CHAPTER 1

The senses

Everything we know about the world comes to us through our senses. We experience the world as we do because our organs of sight, hearing, and smell are constructed in a certain way. We could not see color unless we had more than one kind of visual pigment, perceive pitch unless the peak of the traveling wave of the basilar membrane varied with position in the cochlea, or smell different odors unless the nose contained a very large number of olfactory receptor molecules of different selectivity. No biologist would say as Plato did that "the eyes and the ears and the other senses are full of deceit" (*Phaedo*, 83A), that perception is an unreliable pathway to true knowledge (*Theaetetus*, 186C–187A). We could not as a species have survived the hurly-burly of natural selection unless our senses had been and still are fundamentally faithful reporters of the world around us.

Because of the importance of our sense organs in everyday life and the enormous pleasure we derive especially from sight and sound, humans have always been curious how sensation occurs. The ancient Greeks speculated extensively about the nature of the sense organs and were occasionally quite perceptive. Aristotle recognized the five primary senses of sight, hearing, touch, smell, and taste (*de Anima*, Book 3). Plato (*Timaeus* 45B–D) wrongly supposed that the eye emits a kind of fire akin to daylight, which meets a similar fire coming from objects in the world around us. As these fires met, their motion was thought to be communicated to the soul. Aristotle argued against this notion, though he himself gave no clear idea how he thought vision did occur (Johansen, 1997). On the other hand, he recognized the fundamental importance of moisture in olfaction (see for example *de Sensu*, V; *de Anima*, VII). Moisture must be important, Aristotle reasoned, since fish can smell. How did he know? He doesn't say, and we have to suppose that he or his students had seen fish swimming toward bait. But since the sensation comes to the fish through water rather than through the air, why didn't Aristotle say that fish *taste*? What Aristotle could not have known is that fish, in addition to taste receptors in their oral cavity, have an olfactory organ that has a structure and function very much like our nose. The importance of moisture in olfaction is now absolutely clear: even in terrestrial animals, molecules must pass through the watery mucus of the nose before they can bind to and be detected by olfactory receptor cells.

Some of the most remarkable statements about sensation made by Greek and Roman authors are to be found in the first-century BC *De Rerum Natura* of Lucretius, who based much of his poem on the teachings of the Greek Hellenistic philosopher Epicurus. Lucretius claimed that the distinctiveness of different tastes and odors lies in the shapes of the tiny "seeds" or particles given off to the air or into the mouth by objects tasted or smelled. He thought sweet-tasting substances had smooth round particles, and bitter substances had hooked or barbed particles. He also thought that for both taste and smell the shape of the particles must somehow correspond to apertures within the nose or palate, so that sweet tastes are perceived when smooth particles enter correspondingly

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DOI: 10.1093/oso/9780198835028.001.0001

smooth apertures. To account for the variety of taste and odor, he postulated a variety of apertures, some large, some small, some round, and others square or with many angles. This explanation is not too different from our present understanding that scents and many tastes are produced by molecules having different shapes and binding to receptor molecules with appropriately matched binding sites.

Early studies of the anatomy of the sense organs

Although Aristotle and other Greek men of learning certainly performed dissection on animals (Lloyd, 1975), the first systematic anatomical investigations of the human body were undertaken in Alexandria under the reign of the Ptolemies, during the first half of the third century BC (Longrigg, 1988). Herophilus of Chalcedon and Erasistratus of Ceos, taking advantage of a temporary relaxation of religious scruple, first began the dissection of human bodies, and it is to these men that we owe the discovery and first description of the sensory and motor nerves (Solmsen, 1961; Staden, 1989). Much of their work has been preserved—not in their own writings but in the books of Galen written four centuries later. Galen himself also carried out animal dissection (Duckworth et al., 2010), though perhaps not human dissection. Since he lacked even a magnifying glass, his descriptions of the structure of sense organs are rather crude. He understood that hearing is caused by air striking against the ear but seemed not to have noticed the tiny bones of the middle ear and missed altogether the role of the ear drum in transmitting vibrations into the cochlea. He named the principal parts of the eye, probably using terminology borrowed from Herophilus and Erasistratus, and these are the names we still use: sclera, choroid, crystalline lens. As a medical doctor, he knew that if the lens is not perfectly clear and transparent, vision is largely obstructed. He therefore supposed that the lens was the organ of photodetection, containing a "visual spirit" or πνεῦμα that passes down the optic nerve into the brain. The optic nerve as a consequence was supposed to be completely hollow, the only hollow nerve in the body.

Galen's books were probably filled with diagrams, though none has survived. The earliest schema of a sense organ we have is not from Galen himself but rather from a ninth-century AD translation of Galen into the Arab language Syriac. This translation was made by Humain Ibn Is-Hâq, who was born in Mesopotamia, studied medicine, and became an associate of the court physician of the caliph of Baghdad. The drawing in Figure 1.1A is from an English translation of Humain's manuscript (Meyerhoff, 1928). This schema of the eye had an enormous influence, not only on Arab medicine and science but also on the anatomists of the Renaissance, who continued to show the lens in the center of the eye. With a little effort, they should have been able to do the dissection more carefully, preserving the position of the lens in its proper place toward the front. What changed everything was the discovery of the laws of optical refraction and Kepler's solution of the optics of the eye. Kepler explained how images are formed and assigned the primary role in visual detection to the retina instead of to the lens (see Wade, 1998). Once the function of the lens was understood, it became possible for anatomists to do a proper dissection and find the various parts of the eye in their proper places. This is an example of *Lisman's Law*: you have to believe it in order to see it. The cross-section of the eye in Figure 1.1B was made by Descartes (1637/1987), who not only put the lens closer to its actual position but also identified the ciliary muscle and understood its role in changing the shape of the lens during accommodation.

The development of the compound microscope and improved methods for slicing and fixing tissue led to an explosion of information during the nineteenth century about the tissues of the body, including the sense organs (there are useful reviews of older literature in Polyak, 1941; von Békésy, 1960). The most important studies were surely those of the great Spanish neuroanatomist Ramón y Cajal (1911/1998). His clear drawings provided a wealth of information about the shapes of sensory receptors and other cells in sensory organs (Figure 1.2).

The physiology of sensation

These anatomical discoveries helped stimulate the first useful experimentation on the function of the sense organs. The structure of the ear and the role of the ear drum and bones of the middle ear were understood by the middle of the nineteenth century, and Helmholtz (1877/1954) postulated that sound

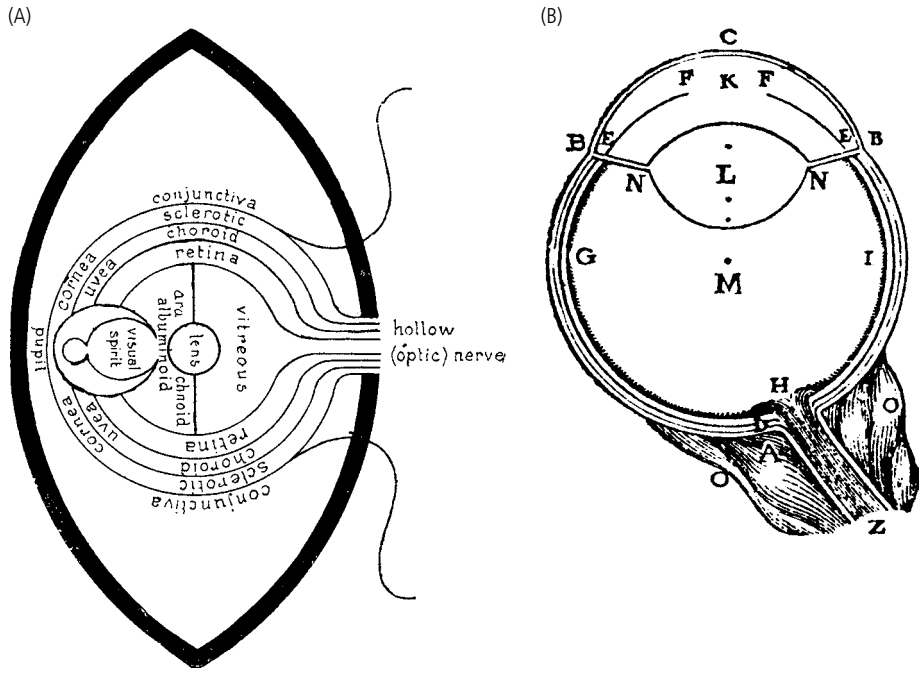

Figure 1.1 Structure of the eye. (A) Diagram of the eye from a ninth-century AD translation of Galen into Syriac by Humain Ibn Is-Hâq, in turn translated into English. (B) More anatomically correct diagram of cross-section of the eye made by René Descartes. ABCB, Cornea and sclera; EF, iris (in actual fact closer to the lens than shown in Descartes' diagram); K, aqueous humor; L, lens; EN, zonule fibers; M, vitreous humor; GHI, retina; H, optic nerve head; O, ocular muscles; and Z, optic nerve. (A from Meyerhoff, 1928; B from Descartes, 1637/1987.)

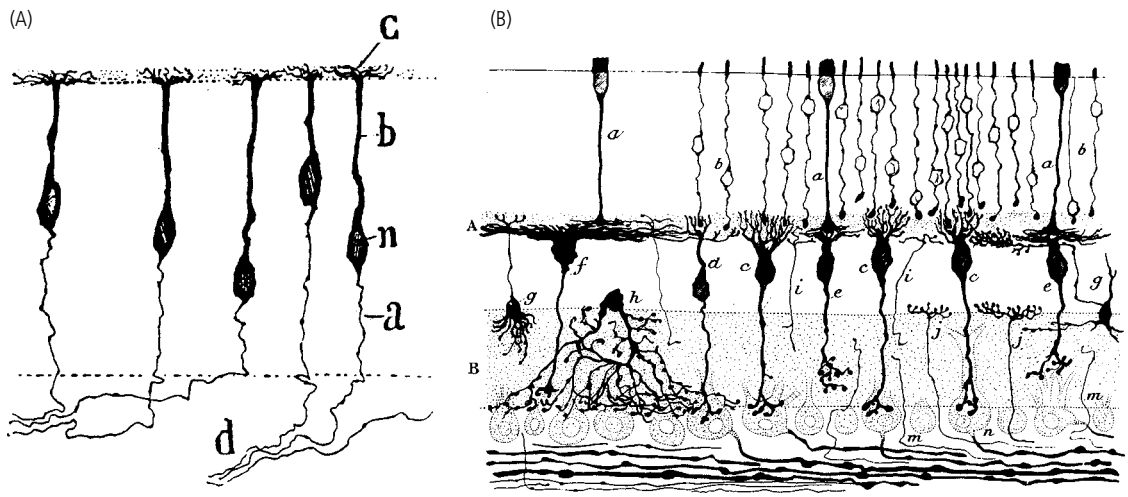

Figure 1.2 Sensory cells from the work of Ramón y Cajal. (A) Bipolar sensory neurons from mammalian olfactory mucosa. a, Axon; b, peripheral process; c, sensory dendrites; d, axon; n, nucleus. (B) Section of retina of an adult dog. A, Outer plexiform (synaptic) layer; B, inner plexiform (synaptic) layer; a, cone fiber; b, rod cell body and fiber; c, rod bipolar cell with vertical dendrites; d, cone bipolar cell with vertical dendrites; e, cone bipolar cell with flattened dendrites; f, giant bipolar cell with flattened dendrites; g, special cells stained very rarely (perhaps inter-plexiform cells); h, diffuse amacrine cell; i, ascendant nerve fibers (probably processes of cell not well stained); j, centrifugal fibers coming from central nervous system; m, nerve fiber (probably again of poorly stained cell); n, ganglion cell. (A and B from Cajal, 1893/1973.)

displaces these structures and causes the basilar membrane in turn to vibrate, with different tones producing vibration in different places. It was, however, von Békésy's actual observations of the movements of the basilar membrane that provided the first experimental evidence for the mechanism of auditory sensation in the mammalian ear (see Chapter 6 and von Békésy, 1960).

The visual pigments of the eye were also first discovered in the nineteenth century (an excellent summary of this early work can be found in Brindley, 1960), and Kühne showed that the molecule rhodopsin, or *sehpurpur* as he called it, changes color (bleaches) when exposed to light. These observations eventually led to the discovery by George Wald and colleagues that it is not the protein component of rhodopsin that absorbs light but rather a relative of vitamin A called 11-*cis* retinal, which is covalently bound to the protein (see Chapter 9 and Wald, 1968).

Some of the first electrical recordings of the responses of sensory receptors were made by E. D. Adrian, who dissected away the axons of single touch receptors from the skin and placed them over a wire electrode to record action potentials (Adrian, 1928, 1931, 1947). A typical result from Adrian's experiments is illustrated in Figure 1.3A. Pressure applied to the skin causes the frequency of action potential firing to increase (upper trace). Pricking the skin (lower trace) is also an effective stimulus but evokes action potentials in more than one kind of mechanoreceptor (note the different amplitudes of the spikes recorded by the electrode). Adrian concluded that action potentials from these receptor cells are communicated to the brain and form the basis of our sensation of touch. Using a similar technique, Hartline recorded action-potential discharges from the compound eye of the horseshoe crab *Limulus* (Hartline and Graham, 1932) and showed (Figure 1.3B) that the frequency of action potential firing depended both on the intensity and duration of the light stimulation (Hartline, 1934). These were the first single-cell responses recorded from an eye, though we now know that they were not produced by the photoreceptors themselves but rather by a cell called the eccentric cell, which receives direct synaptic input from the photoreceptors.

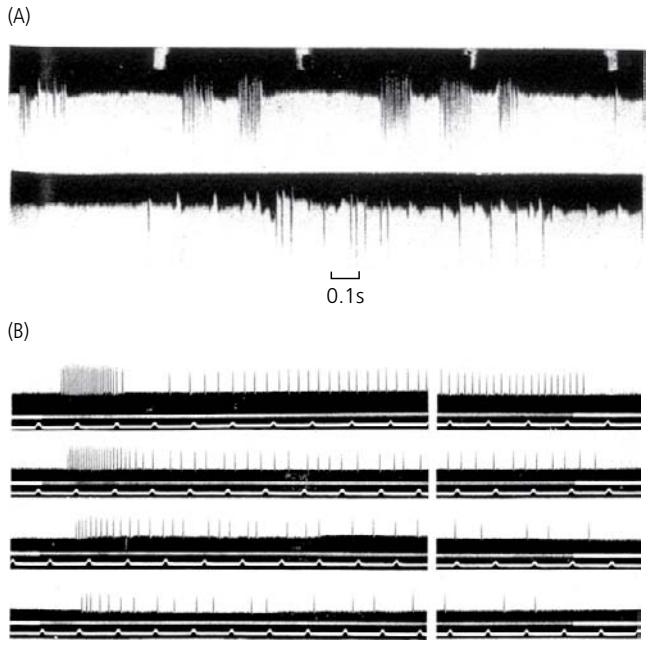

Figure 1.3 Early electrical recordings of sensory responses. (A) Action potentials recorded from single axons dissected from the cutaneous nerve of a frog. (B) Action potentials from the lateral eye of the horseshoe crab *Limulus*. Each trace gives the response to a different light intensity, which was systematically increased by an additional factor of ten from dimmest (bottom) to brightest (top). (A from Adrian, 1947; B from Hartline and Graham, 1932.)

The method of dissection of single nerve fibers is difficult and tedious and was soon replaced by recording with fine metal microelectrodes. These electrodes are made from tungsten or platinum wire exposed and sometimes gold-plated at the tip but insulated along the rest of the length with glass or plastic resin. These metal electrodes can be inserted directly into the tissue to record the small extracellular currents produced by action potentials of single cells. Metal-electrode recording from the nerve coming from the ear established many of the basic properties of auditory responses, such as their time course and dependence on the frequency of the sound (see Kiang, 1965; Evans, 1975). The recordings in Figure 1.4 were collected from a single axon from the ear and show action potentials as a function of sound intensity on the ordinate, with the frequency (pitch) of the sound on the abscissa. As the sound was made progressively weaker, the range of frequencies to which the axon responded became progressively more restricted. The nerve fiber showed greatest sensitivity to a tone near 10 kilohertz (kH), since at this frequency (called the *characteristic frequency*) a response could still be recorded even when the sound was made very weak indeed. Recordings of this kind showed that different axons in the auditory nerve have different characteristic frequencies, spanning the entire range of perceptible sound. The axons are therefore *labeled lines*, each carrying information about a different range of sound frequencies. These experiments showed that the ear must have some way of responding to sounds of different frequencies, so that the different auditory receptors can be tuned each to its own characteristic frequency.

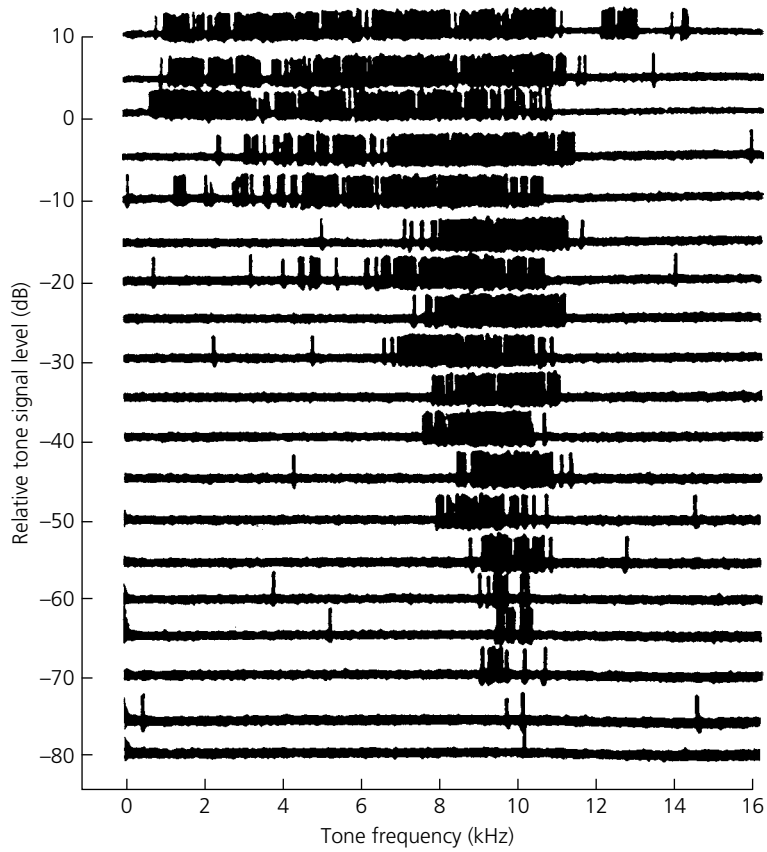

Figure 1.4 Extracellular spike recordings from a single axon from the guinea pig ear. Frequency (pitch) of sound was systematically swept from low to high for a range of different sound intensities. Frequency is plotted on abscissa and intensity is plotted on ordinate in a log scale of decibels (dB). An increase of 20 dB is equivalent to a 100-fold increase in intensity. (From Evans, 1972.)

The first extensive study with metal microelectrodes from olfactory receptors produced a completely different result (Gesteland et al., 1965). There seemed to be no consistent pattern to the responses, with many receptors responding to the same chemicals, sometimes with excitation, sometimes with inhibition. Later recordings confirmed some but not all of these conclusions. They showed that vertebrate olfactory receptors all appear to respond with excitation, producing an increase in spike frequency to stimulation with an odor. Single cells do nevertheless appear to be able to respond to a wide variety of odors. Thus olfactory receptors seem *not* to be labeled lines, at least not in the way originally supposed. I return to this matter in Chapter 7, after I have described the mechanism of olfactory transduction in detail.

These early recordings indicated that receptor cells signal the arrival of sensory stimuli by producing a change in electrical activity. What is the nature of this electrical signal? Is it produced by some change in the cell membrane potential? If so, what is the mechanism that converts the sensory stimulus into an electrical response?

A powerful tool for the investigation of these questions became available with the invention of the intracellular microelectrode in the late 1940s (Ling and Gerard, 1949). An intracellular microelectrode is made from a piece of glass tubing typically 1 mm in diameter. The tubing is melted and pulled to a fine point, in early studies by pulling the glass by hand over a Bunsen burner, but later by placing the glass in a mechanical device that heats the middle of the tubing and pulls at either end to form two electrodes, each with a fine glass tip. The bore of the electrodes is then filled with a concentrated salt solution such as 3M KCl.

The first intracellular recordings from sensory receptors were made by Hartline and collaborators, again from the compound eye of the horseshoe crab *Limulus* (Hartline et al., 1952). Figure 1.5A is from the later study of Millecchia and Mauro (1969b), also from *Limulus*. Light produces a positive-going change in membrane potential, called a depolarization. Similar depolarizing responses were recorded from many other types of sensory receptors, including mechanoreceptors (Eyzaguirre and Kuffler, 1955; Loewenstein and Altamirano-Orrego, 1958) and chemoreceptors of the nose (Getchell, 1977). It came therefore as a great surprise when Tomita and collaborators first showed that the response of a vertebrate photoreceptor to light is a negative-going hyperpolarization (Figure 1.5B and Tomita, 1965).

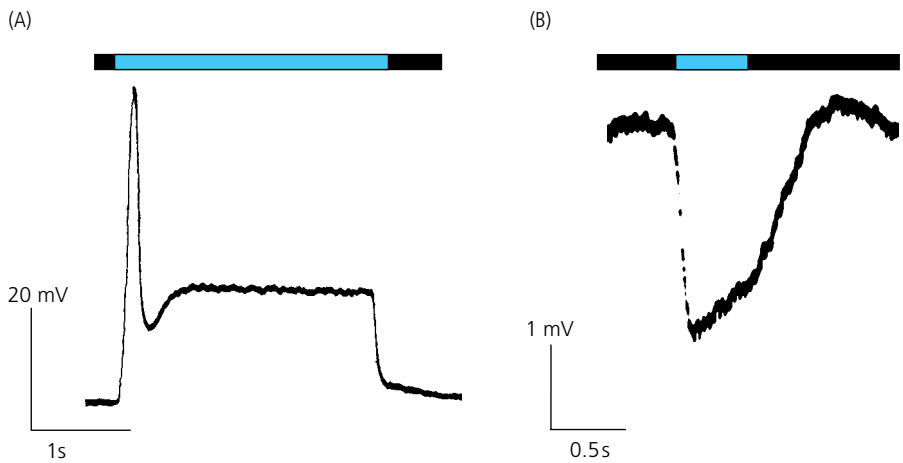

Figure 1.5 Intracellular recordings from sensory receptors. Bars above recordings show timing and duration of light flashes. (A) Depolarizing voltage response from photoreceptor of *Limulus* ventral eye. (B) Hyperpolarizing voltage response from photoreceptor (cone) of a fish. This is the first published recording of the response of a vertebrate photoreceptor. (A from Millecchia and Mauro, 1969b; B after Tomita, 1965.)

Cracking the problem: molecular physiology

Although the important observations of neuroanatomists of the nineteenth and twentieth centuries and the first extracellular and intracellular recordings from receptor cells provided many clues about the early steps in sensory processing, they told us very little about transduction; that is, about the way the electrical signal is generated by light or odor or sound. The physical stimulus received by the sense organ is somehow translated into a change in membrane potential, which is then transmitted into the central nervous system (CNS). The nature of this process remained for a very long time a complete mystery. This puzzle has now been substantially solved for most of the senses in a variety of organisms, providing a fairly clear picture of how sensory signals are produced. These striking advances were greatly facilitated by many years of patient biochemical and electrophysiological investigation, but they were then rather suddenly accelerated by the discovery of the technique of patch-clamp recording and of methods for cloning proteins and expressing their activity.

The invention of the patch electrode by Neher and Sakmann (1976) first made possible direct recordings from the molecules responsible for the electrical activity of nerve cells (see Sakmann and Neher, 1995). A patch electrode is made from fine glass tubing like an intracellular electrode, but the tip of a patch pipette is made very smooth, either by a specialized pipette puller (Brown and Flaming, 1977) or by polishing the end of the pipette with heat under a microscope. The pipette is then pressed against the soma of a cell and slight suction is applied, usually by mouth (Figure 1.6A). The glass of the pipette may then adhere to the cell membrane to form a very tight seal, sometimes called a gigaseal, with a resistance often of the order of 10 gigaohms (10^{10} Ω) or greater. The very high resistance of this seal reduces the electrical noise of the recording and makes it possible to distinguish the opening and closing of single channels in the membrane within the orifice of the pipette (Figure 1.6B). Single-channel responses first of acetylcholine receptors (Neher and Sakmann, 1976) and then of the Na^+ channels of axons (Sigworth and Neher, 1980) were studied

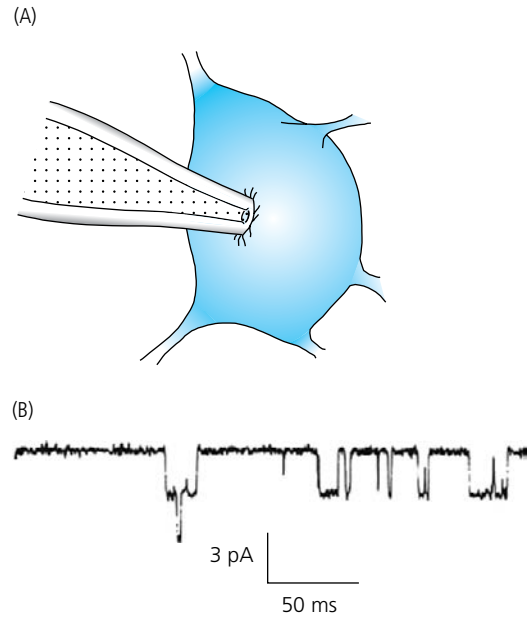

Figure 1.6 Patch-clamp recording from single channels. (A) The tip of a patch pipette is pushed against the cell body of a cell and slight suction is applied to form a seal. (B) Single-channel currents recorded from muscle acetylcholine receptors. The pipette contained 0.3 μM acetylcholine. Downward deflections indicate channel opening. At least two channels were present in this membrane patch. (B from Trautmann, 1982.)

with patch-clamp recording. In a very short time, recordings were obtained from many of the principal kinds of channel molecules of the cells of the nervous system, including those of sensory receptor cells.

Recordings made with patch electrodes sealed to the surface of the plasma membrane as in Figure 1.6 are called on-cell or cell-attached recordings. The extracellular surface of the membrane is exposed to the solution inside the pipette, and the intracellular surface is exposed to the cytosol. If the pipette is sealed in this way and then gently lifted off the cell, the plasma membrane often remains attached to the pipette, forming an excised or inside-out recording (Figure 1.7), so-called because the inside surface of the membrane now faces the outside bathing solution. Inside-out recording makes possible the study of channels that are opened or closed by the binding of some intracellular substance to the cytoplasmic side of a channel protein, such as Ca^{2+}, cyclic nucleotides, and other putative second messengers. As we

shall see, inside-out recording provided crucial evidence establishing the identity of the intracellular second messengers mediating vertebrate visual (Fesenko et al., 1985) and olfactory (Nakamura and Gold, 1987a) transduction.

If, on the other hand, a pipette is sealed onto a cell and additional pressure or a brief voltage pulse is applied, the membrane within the pipette can often be made to break, establishing a direct connection between the inside of the pipette and the inside of the cell. This method of recording is called whole-cell (Figure 1.7, left middle) and is useful for introducing small-molecular-weight molecules from the pipette into the cell. The whole-cell mode of patch clamp is also extensively used to voltage clamp small cells. I describe the method of voltage clamping in more detail in Chapter 3. Whole-cell recording has revolutionized cellular physiology, greatly facilitating the study of electrical responses of a variety of neurons such as pyramidal cells in the cortex and granule cells in the cerebellum, as well as many types of sensory receptor cells, including photoreceptors, auditory hair cells, and the chemosensory receptor cells of the nose and tongue.

A pipette in the whole-cell mode can also be lifted off the cell. As Figure 1.7 shows (lower right), the membrane will often then flip around and reseal, leaving a small patch of excised membrane whose outside surface faces the outside solution. This is called an outside-out recording. The outside-out

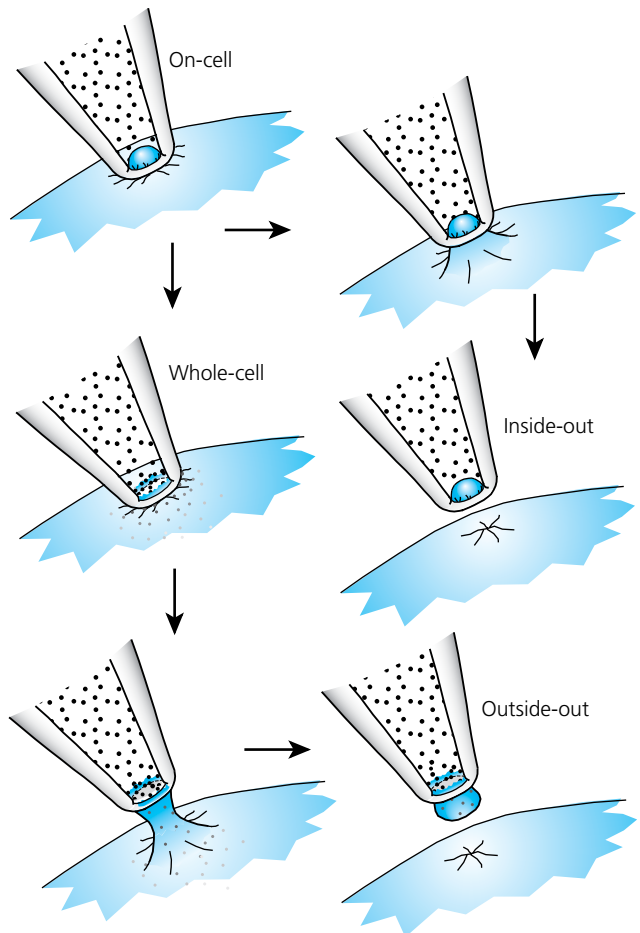

Figure 1.7 Different configurations of recording with patch-pipette technique. On-cell, whole-cell, inside-out, and outside-out recording techniques as described in the text.

mode of the patch-clamp technique has been especially useful for studying ligand-gated channels like those at synapses. These channels have an extracellular binding site for a small-molecular-weight transmitter molecule. A putative transmitter can be added to the bathing solution, and its effect on channel opening can be examined directly.

The revolution of molecular biology

The search for the mechanism of transduction was also greatly facilitated by the development of the techniques of molecular biology. Many of the most important molecules of sensory cells are integral membrane proteins, including the sensory receptor proteins of the nose and tongue, as well as enzymes of second-messenger cascades and the channels that ultimately produce electrical responses. These proteins are firmly embedded in the plasma membrane and difficult to extract and study. In the 1960s and early 1970s, the first attempts were made to isolate these important molecules from neurons and sensory cells and to sequence and study them. In a few favorable cases, it was possible to extract enough of a protein in this way to obtain its complete amino acid sequence (for example rhodopsin, see Artamonov et al., 1983; Hargrave et al., 1983; Hargrave, 2001). In most cases, however, only a very small amount of protein could be extracted—too little to be studied in detail, but enough in many cases to allow the gene of the protein to be cloned.

Many membrane protein genes were first cloned in a similar fashion (Figure 1.8). A small amount of the protein was first extracted and purified, generally with chromatography or electrophoresis. It was then digested with a protease, and a few small-molecular-weight peptides were isolated and sequenced. From these peptides, synthetic nucleotide sequences were synthesized and used to screen a library of clones, made from tissue of the animal from which the protein was originally extracted. Alternatively, an antibody was made to an isolated peptide and used to screen an expression library. From the DNA sequence of the clone, it was possible to infer the

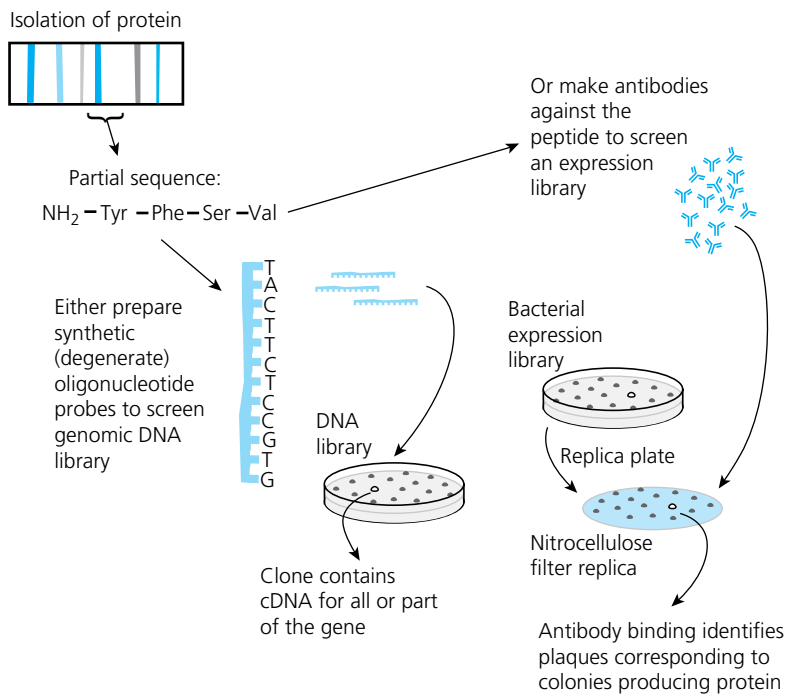

Figure 1.8 Cloning a gene from partial sequence of a protein. Method of cloning used for many of the first proteins whose genes were cloned from the nervous system. The method begins with isolation of partial sequence of a protein, which is then used to prepare oligonucleotides for screening tissue DNA libraries.

amino acid sequence of the protein. It has also been possible to identify families of related proteins within an organism and from organism to organism, by examining complete genomic sequences. We now have complete sequences of the genomes of many model organisms such as the fruit fly *Drosophila*, the mouse, and the zebrafish, as well as of hundreds of other species, from sponges to *Homo sapiens*.

Ultimately, the identification of a DNA sequence as that of a functional protein rests upon the demonstration that the DNA in question can direct the synthesis of a molecule with biological activity. This task can be done by *expressing* the protein. The DNA of the identified clone can be used to make complementary RNA (cRNA), which is then inserted, for example, into an oocyte of the frog *Xenopus* (Figure 1.9A). The oocyte can then be used for voltage-clamp studies of the expressed protein. Alternatively, and now more commonly, the DNA from the clone can be incorporated directly into the DNA of a cultured cell by a process called transfection (Figure 1.9B). DNA packaged into a plasmid or viral vector can be introduced into the cell by a variety of methods, for example by exposing a cell to lipid vesicles containing the DNA, or by giving high-voltage pulses of electricity to pierce holes in the cell membrane. The DNA can then become incorporated into the genome of the cell, and the cells are cultured to select those expressing the DNA of interest. If properly linked to promoters or other regulatory elements, the DNA is transcribed into RNA, which is in turn translated into protein. A stable population of cells may be produced in this way expressing the protein of interest. Transfection is often more convenient than RNA expression in oocytes, because cultured cell lines provide an excellent starting point for producing large quantities of expressed protein for structural or other studies, as well as a convenient preparation for patch-clamp recording.

From the amino acid sequences of the proteins we had our first clues about the structure of the molecules. Many of the most important proteins mediating sensory transduction are integral membrane proteins with extensive sequences lying within the hydrophobic interior of the lipid bilayer. From the sequence alone, reasonable guesses can be made about which amino acids lie within the membrane and which are more likely to face the cytoplasmic or extracellular solution (Figure 1.10). Some amino acids (such as valine and isoleucine) are hydrophobic and much more likely to be surrounded by lipid or other protein, whereas others (such as aspartate and lysine) are hydrophilic or even charged and much more likely to be surrounded by water. By a process known as hydropathy analysis, the sequence of amino acids can be used to make inferences about how the protein folds, indicating the parts of the sequence that are integrated into the membrane and those that are exposed to the intracellular or extracellular solution. Antibodies to specific sequences can then be used to localize parts of the protein on one side of the membrane or the other. Sequences can be identified as substrates for glycosylation or phosphorylation, or can be shown actually to be glycosylated or phosphorylated. These identifications are often helpful in indicating regions that are intracellular or extracellular, because glycosylases and protein kinases only add sugar groups or phosphates at sites accessible to one side of the membrane or the other.

Finally, membrane proteins either isolated or expressed as in Figure 1.9 can be used to form crystals suitable for X-ray crystallography, from which the complete three-dimensional structure of the protein can be determined. Membrane proteins are in general more difficult to crystallize than soluble proteins, but with continued effort crystals were obtained at about the same time for ion channels and G-protein receptors (Doyle et al., 1998; Palczewski et al., 2000). These methods have given us extensive information about mechanisms of ion movement through potassium (Jiang et al., 2003b; Long et al., 2005a, 2005b, 2007) and sodium (Payandeh et al., 2011; Catterall, 2012; McCusker et al., 2012) channels, as well as protein conformation changes producing activation in rhodopsin (Deupi et al., 2012) and other G-protein receptors (see Erlandson et al., 2018).

Structures of near atomic resolution can also be obtained by a newer method called cryogenic electron microscopy (cryo-EM). The protein of interest is expressed in either bacteria or a cell line, and it is then isolated and distributed onto an EM grid. The grid is plunged into liquid ethane and then into liquid nitrogen, to freeze the sample rapidly so as to prevent the formation of ice crystals. The regularities

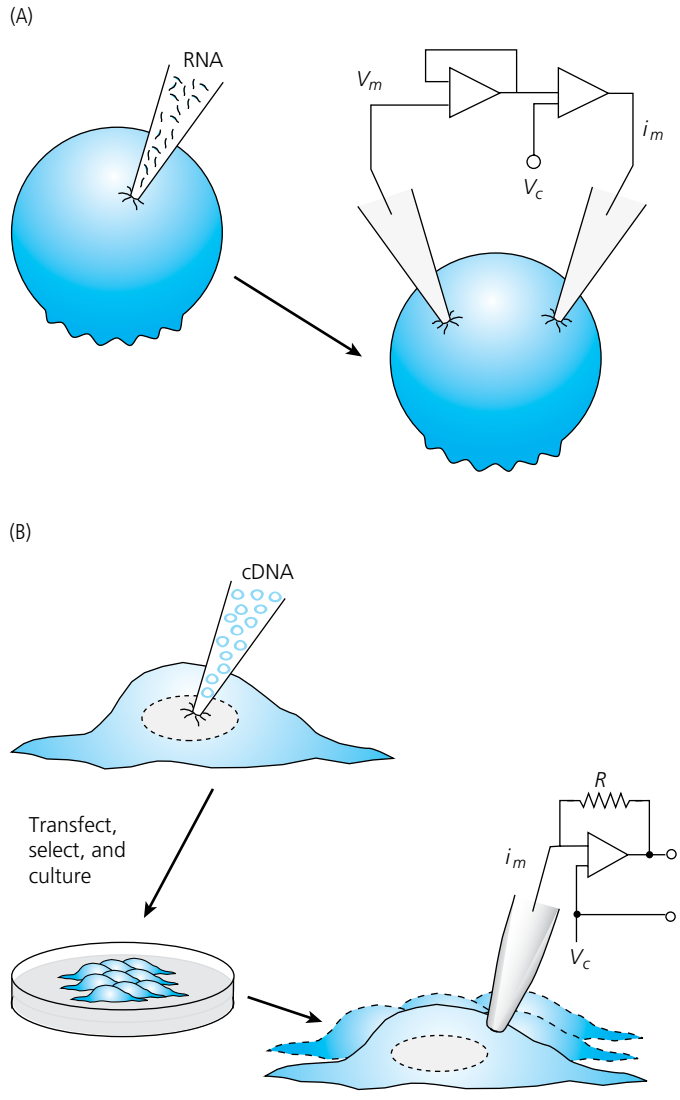

Figure 1.9 Common methods of gene expression for recording electrical activity of ion channels and other membrane proteins. (A) Injection into a *Xenopus* oocyte, which can then be studied by voltage clamping. (B) Transfection. DNA incorporated into a plasmid or viral vector is introduced into the cell by electroporation, Ca^{2+} shock, or direct injection in the nucleus (as shown). The cell line may then be used for patch-clamp recording. V_m, Membrane potential; V_c, command potential; R, feedback resistance of patch amplifier; i_m, membrane current.

of the structure of the frozen particles can then be used to determine the structure of the protein. Large proteins, proteins in solution, and proteins with significant structural heterogeneity can now be visualized with this method.

The techniques of molecular biology can also provide essential information about the function of sensory proteins. Experiments of this kind have been especially informative for receptors that use second-messenger cascades, such as those in the eye and nose. The cloning, for example, of the genes of the family of receptor proteins mediating olfactory transduction in the nose (Buck and Axel, 1991) has led to remarkable insight into the organization and mechanism of transduction in this tissue, which I describe in considerable detail in Chapter 7. Similar

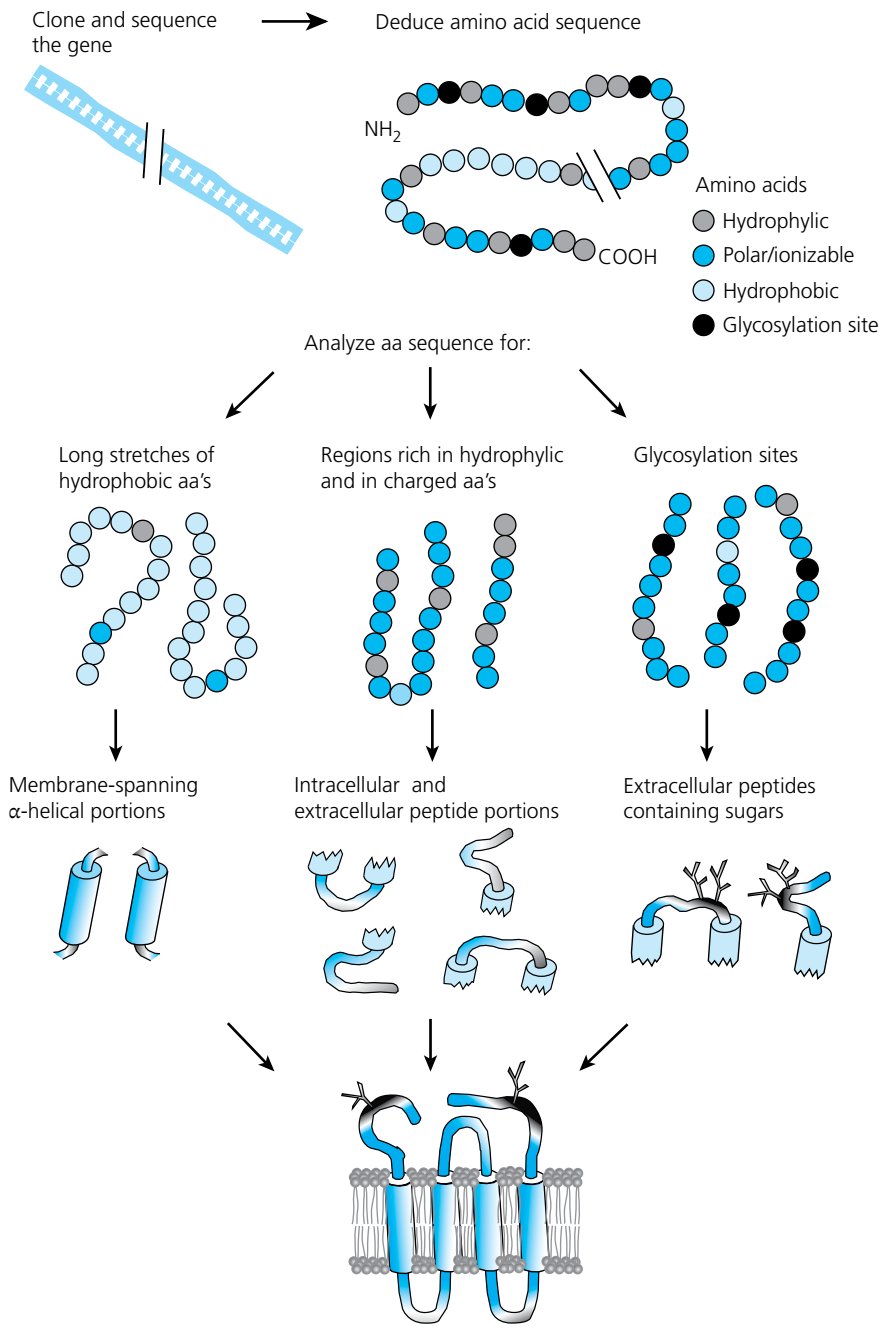

Figure 1.10 Analysis of hydropathy and the folding of membrane proteins. The amino acid sequence of a membrane protein can be used to make inferences about protein structure, as described in the text.

discoveries for the receptors of taste cells (Liman et al., 2014; Roper and Chaudhari, 2017) promise to bring important advances in our understanding of gustatory transduction.

Molecular techniques have provided powerful methods for studying protein structure and function. Site-directed mutations of single amino acids can be made in selected positions in a protein to test the role of specific sequences in substrate binding or catalysis. The DNA can be altered at predetermined locations to produce specific deletions of part of the protein sequence. In this way, whole regions of the protein can be excised. Regions of sequence can even be exchanged between related proteins to produce chimeric molecules, containing part of one protein and part of another. These experimental approaches have been greatly facilitated by new methods of altering the genome, including those based on CRISPR/Cas9 (Doudna and Charpentier, 2014; Doudna and Sternberg, 2017). The CRISPR/Cas9 system is part of a bacterial defense mechanism against viruses, which can be exploited in higher animals including mammals to alter genomic DNA so as to modify or knock out selected genes much more easily and rapidly than was previously possible. These techniques can be combined with structural information to provide remarkable insight into the physiology of the body.

Piezo proteins: channels mediating touch

The power of these methods can be illustrated by the recent isolation and characterization of the piezo proteins (Murthy et al., 2017), which are now known to be essential components of mammalian touch sensation and proprioception (see Chapter 5). These proteins were discovered in the following way. Bertrand Coste in the laboratory of Ardem Patapoutian began to look for channels that might be responsible for tactile sensation. He and his colleagues started screening several commercially available cell lines (Coste et al., 2010), recording from single cells from each of the lines with the whole-cell method of patch clamp while depressing the membrane of the cell with a second pipette (Figure 1.11A). In this way, they discovered that membrane indentation of a Neuro2A cell produces a rapidly decaying inward current, caused by an increase in membrane permeability to cations, principally Na^+. They then isolated messenger RNA from Neuro2A cells and screened for highly expressed membrane proteins, finding nearly 100 candidate sequences. For each of these sequences they constructed a small interfering RNA (siRNA). An siRNA is a nucleotide sequence of twenty to twenty-five base pairs that is complementary to the sequence of the mRNA of the candidate protein. The siRNA binds to the candidate mRNA sequence to inhibit its translation.

The siRNAs were introduced one by one into the Neuro2A cells and the responses of the cells were recorded as in Figure 1.11A, until one of the siRNA sequences was shown to produce a dramatic decrease in the mechanosensitive current. In this way, Coste and colleagues identified a protein called Piezo1 (Figure 1.11B), named from the Greek verb πιέζω, meaning "to press." A second protein called Piezo2 (Figure 1.11C) was identified by its sequence homology and was quickly recognized to be expressed in somatosensory neurons. In a subsequent paper (Ranade et al., 2014), this laboratory used molecular techniques to knock out the *Piezo2* gene in adult mice and showed that it mediates an important component of touch sensitivity (see Chapter 5). Later observations revealed that humans with mutations in *Piezo2* show a selective loss of proprioception and touch perception (Chesler et al., 2016).

Piezo1 and Piezo2 are extremely large multimeric proteins, whose three subunits each have over 2000 amino acids. Hydropathy analysis (Figure 1.12) indicated that each subunit should have nearly forty α-helical hydrophobic regions spanning the membrane (Coste et al., 2015), many more than most ion channels (see Chapter 3). The identification of these regions was confirmed by marking extracellular sites accessible to binding by antibodies against Myc tags, which are small peptide antigens containing ten amino acids. The Myc tags were introduced into the protein structure at selected locations by inserting the Myc sequence into the protein coding region, and the altered protein sequence was then expressed in a cell line. Intracellular sites were identified as sites of phosphorylation with mass spectrometry. Site-directed mutagenesis of single amino acids, followed by expression of the channels as in Figure 1.9, showed that the ion channel of the protein was located near the carboxyl terminus—to the right in Figure 1.12.

Because of its unusual size and sequence, a piezo protein should have quite an interesting structure;

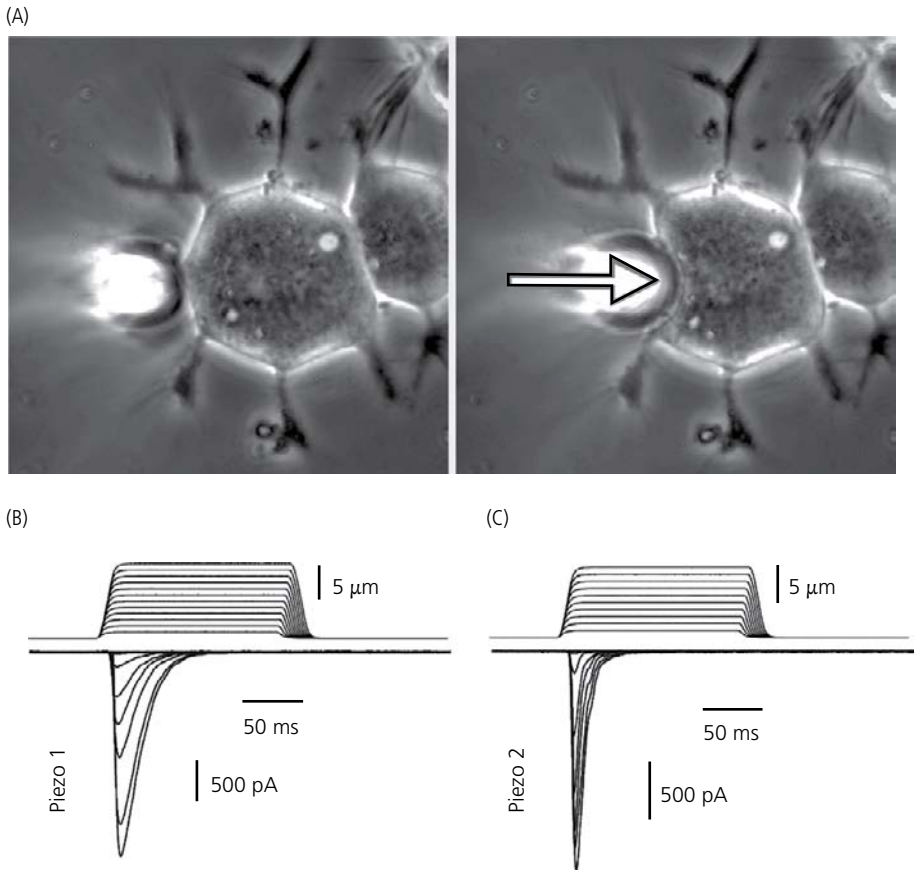

Figure 1.11 Responses of cells transfected with piezo proteins. (A) Method of stimulation. A pipette was mechanically moved against plasma membrane of an N2A mouse neuroblastoma cell during whole-cell recording, visibly deforming the cell membrane. The recording pipette is not shown for clarity. Responses of N2A cells transfected with genes for Piezo1 (B) and Piezo2 (C) to pipette movements of increasing distance in 1-μm increments. Magnitude and waveform of stimuli are shown in upper traces. Lower traces give currents superimposed for each of the stimulus magnitudes, recorded under voltage clamp at a holding potential of –80 mV. (From Coste, 2011.)

but because these proteins are so large, they would be nearly impossible to crystallize for X-ray studies with present techniques. Several groups therefore turned to cryo-EM (Ge et al., 2015; Guo and MacKinnon, 2017; Saotome et al., 2018; Zhao et al., 2018). The protein was expressed in a bacterium or cell line, isolated and solubilized with detergent, and placed on a grid for electron microscopy. Figure 1.13A (from Guo and MacKinnon, 2017) shows an image from such an experiment. Individual proteins are clearly observable, randomly distributed over the grid. Protein images were grouped into classes depending upon their orientation, and the images were then averaged. Representative averages at two different orientations are shown in Figure 1.13B. The averages were refined under software control and assembled into a model to provide a 3D structure of the protein.

These structures have several interesting features. When viewed from above (Figure 1.13C), the channel resembles a propeller with three blades contributed by the three subunits (in red, green, and blue), meeting in the center to form the ion channel. When viewed from the side (Figure 1.13D), the subunits can be seen to have numerous α-helical regions running perpendicular to the plasma membrane and

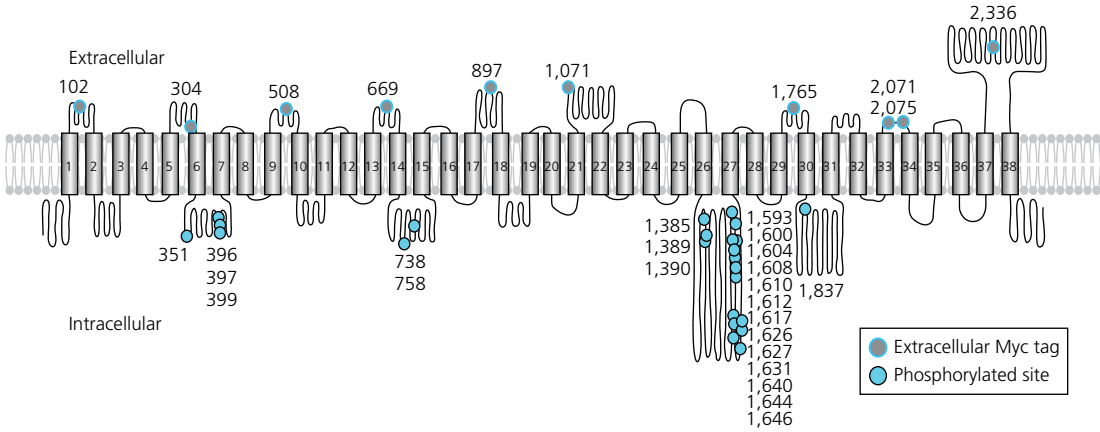

Figure 1.12 Membrane topology of Piezo1 protein. The amino acid sequence of Piezo1 was subjected to hydropathy analysis as in Figure 1.10 in order to predict regions of α helix and provide a preliminary membrane topology of the protein. This preliminary sequence was then tested by identifying regions of the protein that are extracellular or intracellular. Extracellular regions were identified by altering the protein sequence to include Myc tags, which are sequences of ten amino acids inserted in many different places along the protein sequence. Sequences with Myc tags were then expressed one by one in HEK293T cells (a cell line) and exposed to fluorescent antibody against the Myc tag. Cells were only stained if the Myc tag was extracellular and accessible to the antibody. Positions where staining was successful are shown together with the number of the amino acid in the sequence where the Myc tag had been introduced. Intracellular regions of the protein were localized by identifying phosphorylated peptides with mass spectroscopy. Phosphorylated amino acids are shown together with the number of the amino acid in the protein sequence. (From Coste et al., 2015.)

spanning it from one side to the other. Because these regions lie at an angle to one another, they would force the membrane to bend, producing tension. A small cap region on the extracellular side of the membrane lies just above the ion channel, which in the cryo-EM preparations was closed.

How then does membrane tension gate channel opening? The propeller blades of the three subunits are essential, because deletion of any of the groups of helices making up this extended part of the protein produced either a greatly attenuated response or no response at all. The propeller blades could act as a lever, transmitting pressure on adjacent plasma membrane into the middle of the protein to gate the channel (Saotome et al., 2018; Zhao et al., 2018). Alternatively, pressure on the membrane could flatten the channel and its surrounding lipid, which are under tension from the curved shape of the protein (Figure 1.13D). Channel flattening could then produce the change in conformation required to open the channel (Guo and MacKinnon, 2017). Further experimental work may help to distinguish these two alternative explanations.

The code deciphered: sensory transduction

It is remarkable to be able to say that we now understand, at least in outline, how sensation occurs in all of the major senses of the body. This development is, I think, one of the major achievements of modern neuroscience. Though many details of importance still have not been elucidated, it is nevertheless now clear that mechanoreceptors have ion channels like the piezo proteins specialized for the perception of membrane pressure and directly responsible for producing the electrical response of the cell. Vertebrate photoreceptors and olfactory receptors, on the other hand, use complicated signal transduction cascades. Light or odor produces a change in the concentration of one of the cyclic nucleotides which acts as a second messenger and binds to channels to produce the electrical response. An enormous amount of detail has been learned about the proteins responsible for sensation. We have paradoxically much more information about vertebrates for the rather complicated structures of their eyes, ears,

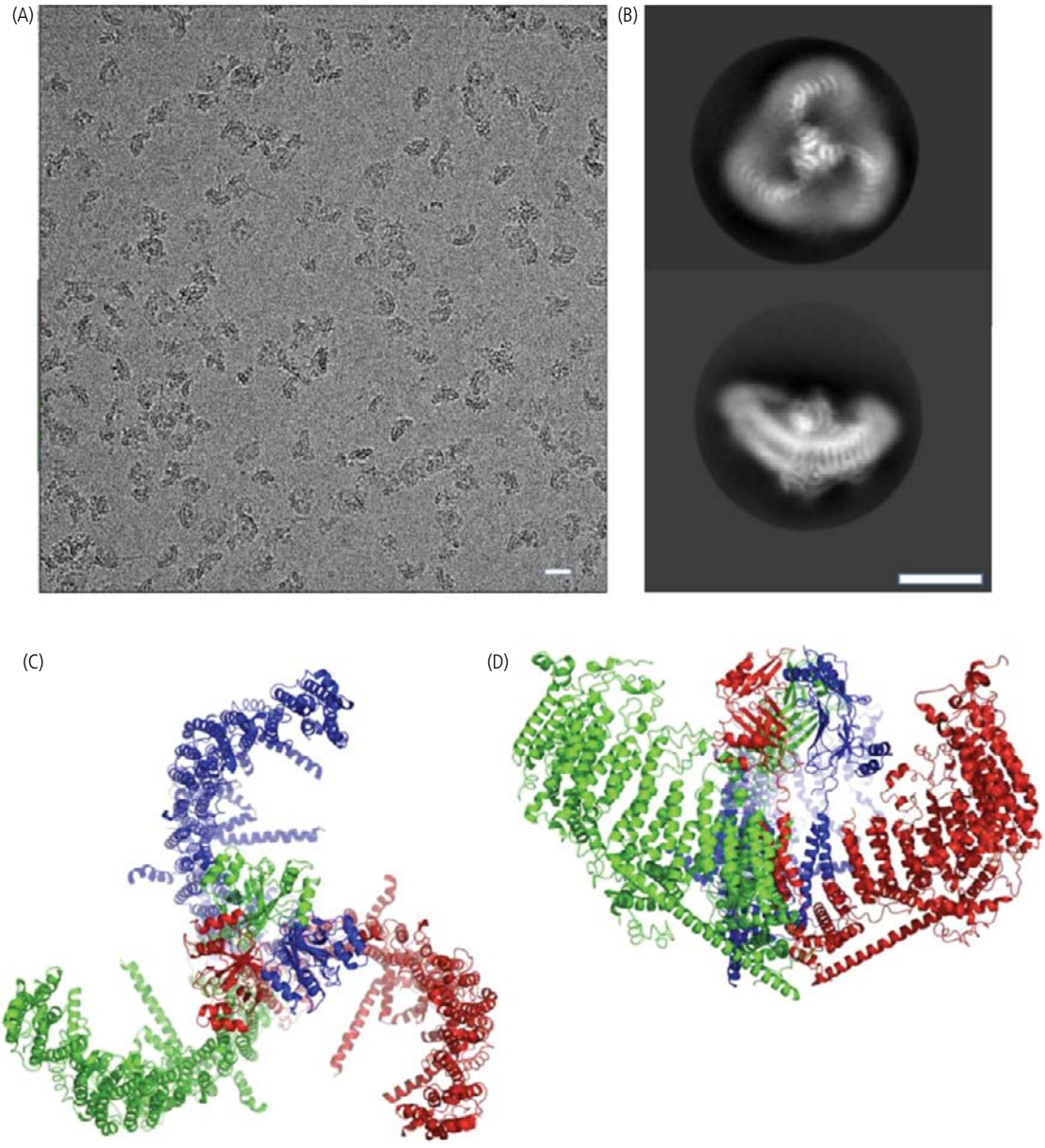

Figure 1.13 Cryo-EM structure of Piezo1. The gene for the Piezo1 protein was expressed in a cell line and purified. The protein was suspended on an electron-microscope grid and rapidly frozen by plunging the grid first into liquid ethane and then into liquid nitrogen. (A) Representative raw micrograph of the protein on the grid; scale bar is 200 Å (20 nM). (B) Protein images like those in (A) were separated into groups according to their orientation, first manually to produce templates and then automatically under computer control. Images in each class were averaged, and representative averaged classes are shown viewed from the top (upper image) and side (lower image); scale bar is 100 Å (10 nM). (C, D) Atomic model of the trimeric channel at an overall resolution of 3.7 Å shown as a ribbon diagram, viewed from the top (C) and side (D). The three subunits have been given different colors. (From Guo and MacKinnon, 2017.)

and noses than for invertebrates and their comparatively much simpler ocelli and sensory hairs. Our understanding of the detection of temperature has made remarkable progress, whether for the hot and cold receptors on the surface of the skin or for the infrared detectors that rattlesnakes use to hunt in darkness. Some species have now been shown to respond to the magnetic field of the earth, though the fundamental nature of sensory transduction in magnetoreception remains a mystery.

The purpose of this book is to describe these major discoveries as well as present areas of uncertainty. Together with an extensive treatment of sensation in mammals, I have included experiments on bacteria and protozoans as well as worms and arthropods. I also describe some mechanisms of sensation like electroreception, for which we as a species have no experience. The focus of this book is the cellular mechanism of transduction: the ion channels, G proteins, enzymes, and second messengers that produce the responses of sensory cells. I also attempt to summarize what we know about the modulation of transduction during adaptation. Where possible, I try to compare the mechanisms used by different sensory receptors. It is remarkable, for example, that adaptation in photoreceptors, olfactory receptors, and hair cells in every case seems to require the calcium ion, though what Ca^{2+} does in each cell is still not clear.

In the first part of the book, I attempt to outline some features common to many kinds of sensory cells, such as mechanisms of elaboration and renewal of sensory membrane, the organization of sensory organs, and the general features of electrical responses. Chapters 3 and 4 provide background information about electrical activity in the nervous system and metabotropic cascades, with sensory cells used to emphasize general principles of cellular physiology. The remaining chapters provide a review of the literature of sensory transduction for the major senses. It would have been possible to write an entire book just on photoreceptors or hair cells, and perhaps even on olfactory receptor cells. It seemed to me much more useful to summarize in one volume the major features of sensory transduction for each of the receptors. In this way, we may more easily appreciate the many similarities in structure and mode of operation of the cells in the different sense organs.

It comes as an enormous source of satisfaction to me personally to be able to write this book in the way now possible. My own laboratory first began working on photoreceptors when I became a member of the faculty at UCLA in the fall of 1975. For nearly as long, I have taught sensory physiology to undergraduates. When I first lectured about the nose, there was almost nothing to say, apart from the anatomy of the receptor cells and the remarkable ability of the sensory epithelium to regenerate. Now it is easily possible to lecture on this subject for many hours without exhausting the fascinating body of knowledge that has since been acquired. Nearly the same could be said for touch and taste. I hope I can successfully convey the excitement of past discovery, and the anticipation I myself feel for the many surprises the future will doubtless bring about these fascinating organs, our senses.