Synapses

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Preface

The introduction of the term *synapse* for the sites at which axons make functional contacts with their target cells marked the beginning of a new era in the study of the nervous system. Sherrington, who coined the term, had become convinced from the work of several neuroanatomists, but principally Ramón y Cajal, that the earlier notion that neuronal processes are in direct "protoplasmic" continuity was untenable. Not only was it difficult to conceive how specific neural functions could be executed if the central nervous system was an elaborate syncitium, but there was also compelling—albeit indirect—evidence from developmental, pathological, and various neuroanatomical studies that argued strongly for the view that neurons are morphologically distinct entities whose processes are contiguous with those of other cells but structurally separated from them. Although the acrimonious debate between those who had espoused the "reticular theory" of neuronal interactions and those who were identified with the "neuron theory" continued well into the twentieth century, for all but the most committed reticularists (like Camillo Golgi and his followers) the issue was effectively settled by 1897, when Sherrington was moved to write that, "As far as our present knowledge goes, we are led to think that the tip of a twig of the [axonal] arborescence is not continuous with but merely in contact with the substance of the dendrite or cell body on which it impinges. Such a special connection of one nerve cell with another might be called '*synapsis.*'" And more than 50 years later he was to say of Cajal's great contribution: "The so-called nerve networks with unfixed direction of travel he swept away. The nerve circuits are valved, he said, and he was able to point out where the valves lie—namely where one nerve cell contacts the next one."

To Cajal's identification of synapses as the mediators of nerve cell interactions, Sherrington was to add a major contribution of his own—the discovery that *inhibition* is as important as *excitation* in determining coordinated neural activity—or, to use his lucent phrase, the "integrative action of the nervous system."

Once the morphological issue of how nerve cells interact had been resolved, attention naturally turned toward understanding the mechanism of synaptic transmission: was it electrical or was it chemical? The fact that there was already considerable evidence to suggest that the transmission of nerve impulses was electrical led most physiologists to espouse the view that transmission at synapses was probably also electrical. But some physiologists and most pharmacologists were convinced—and argued, at times with great vehemence—that it must be chemical. The debate between the two camps—later referred to as the "soup versus spark" controversy—was to continue for more than half a century. It was only

resolved in the late 1950s with the recognition that, although transmission at most central and peripheral synapses is mediated by chemical transmitters, at some sites it is clearly electrical, and, at a few, it is both chemical *and* electrical.

The fascinating history of this debate and how it was finally settled is described at some length in the first chapter of this volume. Here we need only mention that, as in many of the long-lasting controversies in biology, substantial progress was made only when the alternative hypotheses were sufficiently clearly articulated as to be amenable to verification (or, more importantly, falsification), when techniques of adequate "resolving power" had been developed, and when appropriate model systems had been identified.

From among the many examples that could be cited, the following may serve to make these points. Langley's use of nicotine as a chemical probe to analyze the functional organization of the autonomic nervous system led him to postulate the existence of "receptive substances" or neurotransmitter receptors, as we would now refer to them. Loewi's simple yet ingenious experiment of transferring the perfusate from the heart of one frog whose vagus nerve was being stimulated to that of another animal provided the first unequivocal evidence of a chemical mediator—his "Vagusstoff," later identified as acetylcholine. The use of the dorsal muscle of the leech as a biological assay for acetylcholine permitted Feldberg to establish that acetylcholine was released at autonomic preganglionic synapses and later, with Dale and Vogt, to pinpoint its release at the neuromuscular junction. Close arterial injection of acetylcholine (a technique perfected by Brown, Dale, and Feldberg) settled once and for all that transmission at the neuromuscular junction is cholinergic. The isolation of single nerve and muscle fibers by Kuffler permitted the first really critical analysis of the endplate potential, but this approach was soon overtaken by the use of intracellular recording with micropipettes of the type introduced by Ling and Gerard. And this, in turn, led to the discovery by Katz and his colleagues of miniature endplate potentials and in short order the formulation of the "vesicle hypothesis."

The development, at about the same time, of methods for fixing, sectioning, and staining tissues for electron microscopy made it possible not only to establish unequivocally that neurons are morphologically distinct but also to clarify the various cellular elements that make up synapses—the presynaptic process with its complement of vesicles, the synaptic cleft, and the postsynaptic specializations. Intracellular recordings from spinal motoneurons led Eccles to disprove his own most carefully crafted electrical hypothesis for synaptic inhibition and, shortly thereafter, to discover presynaptic inhibition. Using the techniques developed for cell fractionation, Whittaker and his colleagues were able to isolate first synaptosomes and later virtually pure populations of synaptic vesicles. And, finally, there was Fatt's thoughtful prediction that if the anatomical relationships were favorable transmission could be electrical,

which was soon followed by Furshpan and Potter's discovery that this was indeed correct.

Once the basic mechanisms of synaptic transmission had been established, the question arose: is acetylcholine the only neurotransmitter or are there others that remain to be discovered? In the 1970s and early 1980s, this issue was promptly answered: a variety of different transmitter substances was uncovered, beginning with noradrenaline and thereafter including several biogenic amines, a number of excitatory and inhibitory amino acids, a host of neuropeptides, and, most recently, certain gases. For most of these transmitters the relevant receptors were in time identified, their genes were cloned, and the way in which the transmitter actions are terminated was discovered. The cloning of the genes for the various receptors coincided with the emergence of molecular neurobiology as an important subdiscipline within neuroscience, and it coincidentally marked the beginning of molecular and genetic approaches to clinical neurology and psychiatry.

The cloning and sequencing of the genes that encode the many proteins involved in each aspect of synaptic transmission continue to be among the central activities in the field and appropriately form the major part of this volume. Among these proteins are the channels that permit the influx of Ca^{2+} into the axon terminal, the kinases that activate the vesicle release mechanism, the proteins that compose the vesicles themselves (including the transporters involved in neurotransmitter loading and the fusion machinery), the proteins responsible for postrelease endocytosis, those that serve to link the pre- and postsynaptic processes, the various receptors associated with the postsynaptic membrane and the proteins that interact with the receptors to bring about their localization and mediate their signaling, and finally the enzymes and transmitter reuptake mechanisms that bring to an end transmitter action.

Although the molecular biology of synapses had its origins in the late 1970s, it was only in the 1980s that it moved to center stage, and it has been responsible for the greatest progress in the past 15 years. However, other unresolved issues still command attention, including, perhaps most importantly, synaptogenesis and synaptic plasticity. Much has been learned in the past two decades about the general development of synapses and the factors responsible for their stabilization or elimination. And, ever since Bliss and Lømo discovered what they termed "long-term potentiation" in the hippocampus in 1973, behavioral neuroscientists and neurophysiologists have considered the elucidation of the mechanisms responsible for long-lasting changes in synaptic strength as critical for our understanding of the cellular basis of learning and memory.

The impetus for the present volume, in which most of these topics are reviewed, came from a workshop on synapses held at the Howard Hughes Medical Institute in June 1999. Since the workshop had brought together more than 40 of the leading figures in the field, whose work collectively covered nearly every topic of current interest to synaptologists, it seemed to us appropriate to extend this effort to a wider audience in

the form of a monograph—one that would not be simply the proceedings of the workshop but rather a collection of authoritative reviews covering most aspects of the subject. Fortunately several participants agreed to write chapters in their areas of special expertise. Only one constraint was imposed upon the authors: they were asked to present a balanced view of the current state of our knowledge and not just a summary of their own work. Since it was expected that each chapter would stand on its own, it was understood that there would be some degree of overlap among them. Where the overlap was extensive, we attempted to reduce it while, at the same time, allowing the authors' distinctive voices and viewpoints to come through with as little attenuation as possible.

We are especially grateful to our colleague Dr. Kevin Davies, science editor at the Howard Hughes Medical Institute, who not only handled most of the logistics for the workshop but also kept reminding the authors of the impending deadlines for the chapters. Kevin has assisted us in immeasurable ways throughout the editorial process, and the appearance of his name on the title page only hints at the extent of his contribution to the volume. Finally our thanks go to the Johns Hopkins University Press, and especially its director, Jim Jordan, for their willingness to publish the book and for the care they have taken to produce such a splendid volume.

W.M.C.
T.C.S.
C.F.S.

This chapter provides a historical account of the development of our current ideas about the structure and function of synapses. Many of the developments that led to our understanding of synapses and of synaptic transmission occurred between the late 1870s and the mid-1970s and revolved around the resolution of two major controversies. The first of these concerned the morphology of the neuron and more specifically the question: Are individual neurons discrete cells or part of a large syncytium? The second controversy concerned the physiology of the synapse and in particular the question: Is synaptic transmission electrical or chemical? In both cases the controversies arose because the techniques available at the time did not have sufficient analytic power to address the questions that were being asked. However, in each case, the resolution of the disputes revealed new features about the synapse.

In introducing this volume on the modern status of our understanding of the synapse and of synaptic transmission we begin at the beginning and trace the origin and evolution of these controversies, highlighting the methodological improvements that led to their resolution. In an appendix we provide a chronology of the major discoveries that paved the way for the work of the past two decades, together with the names of the investigators who made them.

Galvani, Volta, and Animal Electricity

Although the term *synapse* was not introduced until 1897, the history of what we now refer to as *synaptic transmission* extends back at least until the middle of the nineteenth century and, in one sense, as far back as the end of the eighteenth century and Luigi Galvani's discovery of "animal electricity."[1] In his great treatise *De viribus Electricitatis in Moto Musculari: Commentarius* of 1791, Galvani summarized his experiments on the contractions induced in limb muscles when he inserted one end of a metal hook into the medulla of a frog and attached the other end to an iron railing. These experiments, Galvani wrote, "worked no little wonderment within us and began to give rise to a suspicion that electricity was inherent in the animal itself. . . . [The muscular contractions] were increased by the flow, so to speak, of a very fine fluid from the nerves to the muscles, which we notice took place during the phenomenon, in the same way as the electric fluid is set free in a Leyden jar."

Galvani's contemporary and rival Alessandro Volta was later to challenge this interpretation, but his immediate reaction was one of admiration: "Signor Galvani['s] . . . brilliant discoveries . . . mark a new era in the annals of physics and medicine. The existence of a real and inherent animal electricity . . . is preserved and continues in the dissected limbs so long as some vitality is there, the play and movement of which takes place primarily between nerve and muscles" (Volta, 1792; cited in Stevens, 1971).

Volta's later claim that Galvani's frogs were simply serving as a sensitive galvanometer, reacting to the currents set up by the contact between the different metals in the hook and the railing, was soon shown to be correct. Nevertheless, the activation of the nerve-muscle synapse and the contraction of the limb musculature that followed the electrical stimulation of the brain (and the consequent activation of the synapses between the motor nerve fibers and the muscles) may rightly be regarded as the first experimental demonstration of synaptic transmission.

Bernard, Curare, and the Early Analysis of Synaptic Transmission

More direct evidence bearing on the transmission of activity from nerves to muscles came almost 90 years later, in experiments by Claude Bernard that still have a surprisingly modern ring (Bernard, 1878). Bernard's primary objective was to determine whether a muscle could be caused to contract, independent of its nerve supply. Taking advantage of the recently introduced South American Indian arrow poison curare, Bernard isolated a nerve-muscle preparation and found that, whereas an electrical stimulus to the nerve was ineffective after administering curare, a contraction could still be obtained if the stimulus was applied directly to the muscle. He carried this study one step further by preparing a frog with a ligature that interrupted the blood supply to the lower part of the body but did not interfere with the innervation of the hind limbs. When curare was now introduced above the level of the ligature, a paralysis developed that affected only the upper parts of the body, including the forelimbs. He now found that pinching the skin above the ligature did not produce movements in the upper parts of the body but nevertheless caused normal reflex movements of the hind limbs. From this experiment Bernard drew two conclusions: first, that curare does not cause a loss of sensation, and second, that the effect of the drug must be ascribed to a specific poisoning of the motor nerve or its link to the muscle because, as he had earlier shown, the muscle could still be excited directly even in the presence of curare. Since curare also did not affect the motor nerve in its more central course—from the spinal cord to the level of the ligature—Bernard concluded that the poison acts only on the most distal part of the motor nerve, probably where it makes contact with the muscle.

That a distinct process—*synaptic transmission*—was interposed between nerve and muscle was first recognized by Willy Kühne, Helmholtz's successor in Heidelberg, and by Wilhelm Krause of Göttingen. In the early 1860s Kühne and Krause provided the first good descriptions of the neuromuscular junction (Kühne, 1862; Krause, 1863) and showed a clear separation between the nerve endings and the skeletal muscle fibers. Independently they suggested that a nerve throws a muscle into contraction by means of its "currents of action" (reviewed in Kühne,

1888). The suggestion that neuromuscular transmission was essentially electrical was subjected to a rigorous analysis a few years later by Emil du Bois-Reymond, who is rightly regarded as the father of electrophysiology for his discoveries of the resting potential (which he determined by measuring the demarcation potential) and of the action potential (which he realized was due to current flow). In his two-volume work on the physiology of nerve and muscle, du Bois-Reymond (1877) raised two objections to the electrical transmission hypothesis. The first was that if current flow were responsible for synaptic transmission, it would almost certainly activate adjoining muscle fibers in addition to those innervated directly. Second, if the current were small, its cathodal (excitatory) effect would probably be counteracted by the anodal (inhibitory) current set up in the immediately adjoining area (see Grundfest, 1975, for discussion). Summarizing his views, du Bois-Reymond provided the first proposal that synaptic transmission could be mediated chemically: "Of known natural processes that might pass on excitation, only two are, in my opinion, worth talking about: either there exists at the boundary of the contractile substance a stimulatory secretion in the form of a thin layer of ammonia, lactic acid, or some other powerful stimulatory substance; or the phenomenon is electrical in nature" (1877, 2:700; cited in Davenport, 1991).[2]

We shall return later to the vexing question of electrical versus chemical transmission. But before doing so, we must consider the even more controversial and fundamental issue that dominated the thinking of neuroanatomists and physiologists between about 1870 and 1920—namely, the nature of the contacts between nerve cells. This controversy revolved around the question of whether the nervous system is composed of independent cellular units whose processes contact (but are physically separated from) other cells, or whether the nervous system is a complex syncytium consisting of a network of interlacing fibers that are not physically separate from one another but in direct cytoplasmic continuity. The debate over these two opposing views, generally referred to as the *neuron theory* (or doctrine) and the *reticular theory*, was more prolonged and decidedly more acrimonious than any other in the history of neuroscience (Shepherd, 1991).

Neuron Theory and Reticular Theory: The Remarkable Contributions of Cajal

Rather than conceiving of the processes of nerve cells as being strictly separated from each other by their surrounding surface membranes, as did the advocates of the neuron theory, the reticularists saw axonal and dendritic processes as being continuous with the processes of other cells. In retrospect, it seems difficult to understand how, at the end of the nineteenth century—more than 40 years after Schleiden and Schwann had formulated the cell theory (the idea that cells are the

structural and functional units of all living tissues and organs [see Schwann, 1839])—neuroanatomists would still be questioning whether this theory applied to the nervous system. Yet, until well into the twentieth century, some neuroanatomists continued to question whether the nervous system was made up of morphologically discrete cells. In fact, between 1870 and 1920, the reticular hypothesis had the support of some of the leading figures in the field, including Held (the discoverer of "end feet" and terminal calyces), Apáthy, Dogiel, and especially Camillo Golgi, the great Italian neurohistologist still honored for his discoveries of the chrome silver method and the cytoplasmic organelle that bears his name (Cajal, 1954).

The reticularist view, which challenged both the cell theory in general and the neuron doctrine in particular, dates from Gerlach's discovery in 1872 of a fine network of fibers in sections of the spinal cord, cerebral cortex, and cerebellum, stained with carmine and gold chloride. Gerlach interpreted this network of fibers as being formed by the anastomosis of neuronal processes. This view was reinforced in 1897 by Apáthy's studies on the leech nervous system, from which he proposed that the neurofibrils within nerve processes are continuous from one cell to the next and serve as conductors, much like electrical wires, for the flow of current from one cell to another (Peters et al., 1976).

The persistence of the reticularist view, despite the strong evidence advanced against it, is one of the more remarkable episodes in the early history of neuroscience (see van der Loos, 1967; Clarke and Jacyna, 1987; Shepherd, 1991; and Jacobson, 1993, for reviews), but it is easily accounted for by the inability of the anatomical methods available at the time to resolve cell membranes. As Cajal stated, "To settle the question [of contiguity versus continuity] definitely, it was necessary to demonstrate clearly, precisely, and indisputably the final ramifications of the nerve fibers, which no one had seen, and to determine which parts of the cells made the imagined contacts" (Cajal, 1937).

Since the visualization of cell membranes was well beyond the resolution of the light microscope, this morphological issue could not be definitively settled until electron microscopy was applied to neural tissue in the early 1950s (Bodian, 1966). It is therefore to the great credit of several neuroanatomists working at the turn of the century that, by the late 1890s, all but the most die-hard reticularists were convinced of the morphological discreteness of individual neurons. In the absence of direct microscopic evidence, Wilhelm His, August Forel, van Gehuchten, Waldeyer, Retzius, and especially Santiago Ramón y Cajal were able to adduce several independent lines of evidence for the neuron doctrine.

Waldeyer (1891) is usually credited with having formulated the neuron theory and with having clearly stated that neurons are developmentally, structurally, functionally, and pathologically discrete. However, as Cajal so trenchantly points out, although Waldeyer "supported [the theory] with the prestige of his authority, [he] did not contribute a single personal observation. He limited himself to a short brilliant exposition

[1891] of the objective proofs, adduced by His, Kölliker, Retzius, van Gehuchten and myself, and he invented the fortunate term *neuron*" (Cajal, 1954).[3]

It is important to acknowledge two contributions to the neuron theory that predated Cajal's work. These derived from the studies of His and Forel, who respectively provided developmental and pathological evidence for the individuality of neurons. His provided two types of evidence based on his histological studies of the developing spinal cord in humans and other vertebrates. First, he found that at early stages the wall of the neural tube consists of a single layer (now called the *ventricular zone*), which he described as consisting of two cell types: germinal cells, which he considered to be the progenitors of neurons, and spongioblasts, which he assumed were the precursors of the ependymal and glial elements. We now know that His's interpretation of the cellular composition of the ventricular zone was incorrect—his germinal cells are simply neuroepithelial cells that are in, or have just passed through, the M-phase of the cell cycle, whereas his spongioblasts are cells in G1, S, and G2 (Sauer, 1935). (However, his claim that the cells remained distinct throughout their phase of migration remains correct.) Second, and more important, His recognized that nerve processes are direct outgrowths of young neurons and that they end freely, without fusing with the processes of other cells. With remarkable self-confidence he wrote in 1886, "I consider it as an established principle that each nerve fiber emerges as an outgrowth from a single cell. This is its genetic, trophic and functional center. All other connections of fibers are either indirect or secondary" (cited in Hamburger, 1980).

August Forel's contribution to the neuron/reticular theory debate was based both on the earlier experimental findings of Gudden (1870) and on his own observations of Golgi-stained preparations. Gudden had noted that when a nerve is severed, the resulting neuronal atrophy (or retrograde degeneration, as we now call it) is confined to the relevant cell group and does not spread to involve neighboring populations of neurons, as might be predicted if the cells were physically continuous.[4]

A surprising aspect of Cajal's many contributions to this issue was that he began his studies completely unaware of the earlier contributions of His and Forel. The success of his work, which led him to become the foremost advocate of the neuron theory, derived in large part from his application of the chrome silver impregnation method or "reazione nera" that had been introduced by Golgi in 1873. This method, which was still widely used until recently, offered two advantages. First, the method stains, in an apparently random manner, only about 1% of the cells in any particular region of the brain or spinal cord. This makes it possible to study the morphology of individual nerve cells in isolation from their neighbors. But the method has an additional advantage: the neurons that are stained are often impregnated throughout their entire extent, so that one can clearly visualize cell bodies, axons, axon collaterals, the full dendritic arbor, and, in developing brains, axonal and

dendritic growth cones. As Cajal discovered, the method works especially well in embryonic and immature brains, and because the developing nervous system is considerably less complex than the mature brain, it is much easier to study individual neurons against the background of unstained cells. He stated:

> Since the full grown forest turns out to be impenetrable and indefinable, why not revert to the study of the young wood, in the nursery stage, as we might say? . . . If the stage of development is well chosen . . . the nerve cells, which are still relatively small, stand out complete in each section; the terminal ramifications of the axis cylinder are depicted with the utmost clearness and perfectly free; the pericellular nests, that is the interneuronal articulations, appear simple, gradually acquiring intricacy and extension. (Cajal, 1937:324–325)

By examining in detail nerve cells and their contacts in histological sections of almost every brain region, Cajal was able to describe not only differences between various types of nerve cells but also the great variety of axonal endings found in the central nervous system (CNS). This led him inexorably to conclude that the axon terminals of neurons end freely upon the surfaces of other cells and that at the sites of interaction they are not continuous with their cellular targets and therefore not part of a diffuse network.

We are fortunate in having Cajal's own reminiscences about how he arrived at this view in his delightful—if at times somewhat exuberant—*Recollections of My Life*.[5] In May 1888, he published his first critical observations on the termination of the axons of the stellate cells of the cerebellum:

> [The axons of the stellate neurons] take up a direction transverse to the cerebellar convolution, describing an arc and giving off numerous collateral branches characterized by progressive thickening. Finally both the end of the main fibre and its numerous descending processes break up into terminal fingers or tufts applied closely to the bodies of the cells of Purkinje, about which they form . . . complicated nests or baskets. (*Recollections*, 330; see also Fig. 1.1)

In the same paper he drew attention to the termination in the cerebellar cortex of the mossy fiber afferents, which "exhibit both at [their] ultimate ending and in [their] collateral branches, bunches or rosettes of short tuberous appendages ending freely" (*Recollections*, 331).

Later he was able to show that the rosettes (or "excrescences" as he referred to them) articulate directly with the clawlike ends of the granule cell dendrites. In August of the same year he made two further observations. First he identified the axons of the granule cells, which he named the parallel fibers. Then came the finding that he described in his *Recollections* as "the most beautiful [discovery] which fate vouchsafed to me in that fertile epoch, [that] formed the final proof of the transmission of *nerve impulses by contact*" (*Recollections*, 332; his emphasis). This

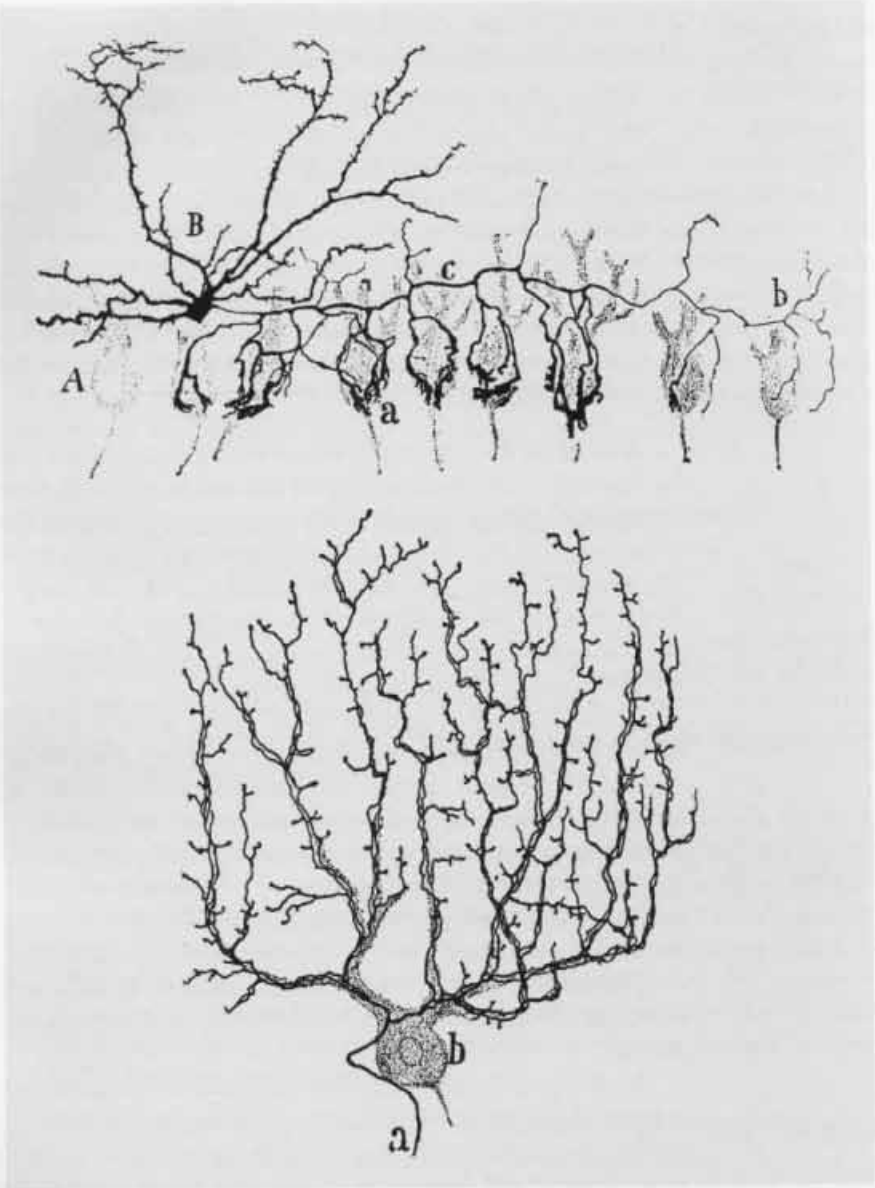


Figure 1.1. Two types of nerve ending that convinced Cajal that axons are not in continuity with their targets. The top panel depicts a cerebellar stellate neuron stained by the Golgi method, showing the basketlike arrangement of its axon collaterals that surround the bodies of the Purkinje cells. The bottom panel shows the pattern of termination of a climbing fiber along the dendrites of a Purkinje cell. Reproduced from Cajal (1995) by permission of Oxford University Press.

was his discovery of the climbing fibers to the cerebellum, which, in characteristic fashion, he described as follows:

> These robust conductors arise in the centres of the pontine region of the brain; invade the white core of the cerebellar lamella; cross the granule layer without branching; afterwards attain the level of the cells of Purkinje; and finally run over the bodies and principal outgrowths of these elements, to which they adapt themselves closely. When they reach the level of the first branches of the dendritic trunks of the Purkinje cells, they break up into twining parallel networks which ascend along the protoplasmic branches, to the contours of which they apply themselves like ivy or lianas to the trunks of trees. (*Recollections*, 332; see also Cajal, 1911)

And, if further evidence were needed that central axons ended freely, he extended his observations to the retina of birds, demonstrating that centrifugal fibers (which arise in the brain) "cross the internal plexiform zone [of the retina] and end by a free varicose arborization among the spongioblasts [i.e., amacrine cells]" and that "in the internal plexiform zone . . . the dendrites of the ganglion cells come into relation with the axons and collaterals of the bipolar cells by contact and not through a diffuse net" (*Recollections*, 339, 340).

We cite these observations at some length to illustrate not only Cajal's justifiable pride in his discoveries but also his pugnacious advocacy of the neuron theory. The defense of this theory was to be a theme to which he would return again and again, and indeed it was the subject of his last major publication, his monograph *¿Neuronismo o Reticularismo?*, published shortly after his death.[6] In this monograph he summarized the extensive body of evidence, both descriptive and experimental, that he had accumulated over the years in support of the neuron theory and listed the many varieties of axosomatic and axodendritic synaptic contacts he and others had observed in different parts of the nervous system.

Until the late 1880s, Cajal's work (which was published in Spanish, in a journal of limited circulation that he had founded) was not widely known or appreciated. But in 1889 he attended a meeting of the German Anatomical Society in Berlin and there attracted the attention of Kölliker, at that time the doyen of European anatomists. Kölliker encouraged Cajal to have his work translated into French or German. As Cajal later recalled, "Although he [Kölliker] had been a supporter of the reticular theory, he abandoned it completely, adapting himself with the flexibility of a young man to the new conceptions of contact and of the morphological independence of the neurons. In his friendliness for me, he carried his goodwill so far as to learn Spanish in order to read my earliest communications" (*Recollections*, 358).

In addition to providing the critical evidence for the neuron doctrine, Cajal also outlined two other rules that governed the functioning

of nerve cells. First, he restated the *principle of dynamic polarization* that had been originally articulated by van Gehuchten. According to this principle signaling within a neuron flows in a single, predictable direction, from the dendrites and cell body that receive inputs from other neurons to the axon and from there to the presynaptic terminals, which contact yet other neurons or effector cells. Second, he outlined the *principle of connectional specificity*, according to which nerve cells do not connect indiscriminately with one another or form random networks. Rather, each cell communicates only with certain postsynaptic targets, but not with others, and always at special points of synaptic contact. Taken together, the principles of dynamic polarization and connectional specificity form the cellular basis for the modern connectionist approach to the brain.

Sherrington and the Integrative Action of Synaptic Transmission

Although he was helped by Kölliker's support, in the long run Cajal's greatest advocate proved to be not a fellow anatomist but the physiologist Charles Sherrington, who coined the term *synapse*. In Part III of the seventh edition of Michael Foster's *Textbook of Physiology*, published in 1897, Sherrington wrote:

> So far as our present knowledge goes we are led to think that the tip of a twig of the [axonal] arborescence is not continuous with but merely in contact with the substance of the dendrite or cell body on which it impinges. Such a special connection of one nerve cell with another might be called "*synapsis*." . . . Each synapsis offers an opportunity for a change in the character of nervous impulses, such that the impulse as it passes over from the terminal arborescence of an axon into the dendrite of another cell, starts in that dendrite an impulse having characters different from its own. (929, 969)

Again we are fortunate to have Sherrington's account of the origin of the term *synapse*, from a letter he wrote to John Fulton:

> You enquire about the introduction of the term "synapse"; it happened thus. M. Foster had asked me to get on with the Nervous System part [Part III] of a new edition of his "Text of Physiol." for him. I had begun it, and had not got far with it before I felt the need of some name to call the junction between nerve-cell and nerve-cell (because the place of junction now entered physiology as carrying functional importance). I wrote him of my difficulty, and my wish to introduce a specific name. I suggested using "syndesm." . . . He consulted his Trinity friend Verrall, the Euripidean scholar, about it, and Verrall suggested "synapse" (from the Greek "clasp") and as that yields a better adjectival form, it was adopted for the book. (Fulton, 1938)

Sherrington was quick to acknowledge his indebtedness to Cajal, whom he had first met in 1885 when, as a young physician/pathologist interested in infectious disease, he had visited Spain to investigate an outbreak of cholera. We have no record of what transpired at their meeting, but from all we know of both men we can be sure that they discussed more than the weather and the differences between Spanish and English academic institutions on issues governing academic tenure. What we do know is that, a few years later, Sherrington successfully persuaded the Royal Society and his mentor Michael Foster (who by then was serving as secretary of the society) to invite Cajal to give the Croonian Lecture for 1894. Sherrington also arranged to have Cajal stay at his home during his visit to London. As Cajal later recalled, "a letter from Sherrington finally decided me [to agree to give the lecture]. . . . [He] claimed generously, as a neurologist, the right to have me stay in his home. At the present time, my host, who was then young [he was, in fact, 36] can be regarded as the leading physiologist in England." In addition to hosting his illustrious guest, Sherrington assisted him in preparing slides and in coloring drawings for his lecture, which was focused largely on his "discoveries concerning the morphology and connections of the nerve cells in the spinal cord, the ganglia, the cerebellum, the retina, the olfactory bulb, etc." (*Recollections*, 419–420).

Writing several years later, Sherrington amusingly described how, during his brief stay at their home, Cajal had succeeded in converting their guest bedroom into a temporary laboratory. He was especially impressed by Cajal's ability to glean so much about the function of nerve cells from looking at their structure, even in the poorest microscope preparation. Cajal's artistic skill enabled him to capture rapidly the essence of an observation, and to describe it with an effective use of anthropomorphisms. As Sherrington later wrote,

> A trait very noticeable in [Cajal] was that in describing what the microscope showed he spoke habitually as though it were a living scene. This was perhaps the more striking because not only were his preparations all dead and fixed, but they were to appearance roughly made and rudely treated—no cover-glass and as many as half a dozen tiny scraps of tissue set in one large blob of balsam and left to dry, the curved and sometimes slightly wrinkled surface of the balsam creating a difficulty for microphotography. . . . Such scanty illustrations as he vouchsafed for the preparations he demonstrated were a few slight, rapid sketches of points taken here and there—depicted, however, by a master's hand.
>
> The intense anthropomorphism of his descriptions of what the preparations showed was at first startling to accept. He treated the microscopic scene as though it were alive. . . . A nerve-cell by its emergent fibre "groped to find another"! Listening to him I asked myself how far this capacity for anthropomorphizing might not contribute to his success as an investigator. I never met anyone else in whom it was so marked.

And, in a more serious vein, Sherrington summarized Cajal's great contribution to synaptic transmission—the subject of this chapter—as follows:

> He solved at a stroke the great question of the direction of nerve currents in their travel through the brain and spinal cord. He showed, for instance, that each nerve path is always a line of one-way traffic only, and that the direction of that traffic is at all times irreversibly the same. The so-called nerve networks with unfixed direction of travel [the reticular theory] he swept away. The nerve-circuits are valved, he said, and he was able to point out where the valves lie—namely where one nerve cell meets the next one. (Sherrington, 1949)

Needless to say, Sherrington's contribution to this topic was not limited to coining the term *synapse* and boosting Cajal's reputation (as if this was necessary). His own work, much of which was summarized in the ten Silliman lectures he gave at Yale University—later published under the title *Integrative Action of the Nervous System*[7] and in the collection entitled *Selected Writings of Sir Charles Sherrington* edited by Denny-Brown (1979)—stands with Cajal's *Histologie du Système Nerveux de l'Homme et des Vertébrés* (1909, 1911) as one of the unquestioned foundation pillars of modern neuroscience.

Something of the flavor of Sherrington's unusual style, and also of the clarity of his thinking, is found in the following quotation from the first Silliman lecture:

> If there exists any surface or separation at the nexus between neurone and neurone, much of what is characteristic of the conduction exhibited by the reflex-arc might be more easily explainable. . . . It seems therefore likely that the nexus between neurone and neurone in the reflex-arc, at least in the spinal arc of the vertebrate, involves a surface of separation between neurone and neurone; and this as a transverse membrane across the conductor must be an important element in intercellular conduction. The characters distinguishing reflex-arc conduction from nerve-trunk conduction may therefore be largely due to intercellular barriers, delicate transverse membranes, in the former.
>
> In view, therefore, of the probable importance physiologically of this mode of nexus between neurone and neurone, it is convenient to have a term for it. The term introduced has been synapse. (Sherrington, 1906)

Sherrington's work on spinal reflexes not only convinced him that the reticular theory was untenable but also led him to delineate several defining features of synaptic transmission. First, he was struck by the valvelike, unidirectional flow of information across a synapse made by different afferent inputs to the spinal motoneurons that form the final common pathway to the muscles. The second feature was what we now call the *synaptic delay*—a measurable delay at the site of interaction beyond that attributable to the conduction time in the afferent fibers. The methods available to Sherrington were too insensitive to measure the

delay at individual synapses, but, as we shall see later, others have since provided such measurements, ranging from less than 0.5 msec to 1.3 msec (Eccles, 1964). The third feature was his conclusion that the interaction between the afferent input and the motoneurons must involve many synapses acting in concert. Central to this notion was the idea that each afferent fiber has only a small effect on a given motoneuron and that many afferent fibers need to sum, both spatially and temporally, to cause the motoneuron to discharge. Finally, Sherrington made the fundamental discovery that not all synaptic actions are excitatory: there are also inhibitory inputs, and, from a functional point of view, these are at least as important as those that lead to excitation.

The recognition of inhibition as an independent and active process was one of Sherrington's greatest accomplishments. It is all the more remarkable when one considers that neither Cajal nor any of his neuroanatomical contemporaries considered the possibility of inhibition in any of their writings. In fact—and this is especially surprising—they continued to ignore the role of inhibition and to make no reference to it long after it had been generally recognized by physiologists.

The concept of central inhibition derived largely from Sherrington's studies on what he termed the "principle of reciprocal innervation." This principle—which, as Adrian (1957) remarked, "was the clue to the whole system of traffic control in the spinal cord and throughout the central pathways"—emerged from Sherrington's experiments on flexor and extensor reflexes. These experiments demonstrated that reflex excitation of motoneurons that activate one group of muscles (e.g., the extensor muscles of a limb) is always accompanied by the inhibition of the motoneurons that innervate the antagonistic group of muscles (in this case, the limb flexors; see Creed et al., 1932, and Fig. 1.2). Writing in 1908, Sherrington addressed the issue of the interaction of excitatory and inhibitory actions on a group of motoneurons in these terms:

> It seems clear that the reflex effect of concurrent stimulation of [an] excitatory afferent nerve with [an] inhibitory afferent nerve on the vastocrureus nerve-muscle preparation is an algebraic summation of the effects obtainable from the two nerves singly. . . . One inference allowable from this is that . . . the two afferent arcs employed act in opposite direction at one and the same point of application in the excitable apparatus. . . . As to the common locus of operation, the point of collision of the antagonistic influences, it seems permissible to suppose either that it lies at a synapse . . . or that it lies in the substance of the "central" portion of a neurone. The net change which results there when the two areas are stimulated concurrently is an algebraic sum of the *plus* and *minus* effects producible separately by stimulating singly the two antagonistic nerves. (Cited in Eccles, 1964)

Sherrington was to remain interested in the nature of central inhibition throughout his career and chose it as the topic of his 1932 Nobel

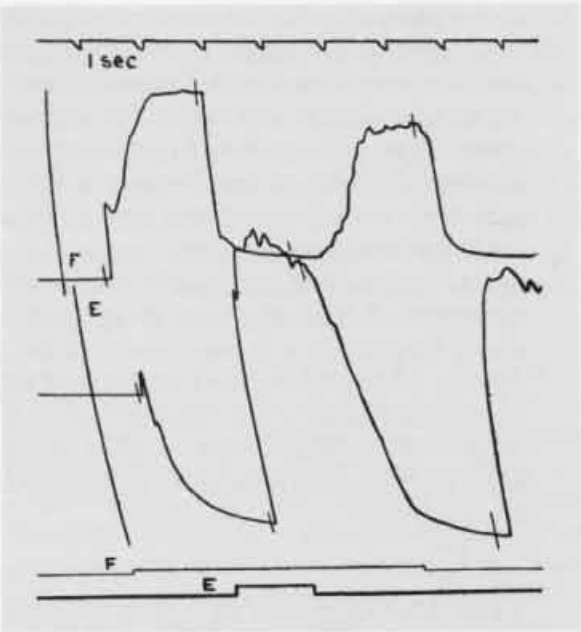


Figure 1.2. An example of reciprocal innervation. Record F is of the contraction of a knee flexor (the semitendinosus) and record E of the contraction of a knee extensor (vasto-crureus) in a decerebrate cat. The upper signal line (F) along the bottom marks the duration of the faradic stimulation of the ipsilateral peroneal nerve, while the lower signal line (E) marks the stimulation of the contralateral peroneal nerve. Note that when the flexor muscle contracts the extensor is inhibited; conversely, when the extensor is thrown into contraction the flexor is inhibited. The lower myograph recording is shifted slightly to the right, for the sake of clarity; in reality the two recordings were synchronous. Reproduced from Denny-Brown (1979) by permission of the Rockefeller Medical Library, Institute of Neurology, London.

Prize address, which he entitled "Inhibition as a Coordinative Factor." He stated with remarkable foresight:

> It is still early to venture any definite view of the intimate nature of "central Inhibition" . . . the suggestion is made that it consists in the temporary stabilization of the surface-membrane which excitation would break down. As tested against a standard excitation the inhibitory stabilization is found to present various degrees of stability. The inhibitory stabilization of the membrane might be pictured as a heightening of the "resting" polarization, somewhat on the lines of an electrotonus. Unlike the excitation-depolarization it would not travel; and, in fact, the inhibitory state does not travel.

Sherrington was, of course, not the first scientist to consider inhibition as an important physiological mechanism. The Weber brothers had described the slowing of the heart on stimulation of the vagus nerve as

early as 1845. Earlier, Sir Charles Bell (1834) had spoken of a "nervous bond" that caused muscles to "conspire in relaxation as well as to combine in contraction," while Fridrich Goltz (in whose laboratory in Strasbourg Sherrington had begun his studies of the nervous system) had demonstrated that strong cutaneous stimuli could inhibit movements in spinal dogs (Goltz and Freusberg, 1874). However, Sherrington not only saw it as an independent physiological process but also put it into the context of synaptic transmission and the integrative action of reflexes. To this extent his contribution went well beyond that of his predecessors, and, in addition, he provided the first quantitative assessment of the magnitude and time course of the inhibitory process. Most important, he correctly placed the locus of inhibition, not at the periphery (as had previously been assumed) but in the spinal cord and more particularly at the level of the synapses upon the motoneurons. In doing so he set the stage for the study of the *mechanisms* whereby synaptic transmission occurs.

The Stimulation of Certain Forms of Synaptic Transmission by Known Chemicals

While Sherrington was busy studying spinal reflexes and the role of inhibition, his Cambridge colleague (and fellow student of Michael Foster) J. Newport Langley was providing the first conclusive evidence that synaptic transmission may occur by chemical means, by investigating transmission through the peripheral autonomic ganglia. According to Davenport (1991), Langley's interest in this subject was first aroused when he received from Professor Liversidge of Sydney a sample of pituri, an alkaloid extracted from the leaves of an Australian plant. With the help of one of his students, Langley quickly established that pituri's physiological actions were identical to those of nicotine, which he then began to use as a chemical probe to study the autonomic nervous system. Among his first observations was that nicotine, applied to the superior cervical ganglion of an anesthetized cat, caused retraction of the nictitating membrane, dilatation of the pupil, and piloerection on the treated side. He subsequently used this approach to analyze the distribution of each nerve root that contributed to the sympathetic nervous system—work that he summarized in his important monograph *The Autonomic Nervous System*, published in 1921.

Initially Langley was inclined to accept the then-current view that the action of drugs such as nicotine, curare, and atropine was on nerve conduction, and that the preganglionic axons and the ganglion cells were continuous: "In the earlier accounts by Dickenson and myself upon the action of nicotine . . . we spoke of it as first stimulating and then paralyzing the nerve-cells of the ganglia since at that time we held the common view that the axis cylinder of a nerve-fibre which excited a nerve-cell was continuous with it." However, he changed his mind when he discovered

that the application of nicotine to the ganglion produced "effects like those produced by brief stimulation of its preganglionic fibers" even after a ganglion had been completely denervated by cutting the preganglionic fibers and allowing them time to degenerate (cited in Davenport, 1991). It followed that the action of nicotine must be directly on the ganglion cells. Langley came to the same conclusion when he cut the nerves to the leg muscles of a chicken and showed (again after the nerve fibers had degenerated) that injecting nicotine still caused the muscles to contract, and that this contraction could be blocked by curare. Reviewing these findings, he concluded that

> In all cells two constituents at least must be distinguished, (1) substances concerned with carrying out the chief functions of the cells, such as contraction, secretion, the formation of special metabolic products, and (2) receptive substances especially liable to change and capable of setting the chief substance in action. Further, that nicotine, curari . . . as well as the effective material of internal secretions produce their effects by combining with the receptive substance, and not by an action on axon-endings if these are present, nor by a direct action on the chief substance. (Langley, 1905)[8]

As neither nicotine nor curare prevented contraction on direct stimulation of the muscle (as Bernard had earlier reported), he concluded that the drugs must exert their (antagonistic) effects on "the receptive substance," and "this seems in its turn to require that the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by du Bois Reymond" (Langley, 1906).

Langley went on to test his ideas about receptive substances on the neuromuscular synapse. He soaked the tip of a sewing thread in nicotine and carefully touched it to the surface of the muscle fibers so as to stimulate only a small area of the muscle membrane. He found that in the innervated muscle he could produce contraction only when he applied nicotine directly to the region near the nerve endings, a region we now call the *endplate*. This experiment provided the first evidence for the localization of nicotinic acetylcholine (ACh) receptors to the subsynaptic region. Langley subsequently found that, several days after denervation, receptor sensitivity had spread throughout the muscle membrane (Langley, 1907).

In addition to his recognition of molecular receptors (as we now know them)—for which he may, with justification, be considered the "father of neuropharmacology"—Langley's earlier work on the regeneration of preganglionic fibers to the superior cervical ganglion provided the first convincing evidence for synaptic specificity and for what would later be known as the *chemoaffinity hypothesis*. Briefly, he showed that following regeneration of the preganglionic afferents, the end-organ responses to stimulating different thoracic roots (e.g., dilatation of the pupil on stimulating T_2 and constriction of the blood vessels to the ears

and piloerection on stimulating T_4) were restored to their normal pattern, from which he concluded that there must be a special (chemical) relationship between the different preganglionic fibers and the related subsets of ganglion cells (see Purves and Lichtman, 1985, for discussion).

A short while before Langley published his studies on the effects of nicotine on sympathetic ganglia and striated muscle, Oliver and Schäffer reported that intravenous injections of a glycerol extract of the adrenal gland produce a marked increase in arterial blood pressure (Oliver and Schäfer, 1895). At about the same time, a Czech scientist, Szymonowicz, made essentially the same observation but found, in addition, that if the vagus nerve was cut the extracts of the adrenal medulla also produced a marked increase in heart rate (Szymonowicz, 1896; cited in Davenport, 1991). Although it was to be some years before this work was extended to the postganglionic sympathetic outflow, these findings were most easily accounted for by postulating that the actions of the adrenal extracts were mediated by a second class of Langley's postulated "receptive substances."

Shortly after "adrenalin" was purified and its chemical structure identified by the Japanese scientist Jokichi Takamine (Davenport, 1982), one of Langley's students, T. R. Elliott, examined its effects on the peripheral target tissues innervated by the sympathetic nervous system. In short order, Elliott was able to show that the effects of adrenaline, whether excitatory or inhibitory, closely paralleled those observed on stimulating the relevant postganglionic fibers. And (again echoing Langley), Elliott found that degeneration of the target tissues' sympathetic input had no effect on (or, on occasion, even accentuated) adrenaline's effect on the target tissues. This led Elliott to conclude that impulses in sympathetic nerves lead to the release of a small quantity of adrenaline or a related compound. In a paper presented to the British Physiological Society, he wrote that "Adrenaline might . . . be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery" (Elliott, 1904).

Prompted by this success, Elliott turned his attention to striated muscle. However, apart from demonstrating that it was not stimulated by adrenaline, he made little progress and regretfully concluded that he had "tried in vain to discover an active substance in the muscle plates of striped muscle" (cited in Davenport, 1991).

Inspired by Elliott's findings on adrenaline, Walter E. Dixon turned his attention to the mediator of the known inhibition of the heart on stimulation of the vagus nerve. To study this phenomenon he removed the heart of a dog (while it was inhibited by stimulating the vagus) and rapidly made an extract of the heart tissue, which he applied to a beating frog heart. Although the frog heart was clearly inhibited by some factor in the extract, Dixon was never able to purify the active component. But his findings led him to postulate that parasympathetic stimulation acts through the release of a substance whose effects mimic those of the natural alkaloid muscarine (Dixon, 1906).

Ironically, at a meeting of the British Medical Association in Toronto where Dixon reported his findings, Reid Hunt of the U.S. Public Health and Marine Hospital Service (the precursor to the National Institutes of Health) described his efforts with his colleague René de M. Taveau to isolate agents that slowed the heart and lowered blood pressure. They had found no fewer than 19 derivatives of choline that lowered blood pressure, of which ACh was by far the most potent (Hunt and Taveau, 1906). Yet neither Dixon nor Hunt (nor anyone else who was present) seems to have made the connection between the two sets of observations, and it was not until several years later that ACh was positively identified as the transmitter involved.

Dale, Loewi, and Feldberg: Establishing Acetylcholine as a Chemical Transmitter

At this point another towering figure appeared on the stage of the synaptic transmission saga: Henry Hallett Dale, another of Langley's students and a close friend of Elliott.[9] Working at the Wellcome Physiological Research Laboratories, Dale began to analyze a large number of sympathomimetic amines that had been isolated by his colleague George Barger. He tested not only their effects on blood pressure but also their actions on several organs and tissues (the pupil of the eye, the nictitating membrane, the salivary and lachrymal glands, the bladder, and the nonpregnant uterus). In this way, Dale identified αl-aminoethyl catechol (later known as noradrenaline) as a particularly potent vasopressor that, unlike αl-adrenaline, did not inhibit contractions of the nonpregnant uterus. Concurrently he began a long series of studies on the actions of ergot, another naturally occurring alkaloid whose effects on the pregnant uterus had been known for almost 2000 years (Gilman et al., 1990). Again he turned to one of his colleagues, Arthur Ewins, to isolate components from ergot mixtures, and he began to look for components with pharmacological properties similar to those of muscarine (which he originally thought might be in the ergot preparations). Although they failed to find muscarine in any of the isolates, they discovered what later proved to be ACh. They found that each preparation, which had a muscarinelike action in their standard assay (loops of rabbit intestine), was associated with choline. And the fact that the active factor was alkaline-sensitive suggested to Dale that it might be ACh. In 1914 he described his experiences with various choline esters:

> The question of a possible physiological significance, in the resemblance between the action of choline esters and the effects of certain divisions of the involuntary nervous system, is one of great interest, but one for the discussion of which little evidence is available. Acetylcholine is, of all the substances examined, the one whose action is most suggestive in this direction. The fact that its action surpasses even that of

> adrenine, both in intensity and evanescence, when considered in conjunction with the fact that each of these two bases reproduces those effects of involuntary nerves which are absent from the action of the other, so that the two actions are in many directions at once complementary and antagonistic, gives plenty of scope for speculation. (Dale, 1914)

Later, looking back on this time, he remarked:

> Such was the position in 1914. Two substances were known, with actions very suggestively reproducing those of the two main divisions of the autonomic system; both, for different reasons, were very unstable in the body, and their actions were in consequence of a fleeting character; and one of them was already known to occur as a natural hormone. These properties would fit them very precisely to act as mediators of the effects of autonomic impulses to effector cells, if there were any acceptable evidence of their liberation at the nerve endings. The actors were named, and the parts allotted; a preliminary hint of the plot had, indeed, been given ten years earlier, and almost forgotten; but only direct and unequivocal evidence could ring up the curtain, and this was not to come till 1921. (Dale, 1938)

What happened in 1921, of course, was Otto Loewi's great discovery of "Vagusstoff." The story of this discovery and of the dreams that led him to perform the critical experiment have become part of the folklore of neuropharmacology. In his own words:

> The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again; it occurred to me at six o'clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o'clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design.

Like most remembered dreams, Loewi's account is distorted. It misstated the chronology (the experiment was performed in 1921, not 1920 as he first reported) while, at the same time, exaggerating the most dramatic event: "[I went to the laboratory at three o'clock in the morning] and at five o'clock the chemical transmission of [the] nervous impulse was proved" (Loewi, 1953).

A more prosaic account would simply recall that, using a readily available technique, Loewi carried out 14 experiments on two species of frogs and on four toads over a period of several days. The experiment involved isolating two hearts and perfusing each through a glass cannula with Ringer's solution. After the vagus nerve to one of the hearts was stimulated and its well-known inhibitory effect observed, the fluid from that heart was transferred (probably using a glass pipette) to the

second heart, where it promptly caused a decrease in the strength and frequency of beating.

In many respects Loewi was fortunate in both the timing of the experiments and his choice of preparation. The frog vagus contains both stimulatory and inhibitory fibers, but in winter the inhibitory fibers predominate (his experiments were carried out in February or March). And the cholinesterase content of the frog heart is low (compared with that of the mammalian heart), so that the released transmitter remained active (at the low temperature of the unheated laboratory) long enough for its effects on the second heart to be observable. In an important control experiment showing that the inhibitory action of Vagusstoff could be completely blocked by the prior administration of atropine, Loewi was able to rule out an alternative possible explanation for his findings—namely that they were due to the release of potassium, as Howell and Duke (1908) had suggested.

Davenport (1991) has discussed at some length the inconsistencies in Loewi's account and the difficulties encountered by others who attempted to replicate his experiments. However, the issue was finally resolved in 1933, when Wilhelm Feldberg and Otto Krayer conclusively demonstrated (with appropriate controls) that stimulating the vagus nerve of a dog released an ACh-like substance into the coronary sinus. This caused the dorsal muscle of a leech to contract and the blood pressure of a cat to fall, provided that the ACh-degrading enzyme, cholinesterase, was blocked by the prior administration of eserine (Feldberg and Krayer, 1933).

Feldberg was to dominate the early thinking on ACh and on chemical synaptic transmission in the period 1930–1950 much as Bernard Katz was later to dominate the field from 1950 to 1970. Feldberg's experiments with Krayer were only the first of a long series of studies he carried out on the role of ACh as a neurotransmitter (Feldberg, 1950, 1977; Bisset and Bliss, 1997). As a medical student in Berlin he had begun working during his vacations at the Physiological Institute of the university and had become fascinated by Langley's book on the autonomic nervous system. His mentor, Schilf, accordingly arranged for him to work with Langley at Cambridge, but unfortunately Langley died within six months of Feldberg's arrival. Yet the two years Feldberg spent at Cambridge had a lasting effect on his career: "I read and re-read all of Dale's papers," he wrote. In 1927 he returned to the Physiological Institute in Berlin but was summarily dismissed from his position in the university in 1933, at the outset of the Nazi purge of Jewish academics.[10] Hearing of his plight, Dale invited Feldberg to join him at the National Institute for Medical Research in London, and it was here that he carried out much of his later work on ACh as a transmitter.

While still in Berlin, Feldberg had undertaken a series of experiments to clarify what was known as the Vulpian-Heidenhain paradox—contraction of the muscles of the tongue on stimulating the parasympathetic outflow in the lingual nerve, after interruption of the hypoglossal

nerve. Dale had suggested that this might be due to the release of ACh from the endings of the parasympathetic fibers to the tongue. To prove this, Feldberg (1933) succeeded in collecting fluid from the cannulated lingual vein and passing it over his favorite assay, the dorsal muscle of the Hungarian leech, which Fühner (1917; cited in Davenport, 1991) had shown to be exquisitely sensitive to ACh.

Shortly after moving to London, Feldberg succeeded (with Gaddum) in perfusing the superior cervical ganglion with eserinized Locke's solution, and he was able to show that stimulation of the preganglionic fibers (which caused retraction of the nictitating membrane) resulted in the release of ACh into the perfusate (Feldberg and Gaddum, 1934). Over the next 15 years he carried out many other studies on the role of ACh (see Bisset and Bliss, 1997, for references). These included investigations of the mechanism of transmission by the gastric vagus (with Dale), by the preganglionic fibers to the adrenal medulla (with Mintz and Tsudzimura), and by the postganglionic sympathetic fibers to the sweat glands (again with Dale).

These several actions all belonged to the category called *muscarinic* by Dale, because they were simulated by muscarine and shared several additional features: (1) there was a long delay between the electrical stimulation of the nerve and the onset of the response of the innervated organ; and (2) the response itself was long-lasting and often persisted well after nerve stimulation had come to an end, with only a very gradual return of the organ or tissue to its baseline level of activity. One needed only to look at the results of experiments in which these autonomic nerves were stimulated to become convinced that by far the best general theory that could account for all these slow actions is one that allows a more or less labile substance to be interposed between the nerve endings and the effector cells, be they smooth muscle or glands.

Following the Feldberg-Krayer experiment of 1933, and the subsequent experiments of Feldberg, little doubt remained in the minds of almost everyone—not only the pharmacologists such as Loewi, Dale, and Feldberg, but even the neurophysiologists such as Eccles, Lorente de Nó, and Erlanger—that muscarinic actions were mediated by chemical transmitters and specifically by ACh. Indeed, the elegance of the Feldberg experiments made it seem that muscarinic actions were the very prototype of chemical synaptic actions. However, in addition to the muscarinic actions of ACh, Dale had described a second class of cholinergic actions, which he termed *nicotinic* because they could be elicited when muscarinic actions had been blocked by atropine. Nicotinic actions were found in the adrenal medulla, in the preganglionic neurons of the sympathetic and parasympathetic ganglia, in skeletal muscle, and in the electroplaques of electric fish. But, in contrast to the general acceptance of muscarinic actions, in 1935 it seemed unlikely to almost everyone, but particularly to neurophysiologists, that nicotinic actions could be mediated by a chemical process. Unlike muscarinic actions, which were very

slow, nicotinic actions were fast, and their rapidity did not seem consistent with a chemical mechanism.

There were, of course, some preliminary clues that even nicotinic effects might be chemically mediated. We have mentioned the experiments of Claude Bernard and others that had shown that curare could block neuromuscular transmission. It was subsequently found that curare could also block transmission in autonomic ganglia. Finally, it was known that ACh also caused contraction of isolated striated muscles of frogs and toads and that, as Langley had observed, nicotine first excited and then blocked skeletal muscle and sympathetic postganglionic cells. But these pharmacological actions of nicotine and ACh on sympathetic ganglion cells and on skeletal muscles seemed for the longest time without physiological significance, and it was not until Feldberg turned his attention to the issue of fast synaptic transmission that the tide began to turn.

The first critical step was taken by Feldberg and Mintz as early as 1933 when they found that nicotinic transmission to the adrenal medulla was cholinergic. As the chromaffin cells of the adrenal medulla are homologous to sympathetic ganglion cells and are innervated by preganglionic sympathetic fibers, it seemed likely that preganglionic sympathetic fibers that synapse in sympathetic ganglia would also be cholinergic. As noted previously, in 1934 Feldberg and Gaddum, using a perfusion method, showed beyond question that on preganglionic stimulation ACh is liberated from the superior cervical ganglion, as it is from the adrenal medulla. And with Dale, Feldberg showed that ACh was the mediator of vagus effects on the stomach (Dale and Feldberg, 1934). From here it was only one step further to examine skeletal muscle. This junction was particularly important for the doubting Thomases, because transmission at the neuromuscular junction had long been considered the most critical test of the chemical hypothesis.

In 1936 Dale, Feldberg, and Marthe Vogt studied the effects of stimulation of the hypoglossal nerve (the motor nerve to the tongue) in cats and dogs in which the parasympathetic outflow in the chorda tympani had previously been severed, and also the effect of stimulation of ventral roots on skeletal muscles in the hind leg, after section of the sympathetic chain. These experiments showed unequivocally that stimulation of the motor fibers (but not sensory or sympathetic fibers) resulted in the release of ACh. Such release did not occur on stimulating denervated muscles or muscles treated with tubocurarine. Also, in 1936, G. L. Brown, Dale, and Feldberg were able to induce a muscle twitch, similar to that seen after nerve stimulation, by injecting ACh directly into the artery supplying the gastrocnemius muscles of cats and dogs close to its entry into the muscles. They further showed that in the presence of eserine a single electrical stimulus to the sciatic nerve elicited a brief tetanus (rather than a simple twitch). The response to tetanic stimulation, on the other hand, was depressed under these conditions because of the accumulation of ACh at the neuromuscular junction.

To summarize, by 1936 Feldberg and his colleagues had found that the motor nerve impulse releases ACh at the neuromuscular junction, and that when it is induced by close intraarterial injection, it causes a brief muscle twitch or, following a single electric shock to the nerve, it gives rise to a short tetanic contraction if the breakdown of ACh is prevented by the prior administration of an anticholinesterase drug. Quantitatively, the amounts of ACh liberated by a nerve impulse and the amount injected are of different orders of magnitude, but this is to be expected because the experimental application is not directly to the active site. Since a blocking dose of curare does not affect the liberation of ACh, but does block its ability to cause muscle contraction, the conclusion that ACh is critical for the neural excitation of muscle fibers was inescapable.

Finally, Feldberg crossed the English Channel in 1939, just before World War II broke out, to collaborate in Arcachon, France, with Albert Fessard on the electric organs of the electric ray, *Torpedo*. They found that the nerves to the electroplaques released ACh and that, on injecting the transmitter into the solution perfusing the electric organ, there was an electrical discharge that could be markedly potentiated with eserine (Feldberg and Fessard, 1942). To the British pharmacologists the situation was now crystal clear. Peripheral transmission was obviously chemical, and it seemed very likely that this would be proved to be true also of central transmission (Feldberg, 1945). The only question remaining was: are ACh and adrenaline (or noradrenaline) the sole transmitters or are there others yet to be discovered?

The Soup versus Spark Controversy

Feldberg's pioneering work had convinced many electrophysiologists, especially Bernard Katz, who, like Feldberg, had emigrated from Germany and was working in A. V. Hill's department at University College, London. However, some physiologists still remained skeptical and favored electrical transmission at sites of nicotinic actions. The short latency and the brevity of the postulated transmitter actions (lasting, at most, just a few milliseconds) suggested that transmission was simply too fast to be chemical. Thus began the hotly debated argument between the two factions—facetiously referred to as the "soup versus spark controversy"—that was to govern thinking about synaptic transmission from 1936 until the early 1950s.

The finding that ACh was released by nerve stimulation at sites of nicotinic action and the speed of the synaptic actions were reconciled by John Eccles, one of Sherrington's last students and certainly his most productive. Eccles argued that there are two components to transmission at nicotinic synapses: (1) an initial, fast excitatory action mediated electrically by the presynaptic action currents; and (2) a prolonged resid-

ual action mediated by chemical transmitters such as ACh. Although Eccles was the most forceful advocate of this view and persistently presented it in a number of reviews (Eccles, 1936, 1937, 1946, 1949), he was not alone in this belief. For example, Monnier and Bacq (1935) had shown that the response of smooth muscle to stimulation of the relevant nerves exhibited an initial fast and a later slow phase. In the United States, the electrical hypothesis found strong support among the group of electrophysiologists whom Ralph Gerard referred to as the "axonologists."[11] As documented in the Symposium on the Synapse held in 1939, their consensus view was that the current generated by the presynaptic axon flows into the postsynaptic cell, where it excites an impulse, much as one active segment of an axon excites the next (Fig. 1.3). In a word, the process of cell-to-cell transmission was simply an extension, to the synapse, of Alan Hodgkin's local circuit model for conduction of the nerve impulse (Hodgkin, 1937a,b).

However, two discoveries made it necessary to reconsider these views of electrical transmission. The first came from a quantitative analysis of the amount of current that a presynaptic neuron could inject into a postsynaptic cell, and the second was the discovery of the endplate (and, later, other synaptic) potentials.

Current Flow between Contiguous Axons

In retrospect it is surprising that not one of the proponents of the electrical theory seems to have bothered to ask: is the current from a presynaptic axon quantitatively adequate to excite a postsynaptic cell? The first attempt to estimate this current came in 1940 from studies of *ephapses*, artificial synapses constructed by closely approximating two axons to one another. Some of these studies appeared at first to provide support for electrical transmission by showing that there is an excellent correspondence between the effects predicted by the local circuit theory of nerve conduction and the effects observed in the neighboring axons. It was therefore concluded that the effects are caused by the electrical current flow across the ephapses (Arvanitaki, 1942; Eccles, 1946).

However, Bernard Katz and O. H. Schmitt (1940) pointed out that the penetrating current acting on the resting fiber at an ephapse is virtually a mirror image of the current in the active fiber. As a result the active fiber produces a triphasic excitability change in the inactive fiber—depression, followed by excitation, and then depression. In addition, the excitatory action produced by one fiber in the other is normally much too weak to initiate an impulse in the inactive fiber. For example, in Katz and Schmitt's experiments the maximum excitatory effect never exceeded 20% of the threshold required to initiate an action potential in the second fiber. It followed from this finding that if electrical excitation were to be adequate for synaptic transmission, special conditions would have to prevail at synaptic contacts. In fact, in Arvanitaki's

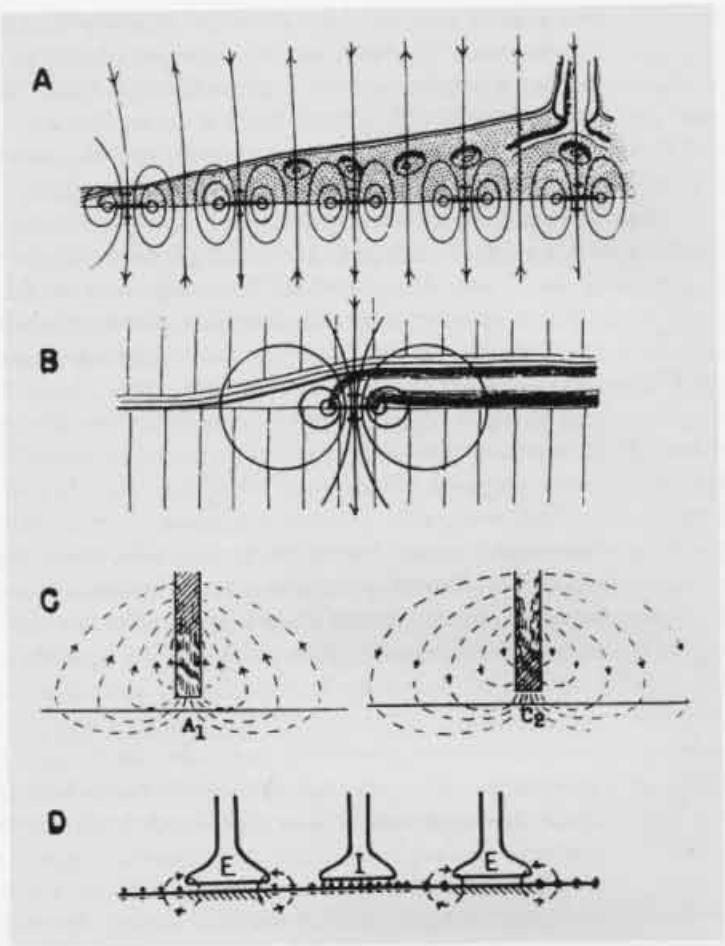


Figure 1.3. Some models of electrical transmission.

(A) Du Bois-Reymond's "modified discharge" hypothesis for the neuromuscular junction.

(B) Du Bois-Reymond's diagram of current flow at the "point of contact" between the motor axon and the muscle fiber.

(C) Eccles's 1946 model for electrical transmission between neurons, showing the pattern of current flow as the nerve impulse approaches the terminal, causing first a hyperpolarization due to inward current flow (A_1) and then, when it reaches the terminal, a depolarization and excitation (C_2).

(D) Brooks and Eccles's (1949) hypothesis for electrical transmission at excitatory (E) and inhibitory (I) synapses. In I the terminal was postulated to be that of a nonspiking Golgi II interneuron, which would produce an anodal focus at its point of contact with the target cell.

Reproduced from Grundfest (1959) by permission of the American Physiological Society.

experiments this was achieved by adjusting the Ca^2 concentration of the bathing solution, thereby increasing excitability at the ephapse; under these conditions impulses were initiated across the experimental ephapse, but only when the axons were chelated.

The experiments by Katz and Schmitt made Eccles realize that electrical synaptic transmission could not function as at an ephapse, and in particular that it could not occur at any arbitrary point where a presynaptic fiber happened to contact a postsynaptic cell. Rather, for electric transmission to occur there would have to be some form of membrane specialization where special conditions for current flow would prevail. He therefore modified his earlier view and postulated that the postsynaptic membrane at the synaptic region had what he termed "the special property of electroreception."[12]

Discovery of the Synaptic Potential

Not only did the discovery in 1938 of the endplate potential present a second serious challenge to the electrical transmission hypothesis, but its later analysis by Stephen Kuffler and Katz was to provide the foundation of our current views of chemical synaptic transmission.

In 1938 Göpfert and Schaefer discovered that the action potential in the presynaptic fiber does not lead directly to the initiation of an action potential in the postsynaptic muscle fiber, a finding that was confirmed the following year by Eccles and O'Connor (1939). Both groups observed that the action potential in the muscle cell did not arise directly out of the baseline but was preceded by a smaller and slower transitional potential. This slower potential is normally lost in the much larger action potential, but it can be unmasked by large doses of curare. In the presence of curare, it was evident that the nerve impulse in the presynaptic axon sets up a local depolarization at the muscle endplate. This local depolarization, which soon became known as the *endplate potential* (EPP), seemed to act like an electronic potential produced by a subthreshold current (Eccles et al., 1941). Equally important, Kuffler was soon to show that there is an irreducible delay, a *synaptic delay* (as first suggested by Sherrington), between the action potential in the axon terminals and the start of the endplate potential.

Similar local, graded potentials were soon demonstrated at other synapses—in the cat sympathetic ganglia, the squid stellate ganglion, and spinal motoneurons in frogs and cats. In each case excitation of the presynaptic axons was found to give rise, with a measurable delay, to a slow depolarization of the postsynaptic cell: these local depolarizations were appropriately termed *synaptic potentials*. Thus by the late 1940s it was generally agreed (1) that synaptic potentials probably occur at all sites of synaptic transmission; (2) that they provide an essential functional link between the action potential in the presynaptic terminal and that in the postsynaptic cell; and (3) that the properties of the synaptic potential are distinctly different from those of either the pre- or the

Figure 1.4. Three of the major contributors to synaptic transmission. Photographed at an international scientific meeting in the 1960s are, from left to right, Stephen Kuffler, Bernard Katz, and John Eccles. (Another photograph, taken when they worked together in Sydney in the 1940s, has been reproduced frequently elsewhere.)

postsynaptic spike in that they are much slower than action potentials and are graded rather than all-or-none.

In his continuing rearguard action against the growing evidence for chemical transmission, Eccles interpreted the synaptic potential as a reflection of the special property of electroreception. He argued that the postsynaptic subjunctional membrane is specialized to give only local responses of graded intensity without the sudden all-or-nothing breakdown of resistance that occurs with the initiation of an impulse. According to this view, the presynaptic impulse sets up an electric current that exerts a diphasic effect on the junctional region of the postsynaptic cell, first an inhibitory (or anodal) focus followed by an excitatory (cathodal) focus with an inhibitory (anodal) surround (Fig. 1.4). The excitatory focus would, Eccles believed, set up a brief and intense local response at the synaptic region that would spread electrotonically over the postsynaptic cell membrane. On reaching a certain threshold, the depolarization of the extrajunctional membrane would finally set up a propagated impulse. The subsynaptic specialization of the postsynaptic membrane was thus seen as an amplifier of the small electrical currents that flowed from the presynaptic axons, acting until the synaptic potential was of sufficient amplitude to trigger an action potential in the postsynaptic cell.

The Experiments of Kuffler and of Fatt and Katz Turn the Tide toward Chemical Transmission

Despite Eccles's progressively more ingenious explanations, by the late 1940s the tide was clearly turning against the electrical hypothesis for transmission at peripheral synapses, as Eccles himself later acknowledged. In his 1964 monograph *The Physiology of Synapses,* he wrote that "In the Paris symposium of 1949 there was fairly general agreement that both neuro-muscular and ganglionic transmission were mediated by ACh, particularly as Kuffler . . . reported most convincing experiments against the electrical hypothesis. *However, there was still fairly general agreement that central synaptic transmission was likely to be electrical*" (emphasis added).

The specific experiments of Kuffler to which Eccles alluded were directed toward three key issues: (1) the synaptic delay; (2) the consequence for the EPP of altering the configuration of the action potential in the presynaptic terminals; and (3) the effects of subthreshold stimulation of the nerve terminals.

The earlier measurements of what was referred to as the *synaptic delay* or *neuromuscular delay* had not been precise because they determined only the latency between the action potential in the presynaptic fibers and that of the muscle or postsynaptic ganglion cell, rather than that between the action potential in the axon terminals and the onset of the EPP or synaptic potential. Kuffler was able to address this issue critically for the first time by carefully dissecting single nerve–muscle fiber preparations, which Katz considered "a brilliant technical feat [that] immediately and deservedly put [Kuffler] 'on the map'" (Katz, 1982).

By stimulating within 0.5 mm of the electrode used to record the EPP, Kuffler found that, in frogs at 20°C, the synaptic delay is on the order of 0.8–0.9 msec. This delay was not appreciably reduced even when the stimulating electrode was as close as 50 μm to the endplate region. If the entire delay were attributable to conduction in the presynaptic terminals, this finding would have implied that the presynaptic action potential was slowed by a factor of about 300 from its prior velocity in the distal part of the nerve, which seemed unlikely. Moreover, since the duration of the EPP is long compared to that of the preceding action potential, it would be necessary to assume that current flow in the presynaptic terminals lasts at least 4–5 msec. This could only occur if the action potential was followed by a depolarizing afterpotential of long duration, and only if the postulated potential was important in depolarizing the terminals and effecting the release of the transmitter. To test this idea, Kuffler exposed the nerve terminals to veratrin, an alkaloid that enhances depolarizing afterpotentials, and found that even though the depolarizing afterpotential was greatly increased, it had no effect on the amplitude of the EPP as recorded from the muscle. Finally, when a subthreshold depolarizing current pulse was applied within 0.5 mm of the terminals, Kuffler

found that it had no appreciable effect on the transjunctional potential changes. In a word, the severe attenuation of current spread from the nerve to the muscle effectively ruled out the possibility that transmission at the endplate could be electrical (Kuffler, 1942a,b).

The final blow to the theory of electrical transmission at the neuromuscular junction was delivered in the early 1950s by Bernard Katz and his colleagues in the department of biophysics at University College, London, in a series of experiments that elevated the analysis of synaptic transmission to an entirely new level. Katz was another of that distinguished group of scientists who had been compelled to leave Germany in the 1930s, and for a short period in the early 1940s he had been associated with Eccles and Kuffler in Sydney, Australia. Katz had received a thorough grounding in biophysics in Germany, and as early as 1939 he had published an important monograph, *Electrical Excitation of Nerve.* On returning to England from Australia, he first collaborated with Alan Hodgkin on the study which established that the rising phase and overshoot of the action potential is due to a sudden increase in sodium permeability (Hodgkin and Katz, 1949). He then joined Hodgkin and Huxley in their initial experiments to test the Na^+ hypothesis by carrying out voltage clamp experiments to analyze Na^+ and K^+ currents during and immediately following the action potential (Hodgkin et al., 1952). But it is for his seminal series of studies with José del Castillo, Paul Fatt, and Ricardo Miledi on synaptic transmission that he is perhaps best known. Indeed, it was for this work that he shared the Nobel Prize for Medicine or Physiology in 1970 with Julius Axelrod and Ulf von Euler.

In the first set of studies with Fatt, Katz extended the ionic hypothesis to synaptic transmission by providing a critical analysis of the ions that flow during the synaptic potential. For these experiments Fatt and Katz used sharp-tipped intracellular recording microelectrodes of the type developed by Ling and Gerard (1949) and used earlier to analyze ion fluxes in muscle fibers by Nastuk and Hodgkin (1950). Intracellular microelectrode recording enabled Fatt and Katz to circumvent many of the technical difficulties involved in dissecting single nerve–muscle fiber preparations and the uncertainties associated with extracellular measurements due to the shunting effects of interstitial fluid (Fatt and Katz, 1951, 1952). Also by using curare, they were able to reduce the amplitude of the EPP below the threshold for action potential initiation and in this way to study the EPP in isolation.

Fatt and Katz found that the EPP produced in the muscle cell by the action of the motor nerve was largest when they placed the recording intracellular electrode precisely at the endplate. As the electrode was moved progressively farther away from the endplate region, the amplitude of the EPP decreased systematically (Fig. 1.5). From these findings they concluded that the EPP is generated by inward current that is confined to the endplate and spreads passively away along the muscle fiber from the region of the endplate. They further found that the synaptic potential at the endplate rises rapidly but decays more slowly. They at-

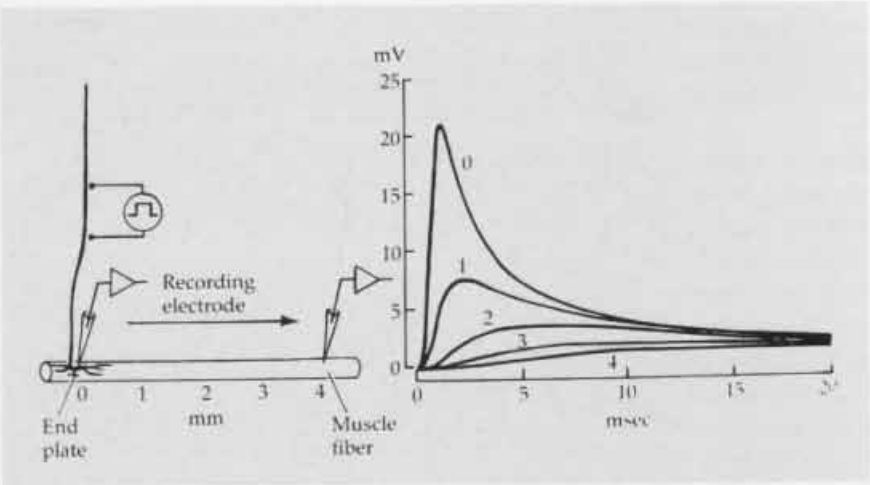


Figure 1.5. Decay of synaptic potentials with distance from the endplate region of a muscle fiber. Records taken by an intracellular electrode at distances of 1, 2, 3, and 4 mm from the endplate show a progressive decrease in size and slowing of rise time of the synaptic potential. Reproduced from Kuffler and Nicholls (1976), after Fatt and Katz (1951), by permission of Sinauer Associates, Inc.

tributed the rapid rise to the sudden release of ACh into the synaptic cleft by the action potential in the presynaptic terminal. Once released, the ACh would diffuse rapidly to the receptors on the surface of the muscle fiber. However, not all the released ACh reaches the postsynaptic receptors, because two processes act to remove it from the cleft: some simply diffuses away out of the synaptic cleft and some is hydrolyzed by the enzyme acetylcholinesterase, which is localized in the intervening basal membrane. Not surprisingly, after treatment with a cholinesterase inhibitor, the EPP is greatly prolonged, and the charge transfer through the endplate can be increased by as much as 50-fold.

Fatt and Katz also examined the mechanism underlying the EPP and suggested that it involved an increase in conductance that was nonselective for Na^+, K^+, and Cl^- and served, as it were, to short-circuit the resting membrane potential. (Functionally it was equivalent to placing a fixed leak resistance across the membrane.) As a result, there was a direct relationship between the value of the resting membrane potential and the amplitude of the EPP. When they examined the reversal potential (i.e., the membrane potential at which the EPP is nullified), they found it to be 14 mV. At more positive potentials, the normally depolarizing EPP reversed its sign and became a hyperpolarizing response. The fact that the values of the postsynaptic membrane potential determined the amplitude, and even the sign, of the synaptic potential indicated that the "battery" responsible for the endplate current must be located in the postsynaptic membrane. This finding effectively excluded a presynaptic source for the current, as had been predicted by the "spark

hypothesis," but was exactly what was to be expected if transmission were chemical.

Since the value of the reversal potential, 14 mV, did not match the equilibrium potential of Na^+, K^+, or Cl^-, the EPP could not be attributed to an increased conductance of any *single* ion species (as is the case for Na^+ influx during an action potential). This reversal potential was instead consistent with a simultaneous increase in the conductance of several ion species.

On the basis of these findings, Fatt and Katz proposed that the release of ACh produced a drastic change in the membrane at the endplate—in effect a short circuit—so that it became transiently permeable to all the major ions: Na^+, K^+, and Cl^-. However, they could not distinguish such a generalized increase in conductance to all ions from a more selective but simultaneous increase in two-ion species such as Na^+ and K^+, or Na^+ and Cl^-. These alternatives were tested several years later by A. Takeuchi and N. Takeuchi (1960) using a voltage clamp technique. They found that in curarized preparations there was an almost linear relationship between the membrane potential and the endplate current, as had earlier been observed by Fatt and Katz. But when the Takeuchis changed the ions in the bathing solution they found that in response to the release of ACh, the endplate became selectively permeable to Na^+ and K^+, but not to Cl^-. The finding of a selective increase in cation permeability, rather than a general permeability breakdown, has subsequently proven to be common at excitatory synapses.

After the appearance of the Fatt and Katz paper, Eccles (1964) rapidly accepted this model for synaptic transmission at the endplate and other peripheral synapses: "It would seem probable," he wrote, "that like the endplate transmitter, the synaptic transmitter [at other peripheral sites] would cause its intense depolarizing action by a large nonselective increase in the ionic permeability of the subsynaptic membrane." He was not, however, ready to accept a similar mechanism for central synaptic transmission.

Eccles's Discovery of Synaptic Inhibition Ends the Soup versus Spark Controversy

As the evidence against electrical transmission at peripheral synapses became incontrovertible, Eccles retreated to the CNS, where he thought that the evidence for electrical transmission was still compelling. In his last major review on this controversy in 1949, entitled "A Review and Restatement of the Electrical Hypothesis of Synaptic Excitatory and Inhibitory Action," written after Kuffler's experiments of 1942, he wrote: "In view of the exclusion of the electrical hypothesis from the neuromuscular junction and the uncertainty of its application to synaptic transmission in ganglia, where acetylcholine transmission also is operative, it would seem expedient to restrict it in the first instance to mono-

synaptic transmission through the spinal cord, where chemical transmission by acetylcholine seems highly improbable, and where the experimental investigation has been more rigorous than elsewhere in the [central] nervous system."

A few years before Eccles wrote this review, he had become friendly with the Viennese philosopher Karl Popper, and he was soon to be much influenced by Popper's way of thinking (Eccles, 1975). Popper argued that since a scientific hypothesis can never be proven—it can only be falsified—the strength of a scientific theory is directly related to the precision with which it is formulated so as to allow it to be falsified by experiment. "The criterion of the scientific status," Popper wrote, "is its falsifiability or refutability" (Eccles, 1975). The falsification of a theory, he stressed, should not be viewed as an embarrassment. On the contrary, it is evidence of the rigor and precision of the hypothesis: to specify a hypothesis so precisely as to allow it to be falsified is the highest goal of science. Popper therefore convinced Eccles to continue to define the electrical hypothesis as rigorously as possible, and this Eccles proceeded to do, not only for excitation but also for synaptic inhibition. In the event, the critical falsification that finally led Eccles to abandon his theories of electrical transmission in the CNS came not from his studies of excitatory synaptic actions but from his discovery of the mechanism underlying synaptic inhibition. As he was to write on a later occasion, "I had been encouraged by Karl Popper to make my hypothesis as precise as possible, so that it would call for experimental attack and falsification. It turned out that it was I who succeeded in this falsification" (Eccles, 1975).

Popper's influence is particularly evident in Eccles's models of synaptic inhibition. Synaptic inhibition had posed enormous difficulties for the proponents of electrical transmission, and over a number of years several imaginative hypotheses had been put forward to account for central inhibition by electrical means. These included (1) the view that impulses in inhibitory fibers blocked the excitatory impulses in the presynaptic terminals, presumably by some hyperpolarizing action; (2) the notion that the inhibitory effect is exerted on the postsynaptic cell because the terminals of the inhibitory fibers end at some special spatial location on the cell, for example around the site of impulse initiation at the axon hillock; and (3) the hypothesis that there are specific inhibitory synapses at which the presynaptic impulses exert an electrical effect that is the functional inverse of excitation.

But the most elegant of the hypotheses for electrical inhibition was proposed in 1947 by Eccles and Chandler Brooks (Brooks and Eccles, 1947). They postulated that inhibitory inputs to the spinal cord ended on short axon cells (like Golgi's type II cells), which formed close electrical contacts with the motoneurons. They further hypothesized that the Golgi cells were non-impulse-generating neurons and that the afferents that impinged upon them would not readily excite the cells because of their high threshold. An incoming volley of impulses would, however,

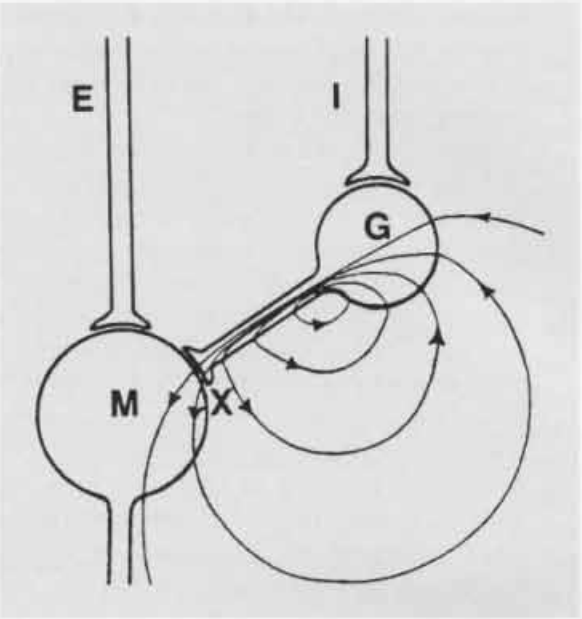


Figure 1.6. Eccles's postulated mechanism for electrical inhibition. The mechanism involves a Golgi II (nonspiking) neuron (G) interposed between the inhibitory input (I) and the motoneuron (M). The inhibitory input subliminally excites the Golgi cell and generates the pattern of current flow indicated by the arrows, which produces an anelectrotonic focus on the motoneuron. Reproduced from Eccles (1982), with permission from the *Annual Review of Neuroscience,* Volume 5, ©1982 by Annual Reviews; http://www.AnnualReviews.org.

set up a synaptic potential in the Golgi cells. Although this synaptic potential would be too weak to initiate an impulse, it would give rise to an inward current that would increase the conductance of the Golgi cells at the site at which the inhibitory fibers ended. Depolarization at this site would then give rise to an outward current flow throughout the rest of the neuron. Because the Golgi cell is small and inactive, and because of the close apposition of its axon terminals to the motoneuron, this outward current flow, it was argued, would penetrate the membrane of the postsynaptic cell and produce an inward current flow at a localized region of the motoneuron membrane. It followed from this hypothesis that synaptic inhibition mediated by the Golgi cell's axon would be diphasic in character—a combined inhibitory-excitatory action (see also Eccles, 1949, and Fig. 1.6).

In the early 1950s Eccles and his colleagues began to apply to motoneurons the same intracellular recording methods used by Fatt and Katz for their studies of transmission at the neuromuscular junction. The initial interest of Eccles's group was to determine if the properties that Hodgkin and Huxley had reported for the giant axons of invertebrates were shared by vertebrate motoneurons (Brock et al., 1951;

Eccles, 1953). But from the beginning they were also interested in the mechanism of excitation and inhibition in the CNS (Brock et al., 1952). When they examined the effects of stimulating inhibitory inputs to the motoneurons they found, to Eccles's surprise, that synaptic inhibition caused a hyperpolarization of the motoneuron without any associated depolarization (Fig. 1.7). This led him to abandon, without reservation, the electrical transmission hypothesis for inhibition that he had so recently espoused. As he now wrote, "The potential change observed is directly opposite to that predicted by the Golgi-cell hypothesis which is thereby falsified" (Brock et al., 1952; see Fig. 1.8). And in the discussion of their 1952 paper he went on to write off electrical excitation in equally strong terms: "Since the experimental evidence has falsified the

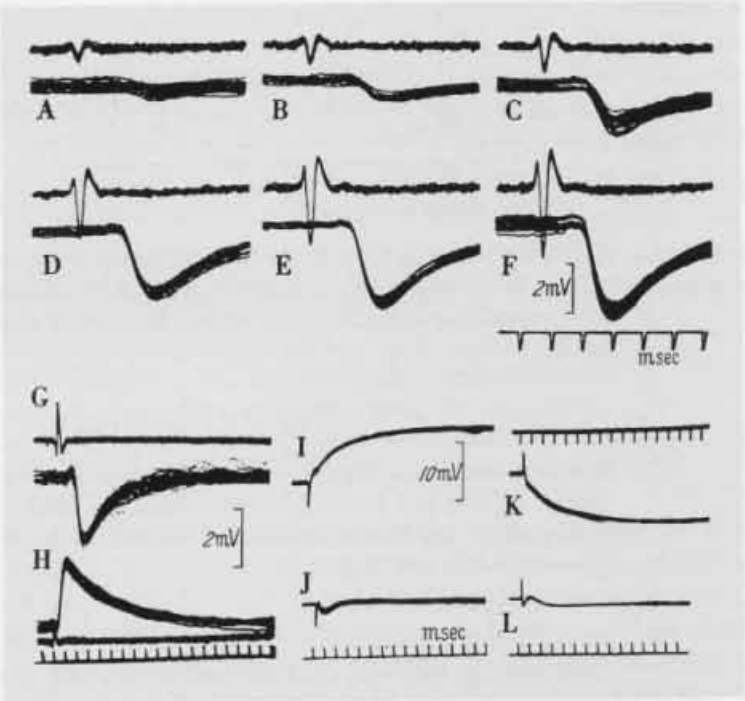


Figure 1.7. Inhibitory postsynaptic potentials. It was IPSPs such as this one that led Eccles to abandon his electrical hypothesis for synaptic transmission. The lower records give intracellular responses of a biceps-semitendinosus motoneuron following stimulation of a quadriceps volley of progressively increasing size, as shown by the upper records, which are recorded from the L_6 dorsal root by a surface electrode (downward deflections signal negativity). All records are formed by the superposition of about 40 faint traces. *G* shows IPSPs similarly generated in another biceps-semitendinosus motoneuron, the monosynaptic EPSPs of this motoneuron being seen in *H*. *I–L* show changes in potential produced by an applied rectangular pulse of 12×10^{-9} A in the depolarizing and hyperpolarizing directions, *I* and *K* being intracellular and *J* and *L* extracellular. Reproduced from Eccles (1964) by permission.

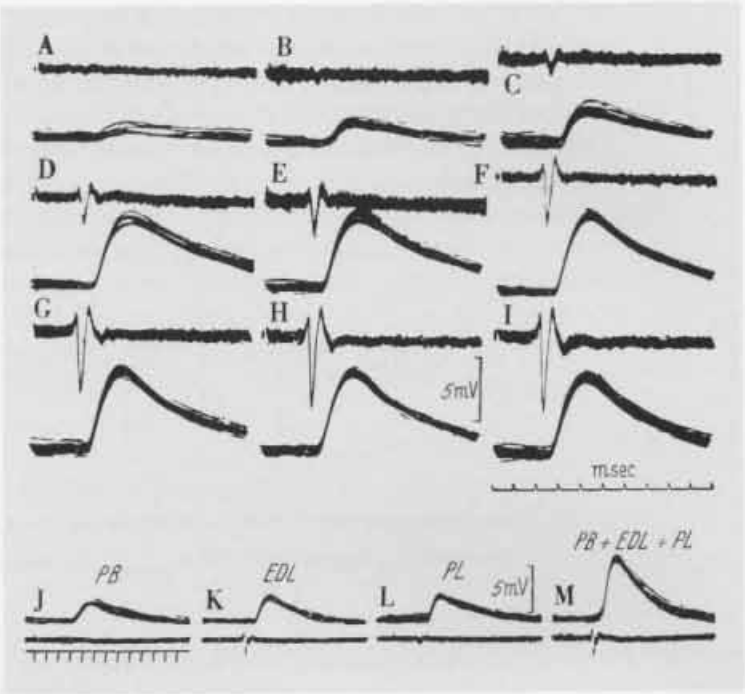


Figure 1.8. Monosynaptic excitatory postsynaptic potentials recorded intracellularly in motoneurons. Each record is formed by the superposition of about 25 faint traces. In *A–I,* the EPSP is generated in a medial gastrocnemius motoneuron by an afferent volley from the medial gastrocnemius nerve of progressively increasing size, as indicated by the spike potentials in the upper records from the dorsal roots. The EPSP attained its maximum in *F,* where the afferent volley was probably maximal for group Ia fibers. In *J–M,* EPSPs are similarly recorded in a peroneus longus motoneuron in response to maximum group Ia volleys from the nerves to peroneus brevis, extensor digitorum longus, and peroneus longus, and by all three volleys together, as indicated by the symbols. Reproduced from Eccles (1964) by permission.

Golgi-cell hypothesis of inhibition and left the chemical transmitter hypothesis as the only likely explanation, it suggests further that the excitatory synaptic action is also mediated by a chemical transmitter" (Brock et al., 1952).

Eccles and his colleagues suggested that the hyperpolarization was inhibitory because it moved the membrane potential from its resting level so that subsequent *excitatory postsynaptic potentials* (EPSPs) acted from a more negative baseline. However, subsequent studies by Fatt and Katz on inhibition in the crayfish showed that inhibitory synaptic actions could occur without a change in membrane potential, simply because of the short-circuit or shunting action of inhibition. Again, as was the case with excitation, the ions responsible for inhibition were at first not clear. Fatt and Katz postulated a nonspecific increase in small ions,

specifically Cl^- and K^+. Later, more detailed studies of a variety of inhibitory actions were to establish that in no case does inhibition involve the simultaneous increase in membrane permeability to more than one ion species. Transmission is due to either chloride permeability or potassium permeability being selectively and independently turned on (Fatt and Katz, 1953).

In reviewing the long struggle leading to the acceptance of chemical transmission in the CNS, Dale (1954) wrote, with more than a little smugness:

> Eccles and his team conclude that this positive variation in the motor horn cell could only be due to the release of a chemical agent from the endings of the afferent fibre making synaptic contacts with its surface, and that, if synaptic inhibition was thus chemically transmitted, synaptic excitation was unlikely to be transmitted by an essentially different process, though the transmitter might probably be a different one. By obvious analogy, it was to be supposed that some chemical agent or other would be effective at all central synapses, and that being accepted, Eccles was naturally ready to take cholinergic transmission in the ganglion in his stride. A remarkable conversion indeed! One is reminded, almost inevitably, of Saul on his way to Damascus, when the sudden light shone and the scales fell from his eyes.

However, although the soup and spark controversy was effectively resolved in 1952, the nature of the excitatory and inhibitory transmitters involved would not be discovered for some years.

The Surprising Discovery of Electrical Transmission

Having been converted to chemical transmission by the discovery of the hyperpolarizing nature of synaptic inhibition, Eccles celebrated the falsification of the electrical hypothesis that he had so vigorously championed by converting wholeheartedly to the chemical hypothesis for synaptic transmission, arguing with equal enthusiasm and vigor for its universality. It was at this point, in October 1954, that Paul Fatt, Katz's collaborator, wrote a masterly review of junctional transmission in which he took a farsighted view of synaptic transmission and presciently pointed out that it was premature to conclude that chemical transmission is in fact universal. He concluded his review as follows:

> Although there is every indication that chemical transmission occurs across those junctions which have been discussed in this review and which are most familiar to the physiologist, *it is probable that electrical transmission occurs at certain other junctions.* The geometry of the junction is decidedly unfavorable for electrical transmission at the junctions which have been mentioned. The pre-junctional structure, which according to the electrical hypothesis would generate the electric current

for transmission, is usually much smaller than the post-junctional structure, which would have its excitatory state altered by those currents. Conditions are much more favorable for transmission in the reverse direction, and, in fact, the excitation of pre-junctional motor nerve fibers by the action currents of post-junctional muscle fibers has been observed to occur in mammalian muscle. This argument does not hold when one considers the synapses between giant nerve fibers where the pre- and post-junctional structures have usually about the same dimensions. In this case some electrical interaction will be expected, its intensity depending on how closely the fibers approach each other. One possible arrangement, which may be envisaged to give a high degree of interaction, is for the two fibers to be actually touching and for the membrane in contact to have a low electric resistance compared with that in neighboring parts of the fibers. The synapse would then serve to direct current between the interior of the two fibers, while active membrane changes would occur in neighboring regions. The ultimate development of such a system would be the elimination of the contacting membrane to secure greatest efficiency of transmission, should other factors permit this. This view of electrical transmission has been taken because it is possible to observe in certain giant fiber preparations a protoplasmic continuity existing between, what in an earlier stage of phylogenetic development must have been independent, synapsing nerve cells. The available evidence, however, does not indicate that a single mechanism of transmission operates at all giant fiber synapses. . . .

A case in which there can be little doubt that electrical transmission operates is in the nervous system of the crayfish, where successive giant nerve cells, each extending along one segment of the thoracic or abdominal region, butt upon each other to form the lateral giant nerve fibers. . . . Transmission takes place in either direction so that an impulse initiated at any level travels over the whole chain of segmental nerve cells, both cranially and caudally. . . . A more perplexing case is the synapses in the crayfish ganglion between the central giant nerve fibers and the motor nerve fibers. The fact that this synapse is polarized to transmit impulses only in the direction from giant fiber to motor fiber cannot be taken as an indication of nonelectrical transmission, since the geometrical arrangement is the reverse of that ordinarily obtaining at synapses: the pre-junctional structure is here larger than the post-junctional structure. (Fatt, 1954)

Three years later, the correctness of Fatt's view was convincingly demonstrated by Edwin Furshpan and David Potter, who analyzed synaptic transmission between the presynaptic giant axon and the postsynaptic motor axon in the crayfish nerve cord and found it to be electrical (Fig. 1.9). The several tests for electrical transmission that Kuffler had carried out at the neuromuscular junction—where he had failed to find evidence for current flowing from the pre- to the postsynaptic cell—turned out positive at this electrical synapse. The latency was extremely short, and even small electrical currents flowed from the pre- to the postsynaptic cell. As Furshpan and Potter (1957) wrote, "It is difficult to

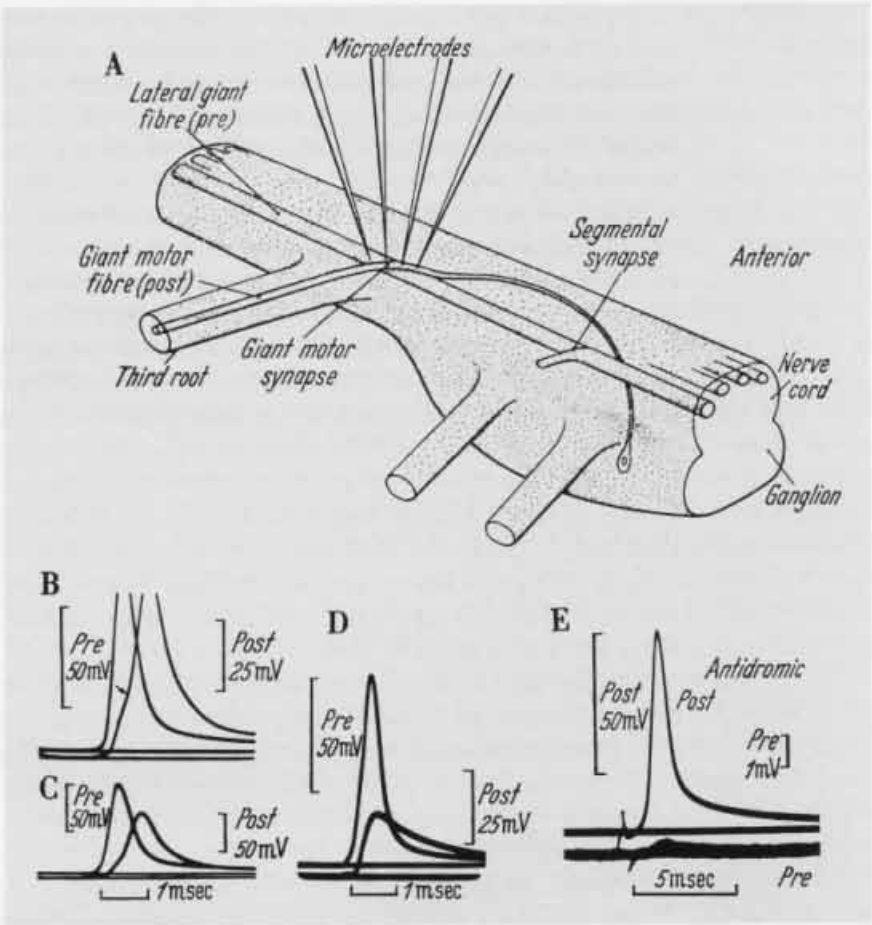


Figure 1.9. The first convincing evidence for electrical transmission.

(A) Semidiagrammatic drawing of a portion of a crayfish abdominal nerve cord, containing one ganglion. The course of a motor giant axon is shown from its cell body in the ventral part of the ganglion until it leaves the third ganglionic root on the opposite side of the cord. Only its junction with the lateral giant prefiber is shown, but its synapses with the two medial giant fibers are located just centrally, where the fibers cross the motor axon. A septal synapse between two segments of the lateral giant fiber is also shown.

(B–D) Orthodromic nerve impulse transmission at the giant synapse shown in *A* with simultaneous intracellular recording from pre- and postfibers, the prefiber potential being recorded in the upper traces. *B* and *C* were recorded from the same synapse at different amplifications, the postspike origin being indicated by the arrow in *B*. In *E* the upper trace is the postfiber antidromic spike potential, which produces a negligible potential in the prefiber (lower trace). Note the separate potential scales for pre- and postfiber records in *B–E*.

Reproduced from Eccles (1964), after Furshpan and Potter (1957), by permission.

assign a value to the response between the pre-spike and the synaptic response. Both potentials seem to arise about the same time but at different rates . . . even subthreshold electric currents passed through one of the internal electrodes can produce appreciable changes in the membrane potential recorded with another electrode on the opposite side of the synapse."

Even more astonishing, several years later Furshpan and Furukawa came up with another surprise—the demonstration that inhibition could occur by electrical means and, in fact, by a mechanism somewhat analogous to that postulated by Eccles several years before (Furshpan and Furukawa, 1964). At the initial segment of the Mauthner cell axon an impulse in the presynaptic fiber generates a positive field in the surrounding extracellular space. This extracellular positivity hyperpolarizes the membrane of the initial segment (which is the point of the lowest threshold for excitation in the Mauthner cell) and causes effective inhibition. As they point out, this is because at any one time the membrane potential is simply the difference between the extracellular and intracellular voltage; if the extracellular voltage becomes more positive, the voltage difference across the membrane of the initial segment would consequently be increased.

Over several years Michael Bennett and his collaborators extended the analysis of electrical synaptic transmission in several important directions (see Bennett, 1966, 1972, for reviews; see also Fig. 1.10). Among

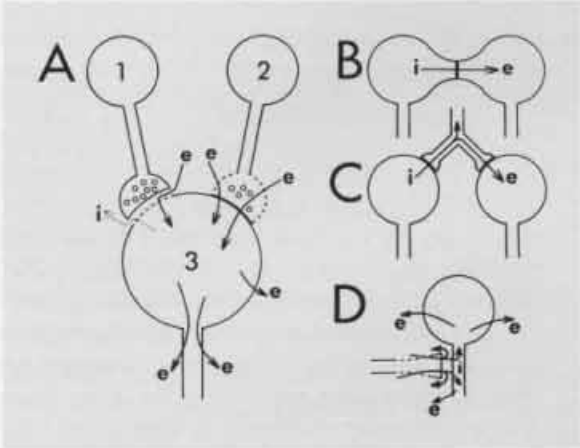


Figure 1.10. Patterns of current flow in a conventional chemical synapse (A_1) *and in an electrical synapse* (A_2). The broken lines indicate the areas active in generating postsynaptic potentials.

(B) A dendrodendritic electronic contact.

(C) Two axosomatic electronic contacts that can synchronize cell firing.

(D) Electrically mediated inhibition found at a Mauthner cell axon hillock. Reproduced from Bennett (1972) by permission.

other things, they discovered that in most cases electrical synapses could pass current in either direction, that is, they were not rectifying (their junctional resistance was constant). This led Bennett to think of them as electrotonic synapses by analogy with electrotonic spread along a core conductor. With Pappas and Nakajima, Bennett (Bennett et al., 1967a–d) carried out a series of electrophysiological and fine structural analyses that enabled them to correlate the biophysical properties of coupling with the occurrence of close membrane appositions, which subsequently came to be known as *gap junctions*.

These studies, culminating in a detailed comparison of electrical and chemical transmission, allowed Bennett to challenge the confidence of those who held that all central synaptic transmission was chemical. Bennett demonstrated that many of the interesting properties that were supposedly the exclusive purview of chemical transmission were also to be found in electrical synapses, and he pointed out that the speed of electrotonic transmission and the reciprocal action of these synapses imparted specific advantages. In particular, Bennett demonstrated that electrotonic synapses are most commonly found in rapidly activated circuits—since they transmit without the delay incurred by the complexities of chemical transmission and in synchronously active ensembles of neurons, in which reciprocity as well as speed is important.

For the sake of completeness we should mention in this context the discovery by Martin and Pilar (1963, 1964) that in the chick ciliary ganglion transmission is both electrical and chemical. Although the preganglionic axons terminate by forming large calyces that embrace much of the surface of ganglion cells, forming extensive, characteristic chemical synapses (De Lorenzo, 1960), there are also more restricted foci of close membrane apposition between the axons and the ganglion cells. De Lorenzo (1966) originally described these as tight junctions, but we now know they are, in fact, typical gap junctions.

Before leaving this topic, we should note also that the discovery of synapses that are based on gap junctions was quickly seized upon by a number of "latent reticularists" who saw in it a modern-day challenge to the neuron doctrine. This view was strengthened when it was shown that although a narrow intercellular cleft exists at gap junctions, this gap is filled with an array of junctional channels that serve to connect the cytoplasm of the two related cells, permitting current to flow freely from one neuron to the other. The gap junction channels are in fact large enough to permit the flow of small organic metabolites between the connected neurons. To this extent, the electronic coupling of cells that are united by such junctions can be regarded as evidence of intercellular continuity and can be considered as an interesting exception to the neuron doctrine as Cajal and others had initially conceived of it. But it is important to appreciate that not only are electrical synapses relatively uncommon in the mammalian CNS, they also provide a form of cell-cell interaction quite different from that conceived of by the reticularists. Each neuron is bounded by its own cell membrane, each has its own

nucleus and array of organelles and, although they may be connected by specialized channels, they function as independent entities in every other respect. Viewed as a whole, the nervous system is unequivocally not a network of cytoplasmically continuous cells.

In summary, by the late 1960s the field had come full circle: both chemical and electrical mechanisms for transmission had been shown to exist, and both mechanisms were known to display a variety of subtypes. Moreover, not only do both mechanisms exist, but some models for synaptic action that had been jettisoned during the soup versus spark controversy had been resurrected during the subsequent détente.

The Quantal Nature of Transmitter Release

With the discovery that transmission at the vertebrate neuromuscular junction and at most synapses in the CNS is chemical in nature, and with the specification of the ionic mechanisms for generating excitatory and inhibitory postsynaptic potentials, attention next turned to the mechanisms whereby the chemical transmitter is released. Here, again, the field was opened up by Katz.

During the course of their experiments, Fatt and Katz (1951) had made a remarkable chance observation: when they recorded from the endplate region of a muscle fiber, there were often small, spontaneous depolarizing potentials even in the complete absence of presynaptic stimulation (Fig. 1.11). These spontaneous potentials were about 0.5–1.0 mV in amplitude and resembled miniature versions of the EPP in their time course and in their response to various drugs. Thus drugs that enhance the action of ACh, such as inhibitors of acetylcholinesterase, prolonged the spontaneous potentials much as they prolonged the EPP, whereas agents that block the ACh receptors, such as curare, also abolished the miniature EPPs. Moreover, as was the case with the EPP, the miniature potentials were recorded only at the endplate, at the point of contact between the nerve and muscle. Fatt and Katz (1952) therefore called these miniature potentials *spontaneous miniature EPPs* (mEPPs).

Fatt and Katz next found that the mEPP frequency could be increased by depolarizing the presynaptic terminal and that the mEPPs disappeared after the presynaptic axons were cut and the motor nerves had degenerated, only to reappear when the muscle was reinnervated. Together these manipulations established that the mEPPs derive from the presynaptic terminals. They also found that removal of Na^+ from the bathing solution abolished both the EPP and the mEPPs, but that reducing the external Ca^{2+} reduced only the size of the EPP but had no effect on the size of the mEPPs.

In 1954 del Castillo and Katz showed that with sufficiently low Ca^{2+} levels the size of the EPP, normally about 70 mV, became no larger than the size of the mEPPs (0.5–1.0 mV). Under these circumstances, succes-

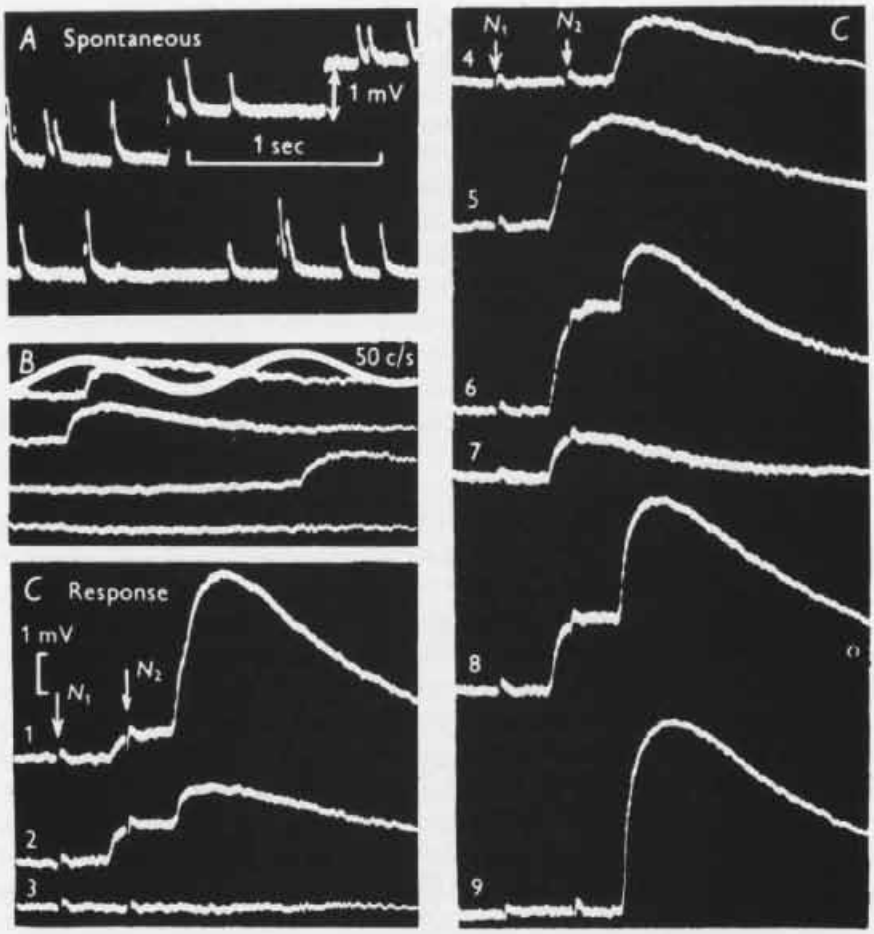


Figure 1.11. Miniature endplate potentials recorded intracellularly from a frog skeletal muscle. The muscle was bathed in a solution containing 0.45 mM Ca^{2+} and 6 mM Mg^{2+}. Records *A* and *B* are of spontaneous mEPPs; record *C* shows the responses to paired nerve impulses with failures to the first impulse (N_1) in records C_4 and C_9, and to the second (N_2) in C_4 and C_9. Reproduced from del Castillo and Katz (1954a) by permission of the Physiological Society.

sive impulses in the motor nerve to the muscle fiber evoked, in a random fashion, EPPs that varied in a stepwise manner so that each EPP was an integral multiple (0, 1, 2, 3, or more) of the mEPP. From this they concluded that the normal EPP also was constituted of an integral number of miniature units and that the effect of lowering the calcium concentration was to reduce the EPP "*in definite quanta*, as though it blocks individual nerve terminals, or active patches within them, in an all-or-none manner" (emphasis added). Thus was born the *quantal hypothesis*, which was to dominate thinking about synaptic transmission for the next three decades.

Quanta: Multimolecular Packets of Transmitter Released from Vesicles by Exocytosis

Following a suggestion by Alan Hodgkin, Fatt and Katz (1952) initially attributed the mEPPs to active localized patches of membrane that generate potentials in fine branches of individual nerve terminals, causing the all-or-none release of a certain number of molecules of transmitter: "We must therefore think of a local mechanism by which acetylcholine is released at random moments in fairly large quantities; and that the most plausible explanation is the occurrence of excitation at individual nerve terminals, evoked by spontaneous fluctuations of their membrane potential . . . but it does not lead to a propagated impulse if the affected area is too small." They were inclined to attribute this spontaneous excitation at the nerve terminals to "electrical noise" generated across the membrane by random fluctuations of the resting potential as a result of thermal agitation of ions within the membrane. This noise, they argued, can become sufficiently large at a small structure occasionally to exceed the threshold for the release of transmitter.

In 1954 Katz abandoned this idea because he and del Castillo had found that the spontaneous release of mEPPs still occurred when all electrical activity had been blocked by the application of an isotonic solution of potassium sulfate. Moreover, extracellular recordings from nerve terminals at branch points showed that the fluctuation of ACh release occurs, even though the action potential invaded the nerve terminals without failure and retained a constant amplitude throughout.

Del Castillo and Katz considered and rejected a second possible basis for the mEPPs: a membrane shutter mechanism. Suppose, they argued, there are specialized areas in the terminal axon membrane that act as ACh gates. In the absence of an action potential these gates would usually be closed. However, the gates could reach a degree of instability when they open for a brief period, during which time a small amount of ACh would be released from the interior of the nerve ending. An action potential would greatly increase the likelihood of an ACh gate opening. A mechanism of this kind is feasible, but it did not seem very attractive, for one would have to explain why such a membrane-controlled flip-flop process leads to a quantal amount of ACh release that is identical for the spontaneous mEPPs and for evoked release, despite the fact that the potential difference across the membrane changes from the resting level to the peak of spike activity during evoked release. Any alteration in the gating action, they argued, would necessarily be reflected in a change in quantal size during the nerve impulse, but this is ruled out by a large body of experimental evidence (Katz and Miledi, 1965).

Finally del Castillo and Katz concluded that the most straightforward explanation for the constancy of quantal amplitude is that the transmitter is released from the axon terminal in discrete multimolecular packets,

which they called *quanta.*[13] The relative constancy of the packets of ACh suggested to them that the size of the quanta is controlled not by the rapidly changing properties of the membrane, but by some cellular process that is not disturbed from the outside. They further proposed that transmitter is stored in preformed submicroscopic packages inside the cell, from which it can be released at the cell surface in an all-or-none fashion (del Castillo and Katz, 1954a–d). *Synaptic vesicles*, which were discovered at about the same time by Palay and Palade (1955) and by de Robertis and Bennett (1955), seemed to provide just the right structural counterpart. The vesicles were of fairly uniform size and were found at the right place within the nerve terminal, whereas no other structures could be seen nearby that would meet the requirements of the quantal hypothesis. In addition, the notion that the membrane-bound vesicles contained small packets of transmitter was consistent with Feldberg's finding that most of the ACh stored in the nervous system is protected or bound within subcellular organelles, to which it remains attached even during processes of homogenization and high-speed centrifugation.

Del Castillo and Katz further argued that the vesicle could actively accumulate the transmitter substance and maintain it at a much higher concentration than exists in the surrounding axoplasm. Moreover, when packaged within vesicles, the transmitter is separated from its postsynaptic target by two membranes: the membrane surrounding the vesicle itself and the plasma membrane surrounding the axon terminal. For the transmitter to be released so that the entire "quantum" reaches the receptors in the postsynaptic membrane more or less synchronously and at a sufficiently high concentration, del Castillo and Katz assumed that the transmitter is released by an exocytotic process in which the vesicle membrane fuses with the presynaptic membrane and thereby discharges its contents into the synaptic cleft (Katz and Miledi, 1965).

In a series of papers del Castillo and Katz explored the statistical nature of quantal synaptic transmission and developed the modern view of transmitter release (del Castillo and Katz, 1954a–d). According to this view, ACh is released in quanta—made up of multimolecular packets. The release of the quanta is probabilistic. It occurs spontaneously even in the complete absence of action potentials, at a rate of about one quantum released per second per endplate. An action potential transiently increases the probability that quanta of transmitter will be released, so that the normal EPP is generated by the release of, on average, about 150 quanta in less than 1 msec, with each quantum contributing about 0.5 mV to the EPP. The exact number of quanta released by any given nerve impulse fluctuates in a random fashion that can only be described in statistical terms. Formally del Castillo and Katz (1954b) expressed this as follows: a nerve terminal contains a large number (n) of quanta, each released in response to an action potential with a probability p:

> The average "quantum content" of the e.p.p. depends on the probability of response of the individual units and this varies with the external Ca and Mg concentration. . . . If one accepts the present results as showing that the miniature e.p.p. is the basic unit of response, then the effect of Ca must be to raise the quantum content of the e.p.p. either by increasing the size of the population n or its probability of responding p.

This line of investigation led to what has come to be known as the *Ca^{2+} hypothesis* (Katz and Miledi, 1965). This hypothesis emerged from a remarkable series of studies of the frog neuromuscular junction and the giant synapse of the squid stellate ganglion by Katz and Ricardo Miledi, who found that the depolarization following an action potential in the nerve terminals opens Ca^{2+} channels and increases the conductance to Ca^{2+}. The entry of Ca^{2+} into the terminal leads, after various delays, to the release of transmitter. They next showed that neither Na^+ entry nor the K^+ efflux associated with the action potential is required for normal transmitter release. Indeed, the only role of the action potential is to depolarize the terminals and thus open the Ca^{2+} channels. Thus when Na^+ and K^+ channels were blocked by tetrodotoxin and tetraethylammonium, respectively, graded depolarizations of the terminals could activate a graded Ca^{2+} influx, which, in turn, results in the graded release of transmitter.

The finding that depolarization of the terminals by the action potential serves to open voltage-dependent Ca^{2+} channels was later confirmed by Rodolfo Llinas and his colleagues, who also found that the synaptic delay—the time from the onset of the action potential in the presynaptic terminals to the onset of the postsynaptic potential—is due in large part to the time required for the Ca^{2+} channels to open. Because the voltage-dependent Ca^{2+} channels are located very close to the transmitter release sites, they can act to trigger transmitter release within as little as 0.2 msec. It has been estimated that the resultant influx of Ca^{2+} produces localized concentrations of up to 200–300 μM in microdomains within the presynaptic terminal near the release sites. Such local increases in Ca^{2+} concentration greatly enhance the probability of vesicle fusion and transmitter release (Llinas et al., 1972).

In most nerve cells there are at least three (and probably more) classes of voltage-sensitive Ca^{2+} channels. One class (the L-type channel) is characterized by a slow rate of inactivation, so that it remains open during a prolonged depolarization of the membrane. The other two classes (N-type and P-type) inactivate more rapidly, and the available evidence suggests that it is the influx of Ca^{2+} through these latter channels that contributes most directly to transmitter release.

The Ultrastructure of the Synapse Visualized in the Electron Microscope

As noted previously, at the same time as Katz's electrophysiological studies were being carried out, cell biologists and neuroanatomists were begin-

ning to use the electron microscope (EM) to study neural tissue. The EM had been developed in Germany in the 1930s, but its application to biological material was delayed until after World War II and until appropriate methods had been developed for fixing, embedding, and sectioning tissues. Thanks largely to the efforts of George Palade and Keith Porter at the Rockefeller University, most of these difficulties had been overcome by the early 1950s. The first high-quality EM images of neural tissue were published in the mid-1950s (de Robertis and Bennett, 1955; Palay and Palade, 1955; Palay, 1956), including the first observations on the fine structure of neurons and their processes and (most important in the present context) the first descriptions of synapses.

From a historical prospective, these observations were of great significance. By directly visualizing the structural discontinuity of the pre- and postsynaptic elements—a process that was possible only with the increased resolution afforded by the EM—they provided the final unequivocal evidence for the neuron doctrine. In addition, they clarified definitively the characteristics of each of the three elements of the synapse: the pre- and postsynaptic elements and the intervening synaptic cleft. Subsequent EM observations extended these initial observations and led to the discovery of new types of synapses that had not been anticipated in the classical literature, providing a new (albeit tentative) basis for the classification of functional types of synapses on the basis of their fine structure (Gray, 1959; Pappas and Waxman, 1972; Peters et al., 1976).

The Presynaptic Components and the Process of Exocytosis

It became evident from an examination of the presynaptic components of the synapse that they contain many (in some instances, hundreds of) vesicular organelles ranging in size from about 20 to 150 nm in diameter. Palay (1967) aptly likened them to "chocolates [coming] in a variety of shapes and size, and . . . stuffed with different kinds of fillings."

As we have seen, Katz and del Castillo immediately recognized that these might be the organelles that store the quanta of transmitter. Subsequent work on the transmitter content of cholinergic vesicles has provided strong supporting evidence for this correlation. And the finding that other transmitters (such as glutamate and glycine) are also released in quantal fashion has established that this is a general feature of all chemically transmitting synapses (Fig. 1.12).

The most common vesicular forms are small (20–40 nm diameter) round or spherical vesicles with clear (i.e., electron-lucent) centers. They are found at the neuromuscular junction, in autonomic ganglia and several other peripheral synapses, and throughout the CNS. Somewhat larger vesicles, about 40–60 nm in diameter with electron-dense centers,

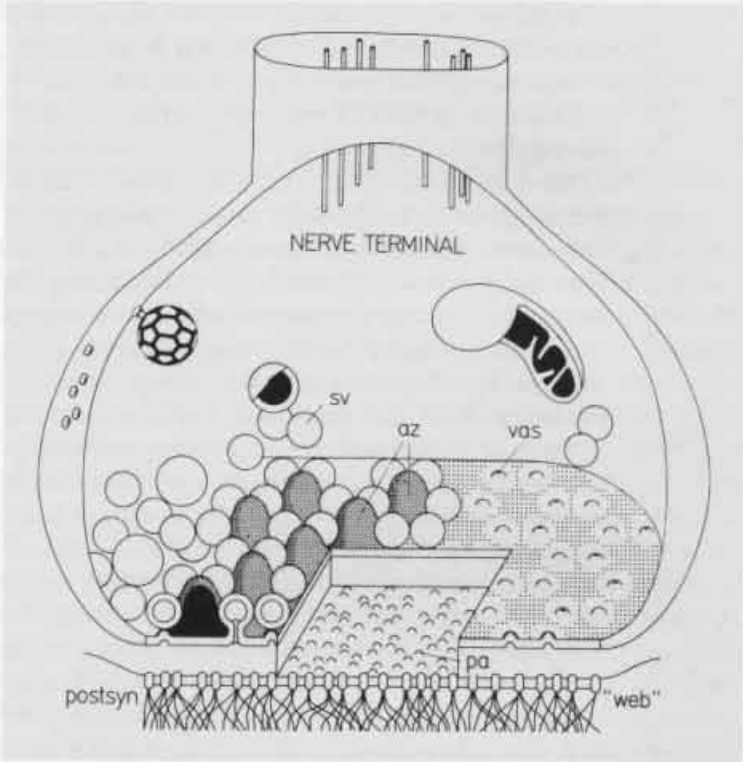


Figure 1.12. Schematic drawing of a "typical" chemically transmitting synapse in the CNS. The illustration is reconstructed from transmission EM and freeze-fracture preparations. az, Active zones; pa, particle aggregate in the postsynaptic membrane; postsyn. "web," postsynaptic density; sv, synaptic vesicle; vas, vesicle attachment site. Reproduced from Akert et al. (1975) by permission of Lippincott, Williams & Wilkins.

are commonly found at sites of aminergic transmission in the CNS and in the peripheral sympathetic system. A third type of synaptic vesicle, characterized by its larger size (80–100 nm diameter) and again possessing a central, dense core, is fairly ubiquitous, but usually occurs in small numbers and always associated with small, clear-centered vesicles. For many years the significance of this third type of vesicle remained uncertain, but they are now thought to be associated with various synaptically released peptides, such as the calcitonin gene related peptide found at the neuromuscular junction and elsewhere. Finally, there is a fourth class of very large vesicles (120–150 nm diameter), characteristic of neurosecretory nerve endings such as those in the neurohypophysis, that contain the peptides oxytocin and vasopressin. These very large vesicles are again commonly found in association with many more small, clear vesicles whose functional significance in neurosecretory terminals is still unknown.

Following the introduction of aldehyde fixation for electron microscopy, several workers observed that in some synapses the small, clear vesicles assumed a flattened or ellipsoidal form. Uchizono (1965) seems to have been the first investigator to have suggested that such flattened (or F-type) vesicles might be associated with the presence of an inhibitory transmitter, having observed them in the terminals of Purkinje cell axons and axon collaterals, as well as in the axons of other known inhibitory neurons in the cerebellum. The physical basis of this vesicle flattening has been shown to be artifactual, in the sense that it is associated with the high osmolarity of the fixing solution. However, its occurrence has proved to be a useful indicator of inhibitory synapses in some (but by no means all) regions of the CNS (e.g., Walberg, 1965; Bodian, 1966).

Del Castillo and Katz postulated that synaptic vesicles discharge their contents by fusing with the presynaptic membrane in the process known as *exocytosis*. This point proved difficult to investigate, even in the EM using conventionally fixed tissue, because the chances of finding a vesicle in the act of opening are relatively small. A thin section through a terminal at the neuromuscular junction of a frog, for example, shows only 1/40,000th of the total presynaptic membrane. As a result, in the 1970s investigators began to apply freeze-fracture techniques to this problem. Heuser and his colleagues (Heuser and Reese, 1973; Heuser et al., 1975) used this technique in an attempt to demonstrate that one vesicle undergoes exocytosis for each quantum of transmitter release. Statistical analysis of the spatial distribution of discharge sites along the active zone showed that individual vesicles fuse with the plasma membrane independently of each other. These results were consistent with the physiological studies indicating that quanta of transmitter are released independently. These freeze-fracture studies therefore provided indirect evidence that synaptic vesicles store the transmitter and that exocytosis is the mechanism by which transmitter is released into the synaptic cleft (see Heuser, 1977, for review).

An alternative approach to the study of synaptic vesicles was pioneered by Viktor Whittaker, who, as early as 1959, had reported the isolation (by homogenization and differential centrifugation of brain tissue) of particles that bound ACh. Later, with George Gray, he was able to show that his fractionation procedure produced *synaptosomes*—pinched-off axon terminals containing synaptic vesicles and mitochondria, attached to postsynaptic densities (Gray and Whittaker, 1962). Two years later Whittaker and his colleagues had further refined the fractionation procedure and obtained preparations of isolated synaptic vesicles that proved to be enriched for ACh (Whittaker et al., 1964). The development of methods for the preparation of relatively pure populations of synaptic vesicles paved the way for the later molecular studies on the characterization of vesicle membrane proteins and the mechanism of exocytosis that are dealt with elsewhere in this volume (see Südhof and Scheller, this volume).

Among the other components of the presynaptic process—such as mitochondria, occasional smooth endoplasmic membranes and cisternae—only three call for special mention here. These are coated vesicles, the presynaptic membrane density, and intermediate filaments. The significance of the *coated vesicles* (we now know that the coat is formed by a meshwork of clathrin) at synapses was not generally appreciated until the freeze-fracture and horseradish peroxidase uptake studies of Heuser and Reese (1973). These experiments showed convincingly that, following the fusion of synaptic vesicles with the presynaptic membrane, the vesicle membrane retains its identity and is endocytotically returned into the presynaptic process, where it can be recycled.

As Fig. 1.13 (which is taken from their work) indicates, the recycling involves several steps: (1) the assembly of a clathrin coat at the site of the invagination of the membrane, usually just beyond the presynaptic membrane density; (2) the movement of the coated vesicle into the presynaptic process; (3) the loss of the clathrin coat; (4) the fusion of the returned vesicle with a membranous cisterna (where it was thought to be reconstituted as a synaptic vesicle); and (5) the recharging of the vesicle with transmitter (now known to be effected through the action of specific

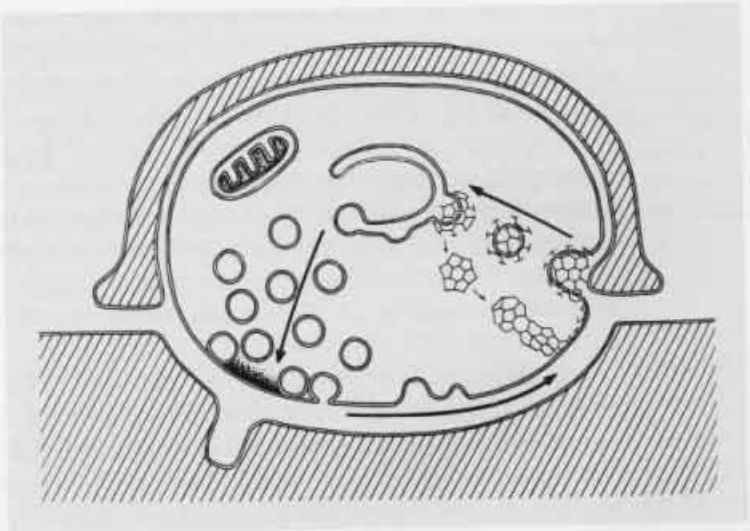


Figure 1.13. Heuser and Reese's synaptic vesicle membrane recycling hypothesis. Based on their studies of the frog neuromuscular junction, they proposed that synaptic vesicles discharge their content of transmitter as they coalesce with the plasma membrane at specific regions adjacent to the muscle. Equal amounts of membrane are then retrieved when coated vesicles pinch off from regions of the plasma membrane adjacent to the Schwann sheath. Finally the coated vesicles lose their coats and coalesce to form cisternae, which accumulate in regions of vesicle depletion and slowly give rise to new synaptic vesicles. Reproduced from Heuser and Reese (1973) by copyright permission of the Rockefeller University Press.

neurotransmitter transporters). More recent work suggests that the fourth step in this process may not always occur and that the vesicles can be refilled with transmitter shortly after losing their clathrin coats.

These findings on the recycling of synaptic vesicles have gone a long way toward clarifying their origin, an issue that had been debated for some time (see Peters et al., 1976, for discussion). Among the many suggestions originally put forward were that they arose (1) from tubular components of the smooth endoplasmic reticulum; (2) from complex multivesicular bodies; or (3) from microtubules. But the most widely held view was that they were transported from the cell body to the axon terminals along microtubules. Since it was known that much intracellular protein trafficking is mediated by vesicles, and that in axons most of the proteins destined for axon terminals are conveyed by fast axonal transport involving microtubules, this view had much to commend it. Moreover, it had been clearly established for the large neurosecretory vesicles in the neurohypophysis that they are assembled within the cell body and transported down the axons that make up the supraopticohypophysial tract (Palay, 1957). However, the current consensus is that most of the smaller vesicles are assembled locally within presynaptic processes from components that either are recycled or were previously transported from the cell soma.

The second component of the presynaptic process that merits comment is the *presynaptic density*, the specialized region of the membrane directly opposed to the postsynaptic element. In most EM preparations, but especially those stained with phosphotungstic acid or bismuth iodide, this portion of the membrane appears to be thicker or denser than others. This appearance is actually due to the presence of a submembranous meshwork of electron-dense material that in some cases has the appearance of a series of pyramidal projections extending into the presynaptic process. En face views of such projections suggest that they may form a regular gridlike arrangement, which Konrad Akert and his colleagues have termed the *presynaptic vesicular grid* (Akert et al., 1972). Their notion is that the spaces between the presynaptic projections are sites at which synaptic vesicles align themselves prior to fusing with the presynaptic membrane. From this hypothesis has emerged the notion that there are specific docking sites for vesicles and specific sites (or *synaptic pores*) where vesicle fusion and transmitter release occur—sites that Couteaux and Pecot-Dechavassine (1970) have termed the *active zones*. In support of this idea is the fact that in nearly every synapse examined there is a small cluster of vesicles closely associated with the presynaptic density (Birks et al., 1960). These vesicles are thought to contain the readily releasable pool of synaptic transmitter, and infrequently Ω-like membrane infoldings can be seen at the active zone, an appearance suggestive of vesicles that had been fixed immediately after fusing with the presynaptic membrane.

In some, but by no means all, presynaptic processes bundles of *intermediate filaments* (or "neurofilaments," as they used to be called) are

evident. These are especially prominent when they are aggregated around clusters of mitochondria[14] or other organelles; this arrangement is thought to account for the ringlike boutons seen in reduced silver preparations, apparently as the result of the deposition of metallic silver on the filament bundles. The presence of such clusters of intermediate filaments is of special interest following axonal injury, when they can become a particularly prominent feature of the degenerating axon terminals (Guillery, 1970).

The Synaptic Cleft

The finding that at all chemical synapses the pre- and postsynaptic processes are bounded by distinct membranes and separated from each other by a clearly defined space—the *synaptic cleft*—was, as we have pointed out, the final vindication of the neuron hypothesis. But the synaptic cleft is of interest in its own right (see Südhof, this volume). In most chemical synapses it is somewhat wider than the usual intercellular spaces, being between 20 and 30 nm in width; however, it is not simply a free space. In EM preparations it can be seen to contain filamentous or dense material that spans the interval between the surrounding membranes and is thought to account for the firm attachment of presynaptic processes to the postsynaptic membrane in synaptosomal preparations (Gray and Whittaker, 1962). In preparations stained with ethanolic phosphotungstic acid the material in the cleft often appears as a distinct intercellular plaque that from cytochemical studies appears to consist of a variety of glycoproteins and glycolipids, similar to the glycocalyx that surrounds most cells (Peters et al., 1976).

The neuromuscular junction is distinctive in this regard, in that there is a well-defined basement membrane (or *basal lamina*) interposed between the longitudinally arranged axon terminals and the muscle membrane (or *sarcolemma*). At the endplate the sarcolemma is marked by a series of deep transverse folds into which the basal lamina extends. The presynaptic densities, and an associated cluster of synaptic vesicles, are aligned opposite the openings of the sarcolemmal folds (Birks et al., 1960), a region that is now known to be densely packed with ACh receptors.

The Postsynaptic Density

The region of the postsynaptic membrane directly opposed to the presynaptic process is marked by the presence on its cytoplasmic face of a zone of electron-dense material. In general this is more prominent than the presynaptic density, and in some synapses it is associated with filamentous material that extends for a short distance into the subjacent cytoplasm. The width of the *postsynaptic density* varies considerably. In

many synapses—especially those found on the somata of neurons or on dendritic shafts—it is not much greater than that of the presynaptic density, leading to the suggestion that such "symmetric synapses" constitute a separate class, distinct from those in which the postsynaptic densities are appreciably thicker and hence appear distinctly "asymmetric." Such asymmetric synapses are especially prominent on dendritic spines. The distinction between the two classes of synapses is, however, not absolute, and considerable variation in postsynaptic densities can be found (Colonnier, 1968).

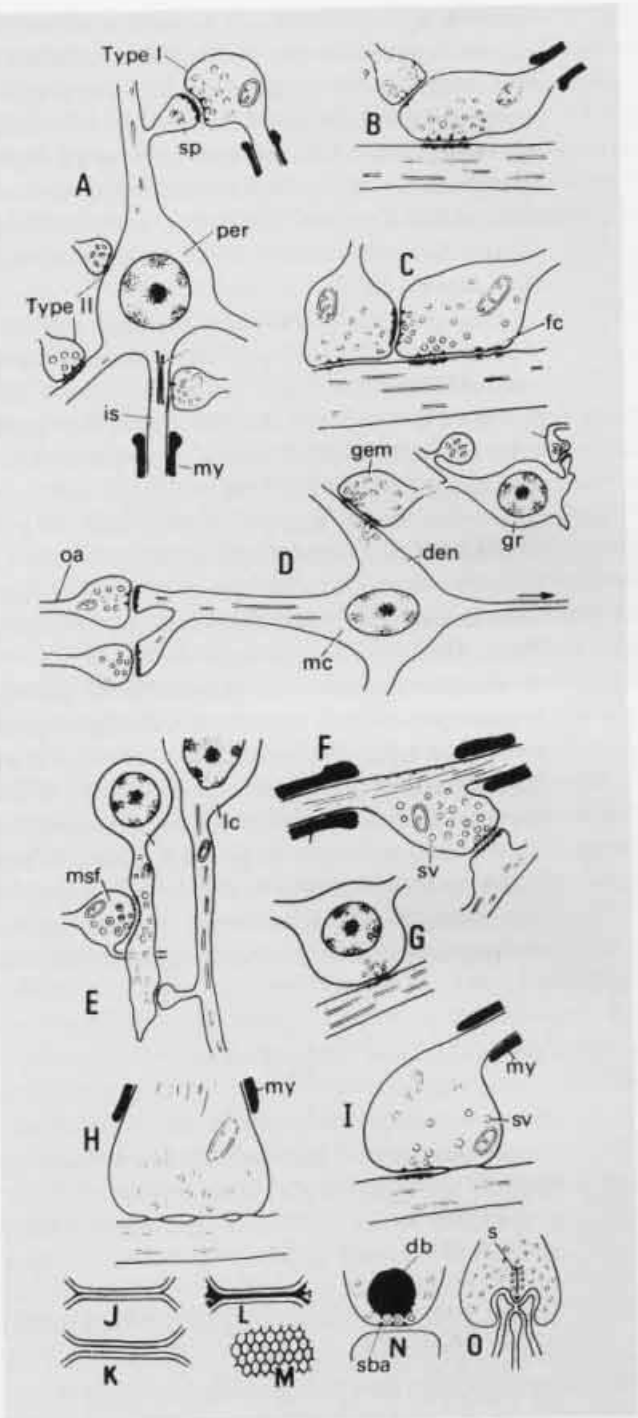
George Gray (1959) was the first electron microscopist to draw attention to the differences between synapses on the basis of their postsynaptic densities. From his studies of the cerebral cortex he suggested that they fall into two classes: type I, with pronounced postsynaptic densities and a somewhat wider synaptic cleft; and type II (corresponding to *symmetric synapses* in later terminology), in which the postsynaptic densities were much less prominent. Not surprisingly, this suggestion was promptly taken up by physiologists, who identified Gray's type I synapses as excitatory and his type II as inhibitory. As with the appearance of the synaptic vesicles, this correlation appears to hold true for many, but not all, regions of the CNS.

Further analysis of the nature of the postsynaptic density had to await the development of techniques for its isolation and chemical characterization, including the characterization of the postsynaptic receptor molecules. Since this research is the subject of later chapters (see especially Sheng, this volume), we will not discuss it further. However, it is worth mentioning here that once molecular probes for receptors (such as the ACh receptor) had been developed, it came as something of a surprise that their density was so high, amounting to as many as 10,000–20,000 receptors/μm^2 at the neuromuscular junction.

Varieties of Synapses

As more and more neural tissues were examined under the EM, it became evident that synapses come in many varieties and that the prototypical synapses of the type considered previously, though common (especially in the CNS), are but one among a host of different forms. A complete account of all the different forms is beyond the scope of this chapter; suffice it to say that variations in each of the principal components—the pre- and postsynaptic elements and the synaptic cleft—have been observed at one or more sites (Fig. 1.14).

We have already mentioned the presence of the basal lamina between the pre- and postsynaptic membranes at the neuromuscular junction. In retinal photoreceptors and at the squid giant synapse, there are prominent ribbonlike structures within the presynaptic processes (Sjostrand, 1958; Dowling and Boycott, 1968; Martin and Miledi, 1972). At spine synapses in the hippocampus and cerebral cortex there is often a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distinct "spine apparatus" consisting of two or more membrane-bound sacs or cisternae separated by plaques of electron-dense material (Hamilyn, 1962). At many synapses in these same regions clusters of polyribosomes, which are now thought to be involved in local protein synthesis, are seen at the base of dendritic spines (Steward and Levy, 1982). In many other regions (including the thalamus, the retina, and the olfactory bulb) both the pre- and postsynaptic elements have the morphological features of dendrites that display "reciprocal synapses," in which one process is presynaptic to another at one point and postsynaptic to that process at an adjoining site (Rall et al., 1966; Price and Powell, 1970; Famiglietti and Peters, 1972). And, most interestingly, in many regions axoaxonic synapses have been observed, with the postsynaptic element being either the axon hillock or the terminal portion of a second axon (see Peters et al., 1976, for a detailed account).

Presynaptic Inhibition

The finding of synapses upon axon terminals in the spinal cord (Gray, 1962, 1963) is of particular historical interest since it provided morphological evidence in support of Eccles's view of the mechanism of presynaptic inhibition. The initial observation of a reduction in the amplitude of an EPSP elicited in a motoneuron by stimulating one afferent when a second afferent is activated (that itself has no effect on the resting potential of the motoneuron) was first made by Frank and Fuortes (1957). They proposed that this was a form of presynaptic inhibition. Later Frank (1959) suggested that the depression of the EPSP, without other detectable changes in the motoneuron, could also be brought about if the terminals of the relevant afferents end on the distal dendrites of the

Figure 1.14. (opposite) Various types of synapses seen in the electron microscope.

(A) Axosomatic, axodendritic, and axoaxonic contacts on a cortical pyramidal cell.

(B) Axoaxonal contacts of the type thought to be involved in presynaptic inhibition of the spinal cord.

(C–D) Serial synapses seen in the thalamus (*C*) and the olfactory bulb.

(E) Synapses between amacrine (i.e., axonless) cells.

(F) En passant synapses at a node of Ranvier.

(G) A somatodendritic synapse.

(H) An electronic contact in the brain of a fish.

(I) A combined electrical and chemically transmitting synapse.

(J–M) Various forms of gap junction.

(O) A photoreceptor synapse in the retina, showing the typical presynaptic ribbon.

Reproduced from Gray (1974) by permission of Oxford University Press.

motoneurons, at a distance too remote to be detected by an electrode within the cell body, and act remotely to shunt the EPSP. He accordingly termed the phenomenon "remote inhibition." In a series of papers published between 1961 and 1962, Eccles reexamined this issue and provided convincing evidence that the observed inhibition is due to a direct action upon the terminals of the primary afferents. As such, it should appropriately be termed *presynaptic inhibition* (Eccles et al., 1961, 1962; Fig. 1.15).[15] This interpretation also served to account for several earlier observations, such as the *dorsal root potential*—an activity-induced depolarization of the dorsal root fibers, studied by Barron and Matthews (1938) and others, that in its time course paralleled presynaptic inhibition. At the time Eccles first proposed it, there was no evidence for the postulated axoaxonal endings, but Eccles was undeterred by this fact. In a seminar at Oxford in 1961, he confidently predicted that such synapses would soon be found "because the anatomists are good boys and always find what they are told to look for."

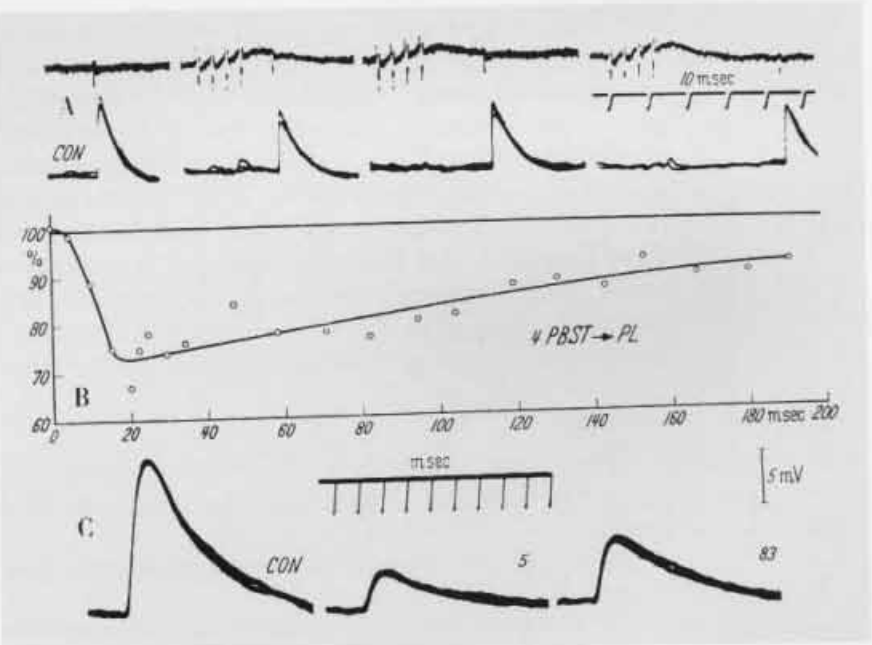

Figure 1.15. Depression of a monosynaptic EPSP by presynaptic inhibition.

(A) The control EPSP (CON) in a plantaris motoneuron is seen to be depressed by four group I conditioning volleys in the nerve to the knee flexors and posterior biceps plus semitendinosus (PBST). The timing of the conditioning and testing afferent volleys is shown in the upper traces (positivity upward in both traces).

(B) The time course of the EPSP depression (expressed as a percentage of control) is shown for the series illustrated in *A*.

(C) The control EPSP (CON) of another experiment is seen to be greatly depressed at both 5 and 83 msec after a conditioning tetanus of 22 group I volleys.

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While these observations on presynaptic inhibition in the mammalian spinal cord were being made, Dudel and Kuffler were studying a closely related phenomenon in the crayfish nerve-muscle system (Dudel and Kuffler, 1961; Dudel, 1962). Here there are two independent innervations of the muscle fiber mediated by a single excitatory and a single inhibitory axon. The essential finding in their work was that when an impulse in an inhibitory fiber preceded an impulse in the excitatory fiber to the same muscle, there was a marked depression of the evoked EPSP. Although there was more than one possible explanation for this phenomenon, Dudel and Kuffler clearly showed the inhibitory impulse acted on the terminals of the excitatory axon to depress the release of transmitter by the excitatory impulse (in addition, of course, to its direct action on the muscle fiber). The inhibitory impulse accomplished this inhibition by reducing the number of quanta released from the terminals of the excitatory nerve.

By contrast, the size of the individual quanta—a measure of receptor sensitivity—was unchanged. Moreover, from the timing and other features of the inhibitory response, Dudel and Kuffler concluded that the observed inhibition was chemically mediated and that the transmitter involved was probably γ-aminobutyric acid (GABA), the same transmitter that the inhibitory axon released directly onto the muscle fiber.

The Search for Neurotransmitters

In an influential review published in 1958, Paton set out five criteria that must be satisfied before a substance can be considered a neurotransmitter (Paton, 1958; see also McLennan, 1963): (1) the enzymes involved in the synthesis of the substance must be present within the presynaptic neurons; (2) the substance must be released from the axon terminals when the presynaptic fibers are stimulated; (3) the action of the substance when applied to the postsynaptic cells must accurately mimic that seen during normal synaptic transmission; (4) a mechanism must be present at the site of the synapses to terminate the action of the putative transmitter; and (5) the effect of drugs (whether agonists or antagonists) on the postsynaptic cells must be the same when the putative transmitter substance is applied to the synapse (usually by microiontophoresis). To this list we would now add a sixth criterion, namely, that the postsynaptic cells must bear the appropriate receptors for the substance.[16]

At the time Paton wrote, only ACh and noradrenaline came close to satisfying these criteria, and until the early 1950s there was considerable skepticism among physiologists that even these substances could be regarded as transmitters in the vertebrate CNS. All this was to change during the next two decades as evidence began to accumulate for a variety of transmitter substances, ranging from simple amino acids such as glutamate and GABA to various biogenic amines such as dopamine, norepinephrine, and serotonin, and, somewhat later, a host of different

neuropeptides. It is impossible within the scope of this chapter to give anything like a full account of the discovery of all the currently recognized transmitters, but some are of particular historical significance and should be mentioned briefly. But before considering these examples, reference should be made to another organizing principle, commonly referred to as *Dale's law.*

Dale (1935), with his usual insight and prescience, had concluded that a neuron would release the same transmitter substance from all its synaptic terminals.[17] Over time this simple and clear statement gave rise to two mistaken notions: first, that the action of a neuron must be the same at all its postsynaptic targets; and second, that a neuron can release only one transmitter. In the 1960s, experiments in *Aplysia* clearly established that a single (cholinergic) neuron could have an excitatory action on one target neuron and an inhibitory action on another (Tauc and Gerschenfeld, 1961; Kandel et al., 1967; Kandel, 1968). And in the 1970s, when appropriate cytochemical markers for different transmitters became available, Hökfelt and his colleagues provided convincing evidence for the co-release of transmitters—usually one or more neuropeptides in association with a so-called "conventional transmitter" (e.g., Lundberg et al., 1979).

Acetylcholine

As we have noted, by the late 1940s it was generally accepted (even by Eccles) that ACh is an excitatory transmitter at several sites in the peripheral nervous system (PNS)—including all autonomic ganglia, parasympathetic postganglionic targets, some sympathetic effector cells, and the neuromuscular junction—and also that it functions to inhibit activity at other sites, such as the heart. However, there was still considerable resistance to the notion that it might also serve as a transmitter within the CNS (Eccles, 1949). This despite the fact that Feldberg and his colleagues had provided rather strong evidence that ACh is a central transmitter (see Feldberg, 1945, 1950, for reviews). The principal evidence for this role of ACh derived from a study that Feldberg had carried out with Marthe Vogt, in which they had described the distribution of the enzyme choline acetylase within the brain and spinal cord, where it appeared to be restricted to certain cranial nerve nuclei and the anterior horn of the spinal cord (Feldberg and Vogt, 1948). Later, with Harris and Lin, Feldberg found that the levels of choline acetylase were lowest in the sensory pathways and also low in the motor cortex. This finding led them to suggest that there might, in many systems, be an alternation between noncholinergic and cholinergic neurons. In the motor system, for example, the so-called "upper motor neurons" in the cortex would be noncholinergic while the lower spinal and cranial motoneurons that they contact are cholinergic (Feldberg et al., 1951). So, as far as Feldberg was concerned, by 1950 "the theory of acetylcholine as [a] central transmitter [was] all but settled" (Feldberg, 1950).

Shortly after Eccles had become convinced that central transmission is chemical, he and his colleagues addressed the mechanism responsible for the recurrent inhibition first described by Renshaw (1946). Since this inhibition is due to collateral branches of the axons of motoneurons and is mediated by the repetitive firing of a population of small interneurons near the ventral margin of the anterior horn (which Eccles had termed *Renshaw cells;* see Fig. 1.16), Eccles thought that the motoneuron axon

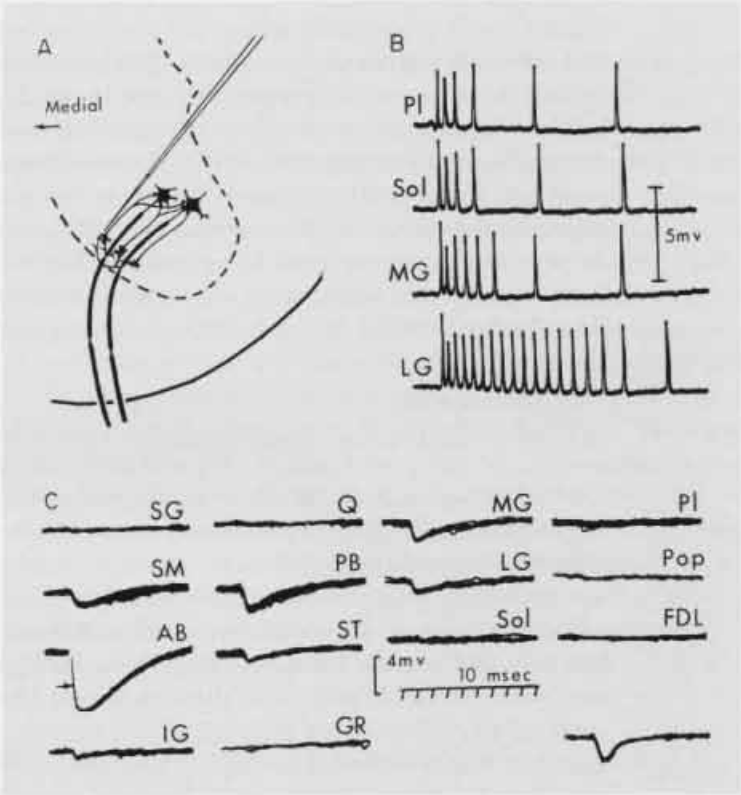

Figure 1.16. Responses of Renshaw cells involved in recurrent inhibition. Renshaw cells are cholinergic interneurons located near the margin of the anterior horn (*A*).

(B) Recordings from a Renshaw cell that fires repetitively to single volleys in the motor fibers to four different muscles.

(C) Intracellular recording reveals that IPSPs of various sizes are produced in an anterior biceps motoneuron by single volleys in the motor fibers supplying eight different muscles of the same hind limb. AB, anterior biceps; FDL, flexor digitorum longus; GR, gracilis; IG, inferior gluteal; LG, lateral gastrocnemius; MG, medial gastrocnemius; PB, posterior biceps; Pl, plantaris; Pop, popliteus; Q, quadriceps; SG, superior gluteal; SM, semimembranosus; Sol, soleus; ST, semitendinosus.

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collateral–Renshaw cell synapse would be a good candidate site at which to test Dale's principle. Since motoneurons release ACh at the neuromuscular junction, their axon collaterals should (if Dale's principle is correct) form cholinergic synapses upon Renshaw cells. In the mid-1950s, Eccles collaborated with his daughter Rosamond, Paul Fatt, and K. Koketsu to demonstrate this point convincingly. They recorded from Renshaw cells and found that local administration of ACh and nicotine increased their firing. Moreover, administration of eserine, an inhibitor of acetylcholinesterase, greatly increased and prolonged the discharge of the Renshaw cells in response to single shocks to the ventral root (Eccles et al., 1954, 1956).

Later studies by others soon demonstrated (1) the presence of cholinergic neurons in several regions of the brain, including the so-called basal nucleus of the forebrain that provides the cholinergic input to the cerebral cortex and hippocampus; (2) the release of ACh in different brain regions after appropriate stimulation of the relevant afferent pathways; (3) the presence of receptors for ACh at sites of termination of the cholinergic fibers; and (4) the release of ACh on electrical or chemical stimulation of brain slices. Since these studies have been discussed extensively elsewhere (Waser, 1975), they need not be considered further here.

Noradrenaline (Norepinephrine)

Dale, who had introduced the term *cholinergic,* used the term *adrenergic* to describe the postganglionic nerves that release an adrenaline-like substance at their terminals. The great interest in adrenaline as a hormone that had been isolated from adrenal extracts in the early part of the century, and the similarities between the actions of adrenaline and those that followed stimulation of postganglionic sympathetic nerves, led to the erroneous assumption that the transmitter liberated by sympathetic nerve endings was in fact adrenaline. However, as early as 1910, Barger and Dale had sounded a cautionary note when they wrote that "the action of some of the other bases, particularly the amino acid and amino-ethyl-bases of the catechol group [noradrenaline] corresponds more closely with that of sympathetic nerves than does that of adrenaline."

Nevertheless the idea that adrenaline was the sympathetic transmitter persisted until the 1940s, in large part because of the report by Cannon and Lassak (1939) that certain organs seem to contain adrenaline in their sympathetic nerves[18] and the finding of Gaddum and Kwiatkowski (1939) that postganglionic stimulation of the nerves to the rabbit ear released what appeared to be adrenaline. It was only in 1946 —when Ulf von Euler succeeded in showing that noradrenaline, not adrenaline, was the principal compound isolated from mammalian sympathetic nerves—that the pharmacological community came around to accepting noradrenaline as the transmitter. Soon thereafter all of Paton's criteria were met as, in rapid succession, the mechanisms of

synthesis, storage, release, and inactivation of noradrenaline at noradrenergic nerve terminals were elucidated. For example, in 1956 von Euler and Hillarp found noradrenaline storage particles in the sympathetic nerve trunk. In 1957 Brown and Gillespie showed that stimulation of the sympathetic nerves to the spleen resulted in the release of noradrenaline into the perfusing fluid. In 1961 de Robertis and Pellegrino De Iraldi described characteristic large, synaptic vesicles with electron-dense cores in sympathetic nerve terminals. And finally, in 1961, Hertting and Axelrod showed that labeled norepinephrine, which is taken up by sympathetic nerves, is released when the nerves are stimulated (see Iversen, 1967, for review).

As early as 1955 Eranko had observed that formaldehyde condensation can cause catecholamines to fluoresce. In 1962 Falck and Hillarp discovered that by freeze-drying and using gaseous formaldehyde they could prevent catecholamine diffusion within tissues, thereby increasing the sensitivity of the method so that it was possible to visualize catecholaminergic neurons and their nerve terminals in histological sections when viewed under a fluorescence microscope (Falck, 1962; Falck et al., 1962). Although all parts of the catechol-containing neurons could be visualized, the strongest fluorescence (and, by inference, the highest concentration of the amine) was found in the nerve terminal and in axonal varicosities. Later work using this method and immunohistochemistry for the enzyme dopamine β-hydroxylase (Swanson and Hartman, 1975) was able to show that most of the noradrenergic neurons in the brain have their cell bodies in a small nucleus of the brainstem, the locus coeruleus, and in two other cell groups in the lower pons and medulla. The locus coeruleus is a remarkable structure; it contains only a few thousand neurons, yet it gives rise to axons that extend over considerable distances to innervate neurons throughout much of the brain (Bloom, 1977; Moore and Bloom, 1979).

GABA and Glycine: The Search for Inhibitory Transmitters

In 1950 Eugene Roberts and Jorge Awapara independently discovered GABA in the brain and determined the mechanism of its biosynthesis from glutamic acid (Awapara et al., 1950; Roberts and Frankel, 1950). Nevertheless its significance remained unclear for some years. However, as early as 1953 Florey had identified in crude brain extracts a fraction, which he termed factor I, that had a powerful inhibitory effect on the slowly adapting neuron of the crayfish abdominal stretch receptor organ (Florey, 1953). Four years later, with Blazemore and Elliott, he succeeded in purifying the inhibitory factor and identified it as GABA (Blazemore et al., 1957). The following year Kuffler and Edwards (1958) demonstrated that GABA could accurately mimic the action of the crayfish inhibitory neuron, and in 1963 Kravitz and his colleagues found

that the concentration of GABA in the inhibitory neuron far exceeded that in the adjoining sensory neuron (Kravitz et al., 1963). Conclusive evidence that inhibition at this site is mediated by GABA was provided by the observation that GABA is released on stimulating the inhibitory nerve in the lobster (Otsuka et al., 1966).

Not long after these studies in crustaceans, several workers demonstrated that GABA played a similar role in the mammalian CNS. In 1966 Krnjevic and Schwartz found that the microiontophoresis of GABA into the cerebral cortex could mimic the action of the local inhibitory neurons. Using much the same approach, Obata and his colleagues showed that this is true also of the action of Purkinje cells on neurons in Deiter's nucleus (Obata et al., 1967). Furthermore, GABA was released into the fourth ventricle on stimulating the axons of Purkinje cells (Obata and Takeda, 1969). Finally, in 1974, Roberts and his colleagues succeeded in raising antibodies against glutamic acid decarboxylase, the key enzyme in the synthesis of GABA, and showed immunocytochemically that they labeled many of the known inhibitory neurons in the brain (Roberts et al., 1976). McGeer et al. (1975) achieved essentially the same result by examining, in autoradiographs, the uptake of ^{3}H-GABA by inhibitory neurons and its transport to their axon terminals.

Glycine

The other known ionotropic inhibitory transmitter in the vertebrate CNS is the amino acid glycine. Unlike GABA, glycine (as a neurotransmitter) is confined to the pons, medulla, and spinal cord, where it is found mainly in interneurons (and their axon terminals) that mediate the inhibition of motoneurons, Renshaw cells, and some of the large neurons of the reticular system (Aprison and Werman, 1965; Aprison et al., 1970). In the period covered by this review much less had been done on glycine than on GABA. However, the finding that iontophoresing glycine onto the spinal motoneurons closely mimicked the naturally occurring inhibition induced by stimulation of group Ia afferents from an antagonist muscle was generally accepted as evidence that glycine is *the* inhibitory transmitter in the lower brainstem and spinal cord (see Aprison et al., 1974, for review).

Glutamate

The surprising discovery that the amino acid glutamate is the major excitatory transmitter in the brain had its origins in the laboratory of David Curtis, one of Eccles's students. In the late 1950s Jeffrey Watkins, working with Curtis and Phillis, found that glutamate and aspartate (and a series of more than 100 analogues) had strong excitatory actions when iontophoresed into the vicinity of spinal neurons in vivo or when added to the bathing solution of isolated spinal cord preparations in vitro. However, when it became clear that glutamate has similar excitatory actions

on virtually every neuron in the nervous system, the balance of opinion swung against the view that glutamate might be a neurotransmitter. How, it was asked, could a molecule that participates in a highly specific signaling mechanism have such widespread and general effects?

In the 1960s and 1970s several different lines of evidence were adduced in an attempt to answer this question. For example, in 1971 Solomon Snyder and colleagues demonstrated high-affinity uptake of glutamate and aspartic acids into a distinctive population of synaptosomes from the brains of rats (Wofsey et al., 1971). In 1967 Aprison and his colleagues had shown that L-glutamate is more concentrated in dorsal than in ventral spinal roots and is found in higher concentrations in the dorsal medulla than in its ventral half. This finding led to the suggestion that glutamate might be the transmitter in primary sensory afferents (Graham et al., 1967). And in the late 1970s, Storm-Mathisen showed that interruption of the perforant path to the hippocampus resulted in a reduction of more than 50% in the uptake of glutamate by the dentate gyrus. Moreover, uptake of ^{3}H-glutamate by mossy fiber and other axon terminals could be readily demonstrated autoradiographically (Storm-Mathisen, 1977; Storm-Mathisen and Iversen, 1979).

Convincing though this type of evidence was to some investigators, many more remained skeptical until Watkins synthesized a number of structurally related analogues of glutamate and aspartate and set out to study the structure-activity relationships of these putative excitatory amino acid receptors. The most notable compound he produced was *N*-methyl-D-aspartate (NMDA). By comparing the excitatory potency of NMDA with that of other analogues (such as kainic acid), Watkins proposed that there must be multiple receptors for glutamate, one of which he named the NMDA receptor.

In 1981 Watkins and Evans published a highly influential review that suggested that glutamate receptors could be divided into two broad categories: NMDA and non-NMDA receptors (the latter including kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate [AMPA] receptors). The NMDA receptors had a number of interesting characteristics. In particular, they were pharmacologically distinct, being blocked by phosphono-substituted amino acid derivatives and also, surprisingly, by Mg^{2+} ions (Watkins and Evans, 1981). The discovery of the blockade of the NMDA receptor by Mg^{2+} was both unique and puzzling. Through the subsequent work of Mark Mayer, Gary Westbook, and Philipe Ascher, it became clear that Mg^{2+} plugged the NMDA channel in a voltage-dependent manner and that, unlike the non-NMDA channels, the NMDA channel was permeable to Ca^{2+}.

In addition to synthesizing agonists, Watkins also synthesized a number of antagonists of the glutamate receptors. His first success was with the NMDA antagonist 2-amino-5-phosphonovalerate. Watkins used these NMDA antagonists to provide the first direct evidence that NMDA receptors are involved in synaptic transmission in the CNS. Others showed that NMDA receptors played key roles in synaptic plasticity (including

long-term potentiation) and in neuropathology (including epilepsy and neuronal cell death; see Bear and Linden, this volume).

It would be difficult to exaggerate the impact of the discovery of the NMDA receptor. Its unique combination of properties allows this receptor to participate in many of the fundamental mechanisms in the brain, of which the following are but a few examples. In 1949 Donald Hebb proposed a theory of associative memory based on the idea that if a presynaptic neuron excites its postsynaptic partner sufficiently strongly, so that it fires an action potential, the synapse would be strengthened. For many years this seemed to be a theory in search of a mechanism, but the discovery of the NMDA receptor provided just the mechanism needed. A large body of evidence now exists supporting the hypothesis that NMDA receptors are critically involved in synaptic plasticity in the hippocampus and elsewhere through an essentially Hebbian mechanism. The essence of the hypothesis is that NMDA receptors usually do not participate in normal synaptic transmission in the hippocampus because, at the resting membrane potential, the channel mouth is blocked by Mg^{2+}. However, when the postsynaptic neuron is sufficiently depolarized by a level of activity through the non-NMDA receptors, the Mg^{2+} blockade of the NMDA receptor channel is relieved. This allows the second defining characteristic of the NMDA channel, its permeability to Ca^{2+}, to come into play. The resulting Ca^{2+} influx triggers a biochemical cascade that ends with the strengthening of transmission at the synapse. Thus the NMDA receptor underlies a highly specific, associative form of synaptic plasticity. This is not all: the same mechanisms allow the NMDA receptor to play a fundamental role in the development of wiring specificity in the nervous system. For example, in the development of ocular dominance columns in the optic tectum of the frog, the NMDA receptor seems to act as a "coincidence detector" that enables neighboring ganglion cells to capture and maintain synaptic contacts with neighboring tectal neurons. Another example of the importance of NMDA receptors comes from their role in excitotoxicity. It is well known that the ischemia caused by a stroke causes many neurons to die and can result in debilitating brain damage. It now appears that the NMDA receptor plays a major role in causing the superadded death of cells outside the immediate zone of ischemic necrosis. The release of glutamate from the oxygen-deprived neurons massively activates NMDA receptors, causing a huge Ca^{2+} influx into nearby neurons, and this Ca^{2+} influx is sufficient to trigger the events that lead to cell death or apoptosis.

Dopamine

In a short but prescient note written in 1939, Herman Blaschko delineated the biosynthetic pathway that leads from the amino acid tyrosine to adrenaline and noradrenaline, in which dopamine is a critical intermediate (Blaschko, 1939, 1942). Until the 1950s this was thought to be dopamine's only role; however, it was during that decade that Arvid

Carlsson noted the marked differences in the regional distribution of dopamine and noradrenaline, both in peripheral tissues and in the mammalian CNS. This led him to suggest that dopamine might act as a transmitter in its own right and have a role quite independent of its function as a precursor to noradrenaline (Carlsson 1959; see Carlsson, 1987, for review).

The introduction of the Falck fluorescence method for mapping the distribution of central aminergic neuronal groups and their projections permitted Dahlstrom and Fuxe in 1964 to delineate, for the first time, the location of dopamine-containing neurons in the brainstem and to show their rostral projections to the hypothalamus, the limbic cortex, and the striatum (caudate nucleus and putamen). The projection to the striatum from the pars compacta of the substantia nigra is of particular interest, since the loss of cells in the substantia nigra had long been recognized as the principal pathological finding in Parkinson's disease. The suggestion by Oleb Hornykiewicz that a loss of dopamine from the striatum might underlie the extrapyramidal motor signs of the disorder, and that some of its clinical features might be relieved by the administration of L-DOPA, is one of the great success stories of clinical neurology (see Hornykiewicz, 1973, for review). Of the other two projections, that to the meso-limbic and neocortex would later be proposed to be important in schizophrenia, and the projection from the tubero-infundibular region of the hypothalamus proved to be critically involved in the regulation of pituitary function. For a general account of the physiological roles played by dopamine and of the various pharmacologically recognizable dopamine receptor subtypes, reference should be made to the review by Gingrich and Caron (1993).

Serotonin

Serotonin, whose role in brain function only began to be understood in the 1980s, was initially isolated from blood platelets. But as early as 1953, Betty Twarog, using a clam heart bioassay, had discovered a high concentration of serotonin in the brain (Twarog and Page, 1953). The following year Amin, Crawford, and Gaddum (1954) were able to show that serotonin was particularly concentrated in the limbic system and hypothalamus. These findings assumed new significance when it was shown the action of serotonin was antagonized by lysergic acid diethylamide (LSD), which had been known for some time to induce mental states reminiscent of those seen in schizophrenia (Woolley and Shaw, 1954).

In the 1960s the distribution of serotonin-containing neurons was mapped by Dahlstrom and Fuxe, using the Falck method. The majority of the cells were found to be confined to two of the raphe nuclei of the brainstem, and their axons could be readily traced rostrally to the hypothalamus and the limbic cortex, and caudally to the spinal cord, where they may act to inhibit the transmission of pain sensibility (Dahlstrom and Fuxe, 1964).

Neuropeptides

Since the discovery of the first neuropeptide—substance P—by von Euler and Gaddum in 1931, a large and continuously expanding number of neuronally active peptides have been identified in both vertebrates and invertebrates. As many of the precursor molecules or prohormones are known to give rise (either by alternative mRNA splicing or peptide cleavage) to two or more biologically active peptides, it is difficult to predict what the total number of such peptide transmitters (or, more correctly in some cases, neuromodulators) is likely to be. Moreover, many peptides that were originally isolated from other tissues, such as the skin and the gastrointestinal tract, have later been shown to be present in particular classes of neurons in the CNS and PNS, and to give rise to distinct neural projections. Some of these peptides now have well-documented physiological and behavioral roles, and many more neurally active peptides of this kind will probably be discovered. Although the primary role of many of these peptides is well known—as in the case of the neurohypophysial hormones vasopressin and oxytocin and some of the hypothalamic releasing hormones—their other functions, as putative neurotransmitters or neuromodulators within the CNS, remain to be determined. And only in a few cases, such as luteinizing hormone releasing hormone (LHRH)—which is responsible for the slow potential changes seen in sympathetic ganglia (Jan and Jan, 1982)—has convincing evidence been adduced about their actions at identified synaptic sites.

As it is impossible in the space available to review the discovery of all the known neuropeptides, we shall limit ourselves to just one—substance P (SP). Here we shall mention only the major historical events that led to its recognition as an important mediator of pain sensibility (see McGeer et al., 1978, for review).

When von Euler and Gaddum first identified the peptide in 1931, they noted that although it resembled ACh in its action on smooth muscle, its effects were not abolished by atropine. It was more than 20 years later before Pernow (1953) showed that it was present in several regions of the brain, including the thalamus, hypothalamus, and basal ganglia, and, interestingly, also in the dorsal roots. This latter observation led Lembeck (1953; cited in McGeer et al., 1978) to propose that SP might be a neurotransmitter. Again, almost 20 years passed before Susan Leeman and her colleagues, while trying to isolate the corticotrophin-releasing factor, discovered a substance that promoted salivary secretion and found that it too was not blocked by atropine. On further study the substance proved to be an undecapeptide that had all the properties of von Euler and Gaddum's SP (Chang and Leeman, 1970). That the peptide had the properties of a neurotransmitter, including its release from neurons in a Ca^{2+}-dependent manner, was subsequently demonstrated by Iversen et al. in 1976. And, at about the same time, Hökfelt and his colleagues, using antibodies against the peptide, were able to map its distribution within the sensory ganglia and in the CNS (Hökfelt et al., 1975).

The history of the discovery of several of the other neuropeptides is at least as interesting as that of SP, and in some cases the discoveries were extremely controversial at the time they were made (Wade, 1981). But here it will suffice simply to mention a number of features that most of the known neuropeptides have in common. First, they are synthesized in the neuronal bodies, packaged into large dense-core vesicles (where they may undergo further processing), and axonally transported along microtubules to their sites of release either at en passant contacts or at axon terminals. Second, at their release sites they are nearly always associated with one of the more conventional neurotransmitters in small, clear vesicles and are co-released with the conventional transmitter. Third, compared with the action of the ionotropic transmitters, their action is slow and long lasting. Fourth, the known receptors for peptide transmitters are of the seven-transmembrane-domain, G-protein-coupled variety, whose intracellular actions on the target cells are mediated by second messengers. Fifth, the relevant receptors may be located at some distance from the release site, and the peptide often has autocrine and paracrine effects. And, finally, unlike conventional transmitters, which are usually present in synaptic vesicles at concentrations in the 100 mM range and whose affinity for the associated receptors is on the order of 100 μM to 1 mM, neuropeptides are present at concentrations of 2–10 mM at most, and they bind to their receptors with affinities in the nanomolar to low micromolar range.

Synaptic Receptors Coupled to Second Messenger Pathways

By the 1970s, it was clear that virtually all the conventional, small-molecule transmitters—ACh, GABA, glutamate, norepinephrine, dopamine, serotonin—not only activate ionotropic receptors and ligand-gated channels to produce rapid synaptic potentials that last for only milliseconds but also interact with a second, even larger class of seven-transmembrane-domain metabotropic receptors that produce slow synaptic responses that can persist for seconds or minutes. Metabotropic receptors consist of a receptor molecule that is coupled to its effector molecule by a nucleotide-binding G protein. G proteins couple the receptors to secondary effectors—such as cAMP, cGMP, diacylglycerol, and metabolites of arachidonic acid—that can activate channels directly. More commonly, however, these second messengers activate a protein kinase that regulates channel function by phosphorylating the channel protein or an associated regulatory protein. This family of receptors is remarkably large, and its members serve not only as receptors for small molecule and peptide transmitters, but also as the sensory receptors for vision and olfaction.

The study of slow synaptic potentials mediated by second messengers has added three new features to our understanding of chemical

transmission. Two of these are particularly important. First, in addition to their action on ion channels, transmitters that act on metabotropic receptors can (by means of their action through second messengers) modify proteins other than the channels, thereby activating a coordinated molecular response within the postsynaptic cell. Second, the second messengers that are activated by these receptors can translocate to the cell nucleus and modify transcriptional regulatory proteins; in this way they are able to regulate gene expression rather directly. Thus second messengers can both produce a covalent modification of preexisting proteins and regulate the synthesis of new proteins. This latter class of synaptic action can lead to long-lasting structural changes at synapses. Finally, fast synaptic transmission is used primarily to mediate behavior; by contrast, slow synaptic actions are often used to modulate behavior.

The Plastic Properties of Synapses

It is perhaps fitting that we should conclude this chapter by returning to an issue that was first clearly articulated by Cajal. Knowing that in most regions of the mammalian brain no additional neurons are generated in postembryonic development, and knowing that the patterns of connectivity that are laid down during development are, of necessity, highly specific, Cajal pondered two fundamental questions: (1) How can the brain acquire new information, in the process usually referred to as learning? (2) How can such information be retained in the form of memory?

In his 1894 Croonian Lecture to the Royal Society, to which we referred earlier, Cajal proposed a possible solution to these problems:

> These observations . . . have suggested to us an hypothesis which will enable us to understand . . . intelligence acquired by good mental training, the inheritance of intelligence . . . and even the creation of . . . artistic ability. . . .
>
> Mental training cannot better the organization of the brain by adding to the number of cells; we know that nervous elements have lost the property of multiplication past embryonic life; but it is possible to imagine that mental exercise facilitates a greater development of the protoplasmic apparatus and of the nervous collaterals in the part of the brain in use. In this way, pre-existing connections between groups of cells could be reinforced by multiplication of the terminal branches of protoplasmic processes and nervous collaterals. But the pre-existing connections could also be reinforced by the formation of new collaterals and protoplasmic expansions. (Cajal, 1894)

No better hypothesis was forthcoming until Donald Hebb and Jerzy Konorski proposed that the strength or effectiveness of specific synapses may be changed as a result of activity:

> The application of a stimulus . . . leads to changes of a two-fold kind in the nervous system. . . . The first property by virtue of which nerve cells

> react to the incoming impulses with certain cycles of changes we call *excitability*, and that changes arising in the centers because of this property we should call *changes due to excitability*. The second property, by virtue of which certain permanent functional transformations arise in particular systems of neurons, as a result of appropriate stimuli or combinations, we shall call *plasticity*, and the corresponding changes *plastic changes*. (Konorski, 1948; his emphasis)

And, to provide a specific neuronal basis for such changes, Hebb noted that "When an axon of cell A . . . excite[s] cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased" (Hebb, 1949).

The idea that learning might produce plastic alterations in synaptic strength, and that the persistence of these changes would give rise to memory storage, was first systematically tested in invertebrates, where studies of synaptic transmission in the neural circuit responsible for the gill-withdrawal reflex in the marine snail *Aplysia* showed that simple forms of learning—habituation, sensitization, and classical conditioning—produce changes in synaptic strength that can persist for one or more days and that parallel the time course of the memory process (Kandel, 1976). This functional plasticity hypothesis was dramatically extended to the mammalian brain by Bliss and Lømo (1973), who found that high-frequency tetani applied to the perforant pathway in the hippocampus—a structure known to be critically involved in memory storage—could produce alterations in synaptic strength, which they termed long-term potentiation (LTP). In brain slices LTP lasts for hours, and in the intact animal, for days.

Since these and several of the other topics we have touched upon are dealt with in detail in the succeeding chapters of this volume, we may end by once again quoting Cajal:

> Functional theories based on the localization of different cortical areas, no matter how good, fail completely to explain mechanisms underlying cognitive activity, which is almost certainly accompanied by molecular changes in neurons, as well as by very complex changes in relationships between neurons. Therefore, to understand cognitive activity, it will be necessary to understand these molecular and connectional changes, not to mention the exact histology of each cortical area and all of their pathways. However, this is still not enough; we also need to understand the properties of neural impulses: What energy transformations are required for their initiation, spread, and involvement in the phenomena accompanying perception and thought, namely consciousness, volition, and emotion?
>
> Our knowledge is far from complete. While waiting for chemistry, cell biology, and histology to help achieve this goal, which will take a very long time, we must be content with hypotheses that occasionally lead to the discovery of a useful observation or formulate a more precise concept. (Cajal, 1995:721–722)

Appendix

A Chronology of the Major Events in the Study of Synapses and Synaptic Transmission

1791 **Galvani** observes the contraction of muscles in the hind limbs of frogs when a metal hook is inserted into the medulla and then attached to an iron railing. He claims to have discovered "animal electricity."

1793 **Volta** recognizes that the source of the current in Galvani's experiments was the interaction of two unlike metals, not the animal itself.

1862–1863 **Kühne** and **Krause** independently describe the structure of the neuromuscular junction and suggest that transmission from nerve to muscle is an electrical process.

1877 **Du Bois-Reymond** calls into question the notion that transmission between nerve and muscle is electrical and suggests that it may be mediated by the release of a chemical substance.

1878 **Bernard** experiments with curare and concludes that it acts to block transmission at or near the neuromuscular junction while having no effect on nerve conduction.

1886 **His** provides strong evidence from his developmental studies for the structural independence of neurons and for the outgrowth of their processes from the cell body.

1887 **Forel** shows that the interruption of neuronal projections leads to the atrophy of only the injured neurons and does not spread to other neuronal populations, thus providing further evidence for neuronal independence.

1888 **Cajal** observes the termination of the axons of the stellate cells of the cerebellum in pericellular "baskets" around Purkinje cells and launches a long series of studies on the mode of axon terminations, in support of the neuron theory.

1891 **Waldeyer** formulates the neuron theory and introduces the term *neuron.*

1897 **Sherrington** coins the term *synapse* for the site at which an axon terminal or collateral makes a functional contact with another cell.

Elliott concludes that adrenaline (epinephrine) is the transmitter released by sympathetic postganglionic fibers.

1905 Based on his experiments on transmission through the superior cervical ganglion, **Langley** suggests that it is mediated by "receptive" substances on the ganglion cells. This is the first clear statement of the concept of receptors for neurotransmitters.

1906 Publication of **Sherrington's** *Integrative Action of the Nervous System,* which summarizes a vast body of experimental work on spinal and other reflexes, including observations on the role of inhibition as an active physiological mechanism.

1909–1911 Publication of the definitive French translation of **Cajal's** great work *Textura del Sistema Nervioso del Hombre y de los Vertebrados* under the title *Histologie du Système Nerveux de l'Homme et des Vertébrés.*

1914 From studies of the actions of various choline esters, **Dale** concludes that acetylcholine is probably the transmitter released at preganglionic synapses and by most parasympathetic postganglionic fibers.

1921 **Loewi** discovers "Vagusstoff" and later shows that it is probably acetylcholine.

1931 **Von Euler** and **Gaddum** discover a depressor substance in various tissues; they name it substance P.

1933 **Feldberg** and **Krayer** repeat Loewi's experiment in dogs and provide clear evidence for the release of acetylcholine on stimulating the vagus nerve. This is the first of what would be a long series of studies that Feldberg was to carry out on acetylcholine as a transmitter in the autonomic nervous system, at the neuromuscular junction, and in the central nervous system (CNS).

1934 Publication of **Cajal**'s last work, *¿Neuronismo o Reticularismo?*, later translated into English as *Neuron Theory or Reticular Theory*, which finally lays to rest the mistaken notion that the nervous system is a syncytium.

1935 **Dale** formulates the principle that neurons release the same transmitter at all their axon terminals.

1936 **Dale, Feldberg,** and **Vogt** demonstrate that acetylcholine is released by motor fibers at the neuromuscular junction.

1938 **Göpfert** and **Schaefer** discover what would later be known as the endplate potential (EPP). Their findings are confirmed by **Eccles** and **O'Connor** (1939).

1939 **Blaschko** identifies dopamine as an intermediate in the biosynthesis of adrenaline and noradrenaline.

1942 **Kuffler** develops the single nerve–muscle fiber preparation and provides the first elementary analysis of the endplate potential.

Arvanitaki describes the properties of artificially constructed synapses, called *ephapses*.

1946 **Von Euler** finds that noradrenaline (not adrenaline) is the transmitter released at most postganglionic sympathetic terminals.

1947 **Brooks** and **Eccles** put forward a rigorously defined electrical hypothesis for both central excitation and inhibition, the latter calling for the interposition of a nonspiking Golgi II cell between the afferent input and the target neuron.

1948 **Konorski** predicts that associative learning may be due to long-term changes in neuronal excitability and defines such changes as "plastic."

1949 **Lloyd** discovers posttetanic potentiation.

Hebb postulates that the near-coincident firing of a presynaptic afferent fiber and its target postsynaptic cell may lead to a strengthening of the synaptic connection. Such synapses become known as "Hebbian."

1950 **Roberts** and **Awapara** independently discover γ-aminobutyric acid (GABA), which is later shown to be the principal inhibitory transmitter in the brain.

1951 **Fatt** and **Katz** extend the analysis of the EPP using intracellular recording. They observe among other things spontaneous discharges, later known as miniature EPPs (mEPPs).

Brock, Coombs, and **Eccles** report the first intracellular recordings from motoneurons in the spinal cord. Their later work (1952) leads to the identification of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively). The finding that inhibition is marked by a simple hyperpolarization causes Eccles to abandon his earlier electrical hypothesis and enthusiastically embrace the view that central synaptic transmission is chemical.

1953 **Twarog** and **Page** find that serotonin is present in high concentration in the brain.

Pernow finds that substance P is present in several regions of the brain, spinal cord, and dorsal root ganglia.

1954 **Del Castillo** and **Katz** describe mEPPs in detail and formulate the quantal hypothesis for transmitter release.

Fatt, in an extensive review of junctional transmission, predicts that at certain sites (especially where the pre- and postjunctional elements are about the same size) transmission may be found to be electrical.

1955 **Palay** and **Palade** as well as **De Robertis** and **Bennett** identify synaptic vesicles in electron microscopic (EM) preparations and relate these to the quantal release hypothesis.

Eranko finds that formaldehyde condensation causes biological amines to fluoresce. In 1962 **Falck** and **Hillarp** develop this as a method for identifying such neurons and their projections in the CNS.

1956 **Eccles** and colleagues establish that transmission at the motoneuron axon collateral–Renshaw cell synapse is cholinergic.

1957 **Frank** and **Fuortes** observe a reduction in the amplitude of EPSPs evoked by stimulation of muscle afferents on stimulation of other spinal inputs. Frank later refers to this as "remote inhibition."

Brazmore, Elliott, and **Florey** purify the inhibitory factor I that Florey had found in brain extracts and show that it is GABA.

1958 **Kuffler** and **Edwards** provide convincing evidence that GABA is the transmitter in the crayfish inhibitory neuron.

Paton sets out five criteria that must be satisfied before any substance can be considered a neurotransmitter.

1959 **Furshpan** and **Potter** discover and provide a detailed analysis of rectifying electrical synapses in the abdominal nerve cord of the crayfish. **DeLorenzo's** EM studies show that the intercellular gap is considerably narrowed at these sites; such contacts are later identified as *gap junctions.*

Curtis, Phillis, and **Watkins** demonstrate that acidic amino acids excite neurons in the spinal cord.

Based on the density of postsynaptic membranes, **Gray** identifies type I and type II synapses, and it is suggested that these are excitatory and inhibitory respectively.

Whittaker identifies particles from homogenized and centrifuged brain tissue that bind acetylcholine.

Carlsson suggests that dopamine may function as a neurotransmitter in its own right.

1961 **Eccles, Eccles,** and **Magni** identify the phenomenon reported by Frank and Fuortes as *presynaptic inhibition* and predict that it is due to endings on the terminals of the excitatory inputs.

Dudel and **Kuffler** find presynaptic inhibition in the crayfish.

Tauc and **Gerschenfeld** show in *Aplysia* that acetylcholine can be excitatory at some neurons and inhibitory at others.

1962 **Gray** and **Whittaker** isolate pinched-off presynaptic terminals with attached postsynaptic densities, which they term *synaptosomes.*

Gray observes axoaxonic synapses in EM studies of the spinal cord and suggests that they may be responsible for presynaptic inhibition, as postulated by Eccles.

1963 **Martin** and **Pilar** show that transmission at single synapses in the chick ciliary ganglion can be both electrical and chemical.

Kravitz and colleagues provide strong evidence that GABA is the transmitter released by the crayfish inhibitory neuron.

1964 **Whittaker** and colleagues succeed in isolating fairly pure populations of synaptic vesicles.

Furukawa and **Furshpan** find that inhibition at the Mauthner cell axon hillock is electrical.

Dahlstrom and **Fuxe** identify cells in the raphe nuclei of the brainstem as the source of serotonin projections to the forebrain and spinal cord.

1965 **Uchizono** observes flattened synaptic vesicles in certain cerebellar synapses and concludes that their presence indicates that the presynaptic fibers are inhibitory. The symmetry of the pre- and postsynaptic specializations at these and in other synapses leads **Colonnier** (1968) to introduce the term *symmetric synapses.*

Aprison and **Werman** provide the first evidence that glycine is an inhibitory transmitter in the spinal cord and brainstem.

1965, 1967 **Katz** and **Miledi** demonstrate the critical role of Ca^{2+} entry into the axon terminals for synaptic vesicle release at the neuromuscular junction.

1966 **Krnjevic** and **Schwartz** show that microiontophoresis of GABA mimics the action of cortical inhibitory neurons.

Rall and colleagues identify "reciprocal synapses" in the olfactory bulb.

1967 **Kandel, Frazier,** and **Coggeshall** demonstrate that different branches of an identified cholinergic interneuron in *Aplysia* can be excitatory at some synapses and inhibitory at others.

Bennett, Pappas, and **Nakajima,** in a series of four papers, provide a detailed account of the ultrastructural appearance and functional characteristics of electrical synapses in the brains of various fish.

Obata and colleagues establish that GABA is the transmitter at sites of termination of cerebellar Purkinje cell axons.

1969 **Katz** and **Miledi** study synaptic transmission in the giant synapse in the squid stellate ganglion, which permits simultaneous intracellular recording from both the pre- and postsynaptic processes.

1970 **Couteaux** and **Pecot-Dechavassine** introduce the term *active zones* for the sites on the presynaptic membrane where vesicle release occurs.

Kandel and his colleagues find that habituation and sensitization, two simple forms of learning, produce alterations in the strength of specific synaptic connections between sensory and motor neurons mediating the gill-withdrawal reflex and that the persistence of these changes contributes to short-term memory storage in *Aplysia*.

Chang and **Leeman** purify an undecapeptide from hypothalamic tissue and identify it as having the properties of substance P.

1972 **Llinas** and his colleagues confirm the findings of Katz and Miledi on the squid stellate ganglion. They also discover that most of the synaptic delay is attributable to the time required for the opening of the Ca^{2+} channels and document the high local concentration of Ca^{2+} near the sites of transmitter release.

1973 **Heuser** and **Reese** provide functional and EM evidence for the recycling of synaptic vesicles.

Hornykiewicz summarizes the evidence that the motor disabilities of Parkinson's disease associated with the death of cells in the substantia nigra are due to the loss of dopamine within the striatum. He proposes the use of L-DOPA to treat the disorder.

Bliss and **Lømo** demonstrate long-lasting changes in synaptic transmission in the dentate gyrus of rabbits following brief tetanic stimulation of the perforant path (one of the major afferent inputs to the dentate). They name this phenomenon *long-term potentiation*.

1976 **Iversen** and colleagues show that substance P is released in a Ca^{2+}-dependent manner from neurons.

1977 **Storm-Mathisen** establishes that glutamate is the excitatory transmitter in the hippocampus by showing that its levels are markedly reduced after interruption of the perforant path and (with **Iversen** in 1979) that ^{3}H-glutamate is taken up by excitatory terminals.

1979 **Lundberg, Hökfelt,** and colleagues demonstrate immunohistochemically that a peptide and a conventional neurotransmitter can coexist in the same presynaptic process and predict that the co-release of such transmitters may be fairly common.

1981 **Watkins** provides evidence for three different types of ionotropic glutamate receptors based on their binding of *N*-methyl-D-aspartate (NMDA), kainic acid, or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). Considerable attention is later paid to NMDA receptors as mediating long-term changes in neurons.

Notes

1. For a succinct account of Galvani's experiments, their antecedents, and the reception they received, see Clarke and Jacyna (1987).

2. As Krnjevic (1974) has pointed out, since du Bois-Reymond wrote at a time when it was generally believed that axons were in direct continuity with the cells they innervated, it is perhaps misleading to suggest that he conceived of chemical transmission in the same way as it is now understood.

3. The reference is to the 1954 translation into English of Ramón y Cajal's last monograph, *¿Neuronismo o Reticularismo?*, which appeared shortly after his death in 1934.

4. Later work showed that in some situations degenerative changes extend to other cell populations. Indeed, in some of Gudden's experiments (which involved lesions of the cerebral cortex in young rabbits), he reported an atrophy of the mammillary body, which we now know to be secondary to the retrograde degeneration in the anterior thalamic nuclei (see Cowan, 1970, for review). But at the time Forel wrote (1887), his interpretation of Gudden's finding was widely considered a significant ancillary line of evidence for the "trophic independence" of neurons.

5. Originally published in Madrid as *Recuerdos De Mi Vida* between 1901 and 1917. References here to *Recollections* are from the English translation by E. Horne Craigie with the assistance of Juan Cano, first published as Volume 8 of *Memoirs of the American Philosophical Society* in 1937. The translation was reissued by MIT Press in 1966 and published in paperback in 1989.

6. This work was translated in part into French and German quite soon after the publication of the original Spanish version but did not appear in English until 1954 (Ramón y Cajal, 1954).

7. The work was first published by Yale University Press in 1906. It was reissued by Cambridge University Press in 1947, on the occasion of the International Congress of Physiology held at Cambridge, and in 1961 as a paperback. Like Darwin's *Origin of Species, Integrative Action* is distinguished for being more frequently cited than read.

8. As Davenport (1991) has pointed out, Langley used the rather cumbersome phrase "receptive substances" rather than *receptors* (which soon became the accepted term) because "receptor" at the time was widely used for sensory receptors in skin, muscles, and the special senses.

9. The list of Langley's students reads like a veritable Who's Who of British physiology, pharmacology, and biophysics in the first half of the twentieth century. It includes three Nobel laureates—A. V. Hill, Edgar Adrian, and Henry Dale—as well as such other giants as Keith Lucas, Joseph Barcroft, and T. R. Elliott.

10. We cannot resist pointing out that many of the critical experiments on synaptic transmission derived from the work of physiologists, pharmacologists, and chemists who left Germany between 1933 and 1937. Among them were Herman Blaschko, Edith Bulbring, Bernard Katz, Otto Krayer, David Nachmanson, Marthe Vogt, and, of course, Wilhelm Feldberg.

11. In addition to Gerard himself, the group included George Bishop, Detlev Bronk, Halowell Davis, Joseph Erlanger, Alexander Forbes, Herbert Gasser, and "Iron Wire" Lillie, as well as various occasional visitors, such as Ragnar Granit, A. Monnier, and William Rushton (Rushton, 1975).

12. In this section we are dealing with electrical transmission as a general synaptic mechanism; we shall consider later some of the special sites at which true electrical synapses occur (see pp. 38–42).

13. Responding to criticisms about his use of the term *quantal*, Katz later wrote:

> Controversies about words, like arguments about priority, are dominated by emotion, and I well remember W. Feldberg's dictum, namely that there is a type of scientist who, if given the choice, would rather use his colleague's toothbrush than his terminology! My colleagues and I were looking for an adjective which would adequately describe the important property of evoked transmitter release, namely, that it occurs in standard "packets" of large multi-molecular size which are identical with the spontaneously occurring units, and whose size is independent of the event (e.g., impulse, local potential change, chemical or osmotic stimuli) which causes the release. We chose the term "quantal" for this purpose, which seems entirely proper and unobjectionable to me. I have, nevertheless, found myself challenged on two grounds: (a) for supposedly basking in the reflected glory of quantum physics, and (b) for applying the term "quantum" to something which is not constant in size, but subject both to random variation and to experimental change. Objection (a) is, of course, impossible to disprove, and to protest would be in vain. All I would say is that I take my authority for the use of the words from an ordinary dictionary (the entry "quantum" in the *Concise Oxford English Dictionary* may serve), and not from books on quantum physics. This may not satisfy the objectors, but I will take that risk rather than discard an adjective which is singularly apt in describing a whole set of characteristic features. (1969:41)

14. The high density of mitochondria in presynaptic processes is a reflection of their high metabolic activity. It also formed the basis of a quasi-selective staining method for synapses.

15. During the 1950s and 1960s, Eccles's laboratory had become a mecca for neurophysiologists from around the world. A partial list (in alphabetical order) of his collaborators during this extraordinary period includes the following: Anderson, Araki, C. McC. Brooks, V. B. Brooks, Coombs, Curtis, Downman, R. Eccles, Fatt, Hubbard, Iggo, Ito, Kostyuk, Krnjevic, Landgren, Liley, Lundberg, McIntyre, Magni, Malcolm, Miledi, Oscarson, Phillis, Rall, Sears, Schmidt, Watkins, and Willis.

16. The recent discovery that carbon monoxide and nitric oxide are released from active neurons and can have both local and more widely distributed effects has challenged the uniqueness of these six criteria.

17. Useful as Dale's principle has been, it is worth noting that it was formulated half a century before it was known that neurons could contain more than one transmitter. It is now known that in some cases different neuropeptides derived from a common prohormone can be targeted to different processes of a cell (Sossin et al., 1990).

18. The question of whether the transmitter released by postganglionic sympathetic fibers is adrenaline or noradrenaline was for a time unnecessarily complicated by Cannon's suggestion that the transmitter (which he termed sympathin) existed in two forms—one excitatory (sympathin E) and another inhibitory (sympathin I). In 1933 he wrote: "Sympathin is defined as the chemical mediator of sympathetic impulses, ME or MI, which in the (effector) cell induces the typical response, contraction or relaxation, and which, escaping from the cell into the blood stream, induces effects elsewhere in organs innervated by the sympathetic" (Cannon and Rosenblueth, 1933; cited by Davenport, 1991).

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