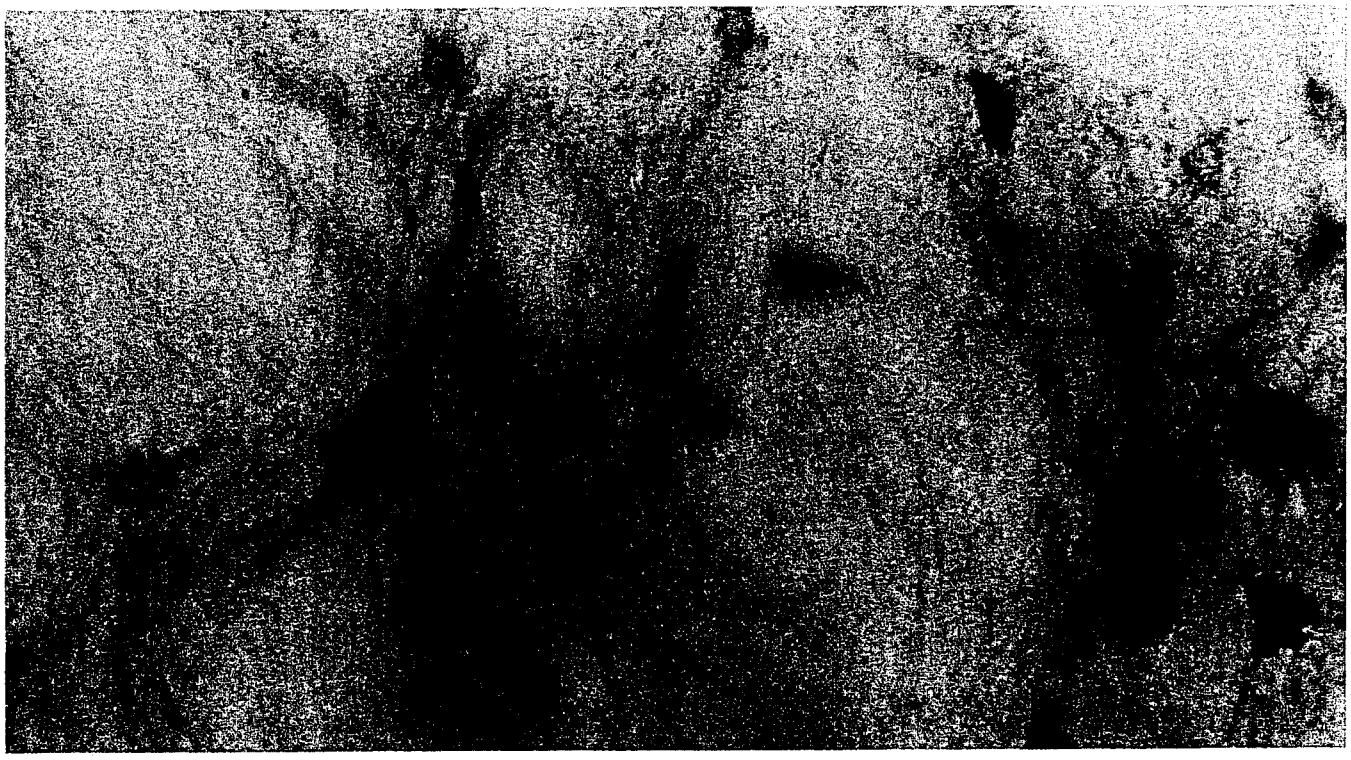

Figure 26: **Retrograde axon tracing** follows axons backward (that is, against the direction of signal conduction) from their terminals to the neuronal cell bodies from which the axons emerge. In this example of retrograde tracing the enzyme horseradish peroxidase (HRP) was injected in minute quantity (.1 microliter of a 10 percent solution) into the subthalamic nucleus of a living rat. The enzyme was absorbed by the axon terminals there and got transported up the axons toward their parent cell bodies. (The two-way transport of molecules is a property of axons.) The micrograph shows the globus pallidus. The pallidal neurons marked as origins of the pallidosubthalamic projection appear as cell bodies of medium size (from 20 to 25 micrometers in shortest diameter) containing a dark, granular pigment well into the dendrites of the cells. The pigment results from the oxidation of tetramethylbenzidine by oxygen liberated from hydrogen peroxide through the intervention of the HRP. To the left a larger neuron shows up in even tones of gray. It was rendered visible by subjecting the tissue to a second histochemical procedure, designed to demonstrate acetylcholinesterase, the enzyme that deactivates the neurotransmitter acetylcholine. The larger neuron is unlabeled by HRP. The tentative conclusion from several such experiments is that pallidal cholinergic neurons do not contribute to the pallidosubthalamic projection.

efforts, to tetramethylbenzidine. Then hydrogen peroxide is added. The hydrogen peroxide is reduced, the liberated oxygen combines with the benzidine compound to make a pigment, and by this relatively simple histochemical reaction the neuronal cell bodies containing the transported HRP stand out by dint of the colored stippling inside them.

Anterograde Tracing

How can axons be followed in the opposite direction—forward to their terminals instead of backward to their parent cell bodies? Again the early investigators destroyed parts of the living nervous system. In some experiments they destroyed cell bodies; in others they cut through a bundle of axons. Either way, the experimental intervention severed axons from the cell bodies that emitted them. The severed lengths of the axons would soon begin to disintegrate (the phenomenon is called anterograde or Wallerian degeneration, after the English physiologist Augustus Waller, who discovered it in the middle of the 19th century), and if the axons were myelinated, the myelin would disintegrate as well. This latter disintegration works a crucial change in the myelin's composition. Normal myelin incorporates quantities of unsaturated fatty acids. Such acids are capable of reducing osmium tetroxide to black metallic osmium. If normal neural tissue is placed, therefore, in a solution of osmium tetroxide, the myelin stains black. Suppose the tissue includes myelin sheaths that are disintegrating because an investigator has made a lesion somewhere in the nervous system. In addition, suppose the tissue is first placed in a solution of potassium dichromate, a powerful oxidant. It stays there for three weeks, and only then is it exposed to osmium tetroxide. Under these circumstances no normal myelin stains. The unsaturated fatty acids have all accepted oxygen from the potassium dichromate, and none remain to take it from the osmium tetroxide. The disintegrating myelin does, however, stain. Even after three weeks in potassium dichromate, it retains its ability to reduce osmium tetroxide to black metallic osmium. In all likelihood, its content of unsaturated fatty acids increases as it breaks down. Since the disintegrating sheaths are alone in turning black, they can be recognized and traced. The method was developed by the Italian physician Vittorio Marchi three decades after Waller's discovery.

Still later it became possible to mark degenerating axons instead of just their myelin sheaths. That represented an advance in the anterograde tracing of neural communication lines, because axons thinner than about half a micrometer could now be stained, and so could axon terminals, although both such structures lack myelin. The advanced techniques, which impregnate axons with silver, were developed in stages, beginning at the turn of this century when Cajal placed blocks—not sections—of fixed neural tissue in a bath of silver nitrate. After several days, he would transfer the blocks to a strong reducing agent: hydroquinone, for example. Nascent metallic silver would form in the tissue, and for an unknown reason it would selectively

aggregate in axons. In the same year — 1901 — a second technique serving the same purpose was introduced by the German neuropathologist Max Bielschowsky. In his technique the tissue would be fixed and sectioned, and the sections would then be soaked in a solution of silver nitrate for a length of time varying between one hour and several days. The sections would next be transferred to a solution of the double salt silver-ammonia nitrate. It is an extremely labile substance. If it stands overnight, nascent silver will slowly precipitate out of solution. Mirrors used to be made by that method. A rather weak reducing agent such as formaldehyde can thus be substituted for the strong reducing agent that would otherwise be required. The silver techniques both of Cajal and of Bielschowsky impregnate normal axons; in this form, therefore, the deposition of silver has only limited utility as a tracing method. To make the Bielschowsky method selective for degenerating axons, an intermediate step is required; it was introduced in the 1940's. Immediately before the first of the two silver baths, the sections are placed in a sequence of mordants — tanning agents, in a rough analogy. The tanning selectively renders degenerating axons receptive to silver.

In the anterograde techniques the direction of the tracing is the direction of forward (or anterograde) axoplasmic transport; thus a technique might well begin by introducing a traceable substance into the cell bodies under investigation. Such a technique, called autoradiographic tracing, came into prominence in the 1970's; it employs amino acids labeled with tritium, the radioactive isotope of hydrogen (Figure 27). Tritiated amino acids are injected, then, into the neural structure whose outgoing connections are to be determined. The cells composing the structure absorb the amino acids from the extracellular medium. Often a cell's appetite for one amino acid is greater than its

Figure 27: **Anterograde axon tracing** follows axons forward, in the direction of signal conduction, from their parent cell bodies to the terminals at which the axons send signals to other neurons. In this example the tracing was autoradiographic. The amino acid leucine was labeled with tritium (radioactive hydrogen) and injected into the internal segment of the globus pallidus of a cat. The leucine was absorbed by neuronal cell bodies there, which made it part of peptides and proteins and dispatched it down their axons. The axons emerge from the internal pallidal segment in a tract called the ansa lenticularis. Four days after the injection the brain was sectioned and the slices were coated with photographic emulsion. Then came six weeks in storage at −20°C. During that time the labeled axons in each slice recorded their position by releasing beta particles (energetic electrons), which reduce the silver halides in a photographic emulsion to grains of metallic silver, much as photic energy does in ordinary photography. The illustration, a dark-field micrograph, shows a section through the caudal thalamus; silver grains show up in white. The wide crescent of grains at center right marks the centrum medianum nucleus, a thalamic destination for the ansa lenticularis. The narrow black oval invading the nucleus from the right is a blood vessel in cross section; it extends some three-fourths of a millimeter into the nucleus. A denser, compact cloud of grains at top left marks the lateral habenular nucleus, a destination unexpected at the outset of the experiment, in 1974. The diffuse cloud toward the lower right is the ansa lenticularis itself, nearing the thalamus from below in what is called field H-1 of Forel. The experiment was done by Haring J. W. Nauta at Case Western Reserve University.

appetite for another. Still, certain amino acids are coveted: an infusion of tritiated leucine or proline almost always is effective. Within hours the neurons at the site of the injection are incorporating the labeled amino acids into the proteins they are making, and many of these proteins are transported down the axon. Some move one or two millimeters per day in the slow form of axoplasmic transport. Others move rapidly: on the order of 400 millimeters per day.

The subsequent treatment of the tissue begins with the fixing and sectioning of the brain. The sections are mounted on glass slides and dried; then, in a darkroom, they are dipped in photographic emulsion, dried again, and placed in light-tight boxes to be stored in a freezer for as long as 12 weeks. During that period, some of the atomic nuclei of the tritium atoms break down. The process is accompanied by the release of free electrons, or beta particles. The ones that fly out of the surface of each section are recorded in the overlying emulsion as grains of black metallic silver. Later, when the emulsion has been developed (in the photographic sense), the grains can be charted under the microscope. An alignment of grains suggests the presence of an axon through which radioactive molecules were passing when the animal was sacrificed; the fixation of the tissue coagulated all the protein in the axon and elsewhere and thereby locked the labels in place. A cloud of grains suggests a terminal arborization. A study of the patterns of grains from slide to slide permits the distribution of the axons arising from the cells at the injection site to be determined quite precisely.

New "Stains"

A crucial point about the axon-tracing techniques we have been describing is that they are all experimental. That is, they require an experiment: the choice of a region whose connections are to be studied, and then the making of a lesion or the injection of a traceable substance at the chosen site in a living animal. In brief, they require a local intervention in the brain. The newest techniques are often quite different. Many of them are complex, yet in a way they are throwbacks: like the Nissl technique, which marks nucleic acids, they reveal the distribution of something inherent in the brain. Thus they require no local intervention. In essence the newest techniques are stains. It is fair to say they derive from the hope that the neurons employing a particular neurotransmitter might be made to show themselves throughout the nervous system.*

*That hope has mostly been thwarted, in that the only simple way to mark a neurotransmitter is the histofluorescence technique. Devised in the 1960's by Bengt Falck of the University of Lund and Nils-Åke Hillarp of the Karolinska Institute, it demonstrates the presence of the monoamine neurotransmitters serotonin, dopamine, norepinephrine, and epinephrine in cell bodies, axons, and axon terminals at the surface of thinly sectioned neural tis-

At bottom the newest techniques exploit the nature of proteins. Notably, proteins are antigenic: if they invade the body of a vertebrate, they are met by defensive measures that constitute what is called the immune response. In one aspect of the response, plasma cells — the offspring of *B* lymphocytes, a type of white blood cell — secrete antibodies: molecules (specifically immunoglobulins) primed to bind to the invader. Each plasma cell secretes a particular antibody. The idea arises that antibodies could be primed to bind to a protein of interest to an investigator, and by that binding could mark its distribution in neural tissue. Thus a "staining" method develops. It is immunohistochemical (Figure 28). First a protein is purified from the brain of one species, say a goat. The protein can be a suspected neurotransmitter that has proved to be a peptide. The endorphin neuropeptides are prominent examples. It can be an enzyme that participates in the synthesis of a neurotransmitter — say choline acetyltransferase, which makes acetylcholine. It can be an enzyme that breaks down a neurotransmitter — say acetylcholinesterase. It can be an enzyme with a wholly different function. It can be a structural protein. Tubulin, a protein in microtubules, is an example. It can be a membrane protein: a receptor or part of an ion-conductance channel. In principle there is no difference; each is a chain of amino acids, and so is potentially antigenic. The protein is injected into the blood of a different species, say a rabbit. The immune system treats it as an invader and primes antibodies against it. The antibodies are collected. Then each antibody is given a label such as radioactive atoms or a fluorescent chemical group. Finally the antibodies are applied to sections of the brain of any species. There the antibodies bind to the antigenic protein intrinsic to the tissue.

Two problems can interfere with the project. In the first place, the immune response is diverse: it primes antibodies against different molecules on the surface of an invader (say the protein capsule of a virus), or even different parts of a molecule. This means the defenses of the body do not characteristically produce a pure strain of antibody, even in response to a single type of invader. In addition a given antibody can bind to different molecules, provided they share the sequence of amino acids against which the antibody is primed. (This broadening of the response is termed cross-reactivity.) In 1975 the problem was overcome, at least in part, when mouse lymphocytes were successfully fused with mouse myeloma (bone-marrow cancer) cells. The resulting hybrid cells — they are known as hybridomas — prove to have hybrid properties: like plasma cells, they secrete antibodies; like cancer cells, they are immortal in cell culture. As before, an animal is exposed to a protein purified

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sue. The technique rests on the happy circumstance that an indoleamine such as serotonin or a catecholamine such as dopamine, norepinephrine, or epinephrine combines with formaldehyde to yield a compound that fluoresces under ultraviolet light. Sections of the tissue are exposed to formaldehyde vapor. Serotonin fluoresces yellow; dopamine, norepinephrine, and epinephrine fluoresce green.

Figure 28: **Immunohistochemistry,** a new "stain" for neural tissue, exploits the immune response of vertebrate animals; in particular it uses antibodies. In its newest version the antibodies are monoclonal and monospecific: they are produced by a clone (the identical offspring of a single parent cell), and they bind to only one protein. The tissue shown is from the nucleus basalis, a part of the substantia innominata, at the base of the forebrain. It was exposed to a monoclonal antibody specific for the enzyme choline acetyltransferase, which makes the neurotransmitter acetylcholine. The antibody had been linked to a peroxidase, so that its binding sites in neural tissue could be marked by a pigment. Several neurons are darkly stained. Their cytoplasm includes choline acetyltransferase; evidently, then, their transmitter is acetylcholine. They are in fact the only known cholinergic neurons that project to the neocortex. The micrograph was made (with tissue from a rhesus monkey) by M. M. Mesulam and his colleagues at the Harvard Medical School.

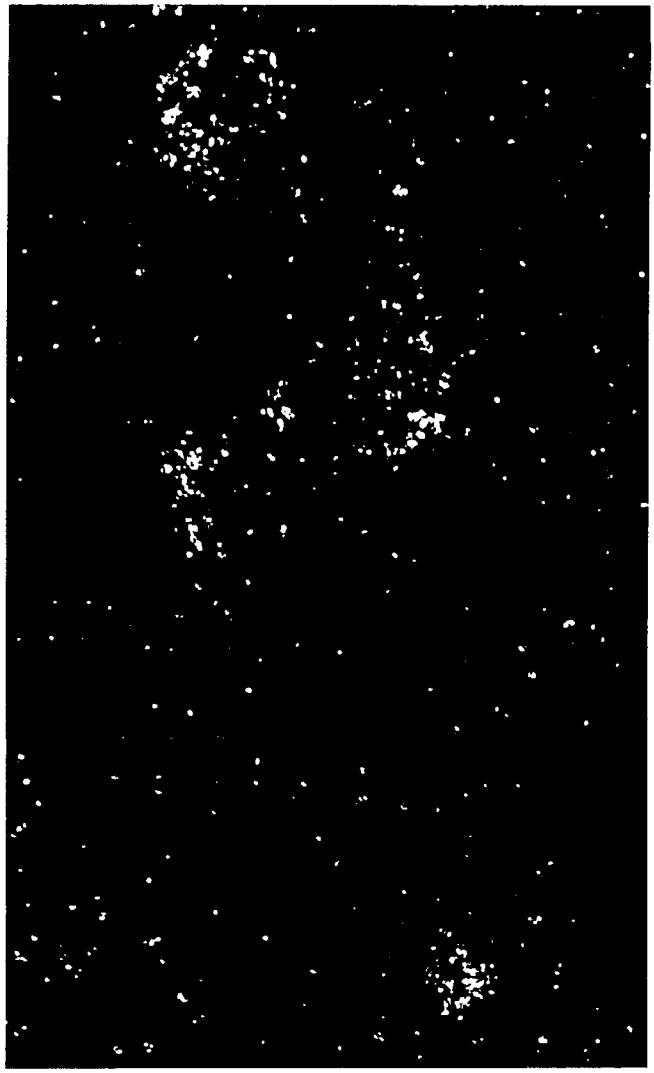

Figure 29: **In situ hybridization,** a second new "stain," exploits the technology of recombinant DNA and genetic engineering. Here the stain is employed to locate neurons that synthesize adrenocorticotropin, or ACTH. The dark-field micrograph is of tissue from the arcuate nucleus of the hypothalamus of a rat. The tissue was exposed to a DNA engineered especially for the experiment. For one thing, the DNA binds to a particular messenger RNA: the one that specifies the structure of ACTH. In addition, the DNA incorporates tritium atoms, whose radioactivity produces metallic silver grains (*white dots*) in the photographic emulsion with which the tissue was coated. Clusters of dots mark neuronal cell bodies in which the genetic instructions for the making of ACTH are being expressed. ACTH, already known to be a hormone released by the anterior lobe of the pituitary gland, is now suspected of being a neurotransmitter, too. The "staining" was done by Josiah N. Wilcox of the College of Physicians and Surgeons of Columbia University.

from a different species. Lymphocytes proliferate, each producing a particular antibody. The lymphocytes are fused with myeloma cells; the fusion makes them immortal. The hybrid cells are then allowed each to give rise to a clone: a colony of identical offspring. The entire colony is devoted to the synthesis of a pure, or monoclonal, antibody.*

The second problem with immunohistochemistry is also inherent in the technique, which demonstrates the distribution of a protein in not only the cells that make it but also the cells that simply accumulate the substance: say target cells for the protein. Here, too, a solution has emerged. It takes the form of a very new "staining" technique; as this is being written, the technique is being refined. Called in situ hybridization (Figure 29), the technique relies on the central dogma of molecular biology, which affirms that a protein is made in a cell by ribosomes following instructions coded first in a gene in the nucleus of the cell and then by a messenger RNA dispatched from the nucleus to a ribosome. (In the language of molecular biology, the genetic material, DNA, is transcribed into messenger RNA, which is translated into protein.) Accordingly, in situ hybridization marks the distribution of an mRNA: the instruction tape for the protein, found only in the cells that actually synthesize the molecule. One begins with a collection, or "library," of mRNA's extracted from cells. Each is a single-stranded nucleic acid. A sequence of manipulations produces a corresponding double-strand DNA, each manufactured in quantity by a bacterial clone. A single clone's product is chosen; then the "staining" itself can begin. First the DNA is made radioactive. Next it is boiled, so that each double strand comes apart into single strands. The single strands are applied to slices of neural tissue. One strand from each pair is "coding": its sequence of nucleotides duplicates part of the messenger under study. It is useless as a marker. The other strand is "anticoding": it is the chemical complement of the messenger, and so the two can bind, producing an in situ hybrid: one strand is mRNA, the other is radioactive DNA. The slices are dipped in photographic emulsion and prepared for autoradiography.

The sequence is complicated. Nevertheless, it is a straightforward exercise in the new technologies of recombinant DNA and genetic engineering. Indeed, one moves almost too readily from a library of proteins to a library of

*An application of immunohistochemistry now promises to supersede autoradiography as a method of anterograde axon tracing. One begins by injecting into a chosen part of the brain or spinal cord a vegetable protein known as a lectin. The one now employed is purified from kidney beans. The lectin is absorbed by cell bodies and transported down their axons. Then, at the axon terminals, it becomes a marker when it binds to an antibody that has been primed against lectin and in addition has been linked to a peroxidase. (In effect the antibody is a tug that takes peroxidase to moorings the investigator has readied in the brain.) Lectin immunohistochemistry is the most sensitive anterograde tracing method yet devised; it reveals a wealth of detail that makes it rival a Golgi preparation. For example, it routinely reveals elaborate synaptic plexuses woven by axons around their target neurons. An example of lectin immunohistochemistry is displayed as the frontispiece to Part II.

monoclonal antibodies, or from a library of messenger RNA's to a library of DNA probes. One can make preparations of labeled neurons without knowing, or even guessing, what substance one has labeled. On the other hand, browsing in a library can be instructive in unexpected ways. Investigators at the Salk Institute and the Scripps Clinic led by Floyd Bloom and by J. Gregor Sutcliffe have undertaken what they term an "unconstrained" study of the mRNA's in neurons in the brain of the rat. They estimate the library of the mRNA's throughout the animal numbers from 50,000 to 100,000. Of these a given neuron has about 1,000. The number may seem small, but then, a neuron is a highly differentiated cell, one that has followed a particular developmental path among the many offered by the organism's genetic makeup. In effect it has renounced a multitude of possible careers and has correspondingly lost the capacity to synthesize a multitude of proteins. Among the 1,000 messengers, some 200 are found in roughly equal quantities in cells of the liver and the kidney. The proteins these messengers specify are equally useful there. Another 200 are found in the liver and the kidney, but in unequal quantities. The remaining 600 are unique to the neuron. This is not to say that the capacity to synthesize a mere 600 proteins is what distinguishes the brain from other organs. Neurons differ not only from liver or kidney cells but also from one another: they have distinctive shapes, distinctive connections, distinctive membrane channels and receptors. They have distinctive messenger libraries as well. Thus the number of messengers unique to the brain may be as great as 30,000. Some specify rather long proteins. Others are quite widely allocated among neurons. Still others are so abundant in certain neurons that they enable such cells to make a protein in great quantity. That leaves roughly 800 mRNA's. Each enables privileged neurons to make a short amino acid chain — a peptide — in small quantity. The 800 peptides are candidates for hitherto unknown neurotransmitters.