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Mastering the Patch Clamp Technique: A Practical Guide

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Foreword

"Patch pipettes will be more useful than initially thought." Fred Sigworth's prediction has indeed come true. Originally, patch pipettes were used to measure "elementary" currents through single ion channels in the membrane of frog muscle fibers. Today, electrophysiologists use patch pipettes to investigate membrane currents, potentials, and capacitances in preparations as diverse as erythrocytes, plant cells, and neurons. Extended applications of the patch clamp technique now allow these processes to be measured with multiple pipettes in natural tissues and even in animals during behavior. Combined with modern imaging techniques, a detailed understanding of signaling in complex networks such as the cerebral cortex is within reach. This book provides a competent overview of the wide range of possibilities for recording electrical signals in living tissue with high resolution and, so to speak, to "eavesdrop" on the "conversations" between cells.

Bert Sakmann

Preface

In 1991, Erwin Neher and Bert Sakmann were awarded the Nobel Prize in Physiology or Medicine, in recognition of their work on the "function of single ion channels in cells". Fifteen years earlier, they had developed a method for measuring currents through single ion channels in the membrane of living cells. A discovery that, according to the German-British neurophysiologist and Nobel laureate Bernard Katz, "is equivalent to the detection of atomic particles 50 years ago in terms of both aesthetic satisfaction and scientific significance for biology".

The patch clamp technique is now one of the most important neurophysiological working methods. Its application has provided significant insights into the function and properties of ion channels in basic biomedical research, and it has also become indispensable in applied pharmacological research. For these reasons, the method plays an important role in the theoretical and practical training of biology, biochemistry, medicine, and pharmacy students, and in the context of bachelor's, master's, and doctoral theses.

This book is aimed at advanced students of the aforementioned subjects as well as scientists who wish to gain a deeper understanding of the technique and/or acquire the skills required to perform it. It provides concrete answers to the most important questions that arise, for example: What exactly is meant by "patching"? Which specific applications does the patch clamp technique offer? How do you set up a patch clamp rig? How do you proceed practically in your patch clamp experiment?

The first edition of this book was published more than 25 years ago. Since then, the patch clamp technique has further evolved. Therefore, almost all illustrations are new, and most chapters had to be largely rewritten. Some new parts, like Chap. 6 on special applications or Chap. 7 on the documentation and processing of data, have been added. Also in this edition, we have tried to facilitate access to the specialized literature by citing particularly relevant classic papers and important recent publications at the end of each chapter.

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The History of the Patch Clamp Technique

1

Electrical processes at biological membranes are universally prevalent. They contribute to elementary functions of osmoregulation, metabolism, and exocytosis, and are of central importance for electrical signaling in neurons, sensory and muscle cells. In short: Without knowledge of electrophysiological mechanisms, numerous life processes (and their dysfunctions in diseases) cannot be understood.

The function of nerve and muscle cells requires, among other things, ionic currents that flow through ion channels. With every movement, sensation, thought, and heartbeat, ion channels open and close. They play an important role not only in electrically excitable cells, but are present in every cell membrane of all organisms, including the membranes of cell organelles. The patch clamp technique, which we will introduce in this book, can be used to study single ion channels, as well as currents across the entire cell membrane, in great detail. Since the beginnings in the 1970s, it has become a widely used standard method for measuring electrical processes in cells. In this book, we want to present its theoretical basis, its practical implementation, and its many applications in a compact and "user-friendly" way.

1.1 Bioelectricity

The insight that nerve and muscle cells work with the help of electrical signals is relatively old. The Italian physician and naturalist Luigi Galvani (1727–1798) already believed in the existence of an "animal electricity" in his book published in 1791 *De viribus electricitatis in motu muscularis commentarius*. Despite numerous experiments, he could never conclusively prove it. He imagined that the surface of the muscle was charged with "one" electricity, its interior with "the other" electricity, and the nerve entering the interior formed a conductive bridge between them. It was not until 1838 that the Italian Carlo Matteucci (1811–1868)

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was able to directly measure the electrical current of a muscle. He claimed that the surface of a muscle had a positive voltage, its interior a negative voltage.

Through the comprehensive investigations on animal electricity (between 1848 and 1884) by Emil du Bois-Reymond (1818–1896), director of the Institute for Physiology at Charité in Berlin, Germany, the scientific field of electrophysiology was finally established. Du Bois-Reymond measured the nerve and muscle current with zinc/zinc sulfate electrodes and showed that when a nerve or muscle is stimulated, a "negative fluctuation" of this current occurs, meaning "that any part of the muscle that is in excitation behaves negatively relative to a resting part" (according to Bernstein 1912). He also disproved the prevailing belief that nerves could only conduct in one direction. Two years later, the German physicist and physiologist Hermann von Helmholtz (1821–1894) determined the speed of stimulus conduction in the frog nerve to be 26–30 m/s. However, how cells generate such electrical phenomena remained unknown for a long time.

1.2 The Ion Theory

By the end of the nineteenth century, it was already postulated that cells have a conductive cytoplasm and a lipid cell membrane, which is hardly electrically conductive, but permeable to water and many low molecular weight substances. Therefore, the Viennese physiologist Ernst von Brücke (1819–1892) suggested that this membrane could contain channels or pores that allow water to pass through, but exclude larger dissolved molecules. Later, the English physiologist William Bayliss (1860–1924) pointed out that such water-filled channels could also conduct ions without them having to lose their hydration shell. Finally, the German physiologist Julius Bernstein (1839–1917) developed the hypothesis that the nonuniform distribution of ions across a selectively permeable cell membrane is responsible for the resting potential. He calculated its amplitude – assuming that potassium plays the main role – to be –68 mV. He further wrote: "The membrane potential decreases with stimulation" and explained this by an increase in the ion permeability of the membrane (Bernstein 1912). His almost visionary "membrane theory of bioelectric currents", which he summarized in his book *Elektrobiologie* in 1912, should not obscure the fact that until the 1930s "the leading axonologists were thoroughly skeptical both of the membrane theory in general and of the local circuit theory in particular." (Hodgkin 1976).

1.2.1 The Voltage Clamp

It was not until the end of the 1930s that the American biophysicists Kenneth S. Cole (1900–1984) and Howard J. Curtis (1906–1972) developed the voltage clamp technique and thus demonstrated that the membrane conductivity of a nerve

cell increases when excited. Cole (1979) later recalled this classic experiment: "Hodgkin visited us in our laboratory when we had just recorded the change in conductivity on the oscilloscope. He was as excited as I've ever seen him before, jumping up and down as we explained it."

Their experiments seemed to confirm Bernstein's hypothesis; they clearly showed that ionic currents were responsible for the electrical signals of nerve cells – the action potentials – but they did not reveal which ions were involved and how these currents were generated. Theoretically, the ions could passively flow through pores in the membrane or be actively moved through the membrane by transporter molecules (carriers, pumps).

1.2.2 Hodgkin and Huxley

The two Englishmen Alan Hodgkin (1914–1998) and Andrew Huxley (1917–2012) assumed a carrier model when, in the mid-1930s, they began to study the origin of the action potential on the giant axon of the squid. After a war-related interruption, they published a series of papers between 1949 and 1952 in which they showed how an action potential actually arises. Using the voltage clamp technique developed by Cole and Curtis, they discovered that neuronal excitation is caused by specific currents of sodium and potassium ions through the cell membrane, and were able to separate these ionic currents from each other using a mathematical model.

Hodgkin and Huxley had conducted their experiments to test the carrier hypothesis, but as Hodgkin later recalled, they were disappointed to find that this model was obviously wrong:

> "We had started off to test a carrier hypothesis and believed that even if that hypothesis was not correct, we should nevertheless be able to deduce a mechanism from the massive amount of electrical data that we had collected. These hopes faded as the analysis progressed. We soon realized that the carrier model could not be made to fit certain results, for example the nearly linear instantaneous current voltage relationship, and that it had to be replaced by some kind of voltage-dependent gate." (Hodgkin 1976)

Assuming such voltage-dependent and ion-selective "gates", Hodgkin and Huxley finally developed a series of equations that could explain the height and time course of the action potential with astonishing accuracy. For this achievement, the two scientists received the 1963 Nobel Prize in Physiology or Medicine (Hodgkin 1963; Huxley 1963). Hodgkin and Huxley were able to predict the most important properties of the voltage-dependent gates with their revolutionary work; however, it took more than 20 years before these gates (or channels, as we call them today) could actually be demonstrated.

1.3 The Development of the Patch Clamp Technique

This was roughly the state of research when Bert Sakmann and Erwin Neher were doing their doctoral theses at the Max Planck Institute for Psychiatry in Munich. In 1969 and 1970, as both later recalled (Neher and Sakmann 1992), "two fascinating papers" by Ross Bean, Steven Hladky and Denis Haydon appeared. They had observed the first ion channels in artificial membranes.

1.3.1 The First Channels using the Black-Film Technique

Bean, Hladky and Haydon constructed a measuring apparatus with two chambers separated by a Teflon wall with a small hole in it. They filled the two compartments with salt solution and then spread a drop of phospholipid solution over the hole in the Teflon wall. The lipid forms a thin film over the hole, similar to a soap bubble, which is compressed into a lipid bilayer by the external forces (buoyancy of the oil, water pressure and electrostatic attraction of the two bodies of water). Since this membrane appears optically black, it is called a black film or also after the inventors Müller-Rudin membrane. The hole is thus closed by an artificial lipid bilayer, which forms a very high electrical resistance for ions. However, if certain proteins, such as the bacterial antibiotic gramicidin A, are added to the salt solution in one of the two chambers, the ionic conductivity of the membrane increases abruptly: The peptide gramicidin A is incorporated into the artificial membrane and spontaneously forms ion channels. These open and close in an all-or-none fashion, thus causing short-term, abrupt changes in the current through the membrane.

These experiments showed for the first time the behavior of single ion channels. However, it was an artificial model and not an observation of natural channels from nerve or muscle cells. The first measurements of such channels came from the laboratory of Bernard Katz (1911–2003), a native of Leipzig, Germany, who – being persecuted by the Nazis as a Jew – emigrated to England in 1935 and later worked at University College in London (Katz 1986).

1.3.2 Noise Analysis

Katz had participated in the experiments of Hodgkin and Huxley in the 1930s and later discovered the processes underlying synaptic transmission at the neuromuscular junction, for which he received the 1970 Nobel Prize in Physiology or Medicine (Katz 1970). At the beginning of the 1970s, he and the Mexican neuroscientist Ricardo Miledi (1927–2017) were able to measure the properties of ion channels in the membrane of excitable cells for the first time. The two researchers succeeded in using an indirect and rather difficult-to-understand method, known as noise analysis, to determine some of the properties of the nicotinic acetylcholine receptor at the neuromuscular synapse, the motor endplate.

By analyzing the electrical noise (by "noise" we mean the random fluctuations in the ionic current induced by acetylcholine) the researchers were able to determine that a single acetylcholine receptor conducts about 10 million ions per second. This was an important clue to the underlying mechanism. If the acetylcholine receptor functioned like a transporter, it would have to move an ion across the membrane in 0.1 μs, a speed that seemed much too high for such a transport. Therefore, Katz and Miledi concluded that the current measured after acetylcholine application could not be caused by an active transport process, but that the ionic current must flow passively through channels.

The work of Katz and Miledi showed that ion channels in biological membranes have very similar properties to the gramicidin channels that had been observed in artificial membranes. However, their experiments were based on the simultaneous measurement of the current through a large number of ion channels. Therefore, they were not able to directly observe the opening and closing of single channels. As a result, Katz and Miledi could only draw indirect conclusions about the properties of the channels, such as the average time that each channel stays open (open time), and the average size of the current through each individual channel (current amplitude or single channel conductance). The direct observation of the activity of single channels was first achieved by Erwin Neher (*1944) and Bert Sakmann (*1942), who were awarded the Nobel Prize in Physiology or Medicine for this work in 1991 (Neher 1991; Sakmann 1991).

1.3.3 The First Patch Clamp Experiments

Sakmann had worked at University College in London with Bernard Katz from 1970 to 1973. After that, he went to the Max Planck Institute for Biophysical Chemistry in Göttingen, where Erwin Neher in the department of Hans Kuhn had already begun to investigate single channels in artificial membranes. Neher and Sakmann decided to study the acetylcholine receptors of frog muscle cells in addition to their current projects. They wanted to detect the single channels directly on the biological preparation!

However, this was not as easy as it sounds. The main problem was that the current through a single ion channel had to be extremely small, as expected from Katz's and Miledi's calculations. Even the electrical background noise was about 100 times greater than the current that Neher and Sakmann wanted to measure with the methods then in use. This background noise is mainly caused by the countless channels and ion transporters on the entire surface of the cell. How small the currents through individual channels are can, perhaps, be best illustrated by looking at the different current amplitudes in physiological processes (Fig. 1.1).

Neher and Sakmann wanted to extract the tiny single-channel currents from the background noise by electrically isolating a very small section of the cell membrane, a patch, from its surroundings. They therefore decided to place a very thin glass tube as a measuring electrode on the cell surface. The idea was to place this