Many generations of experiments then were carried out to see whether direct CP violation exists. The measurement required extremely high precision, and after many improvements over 25 years, direct CP violation was finally confirmed[15,16]. Together with the observation of CP-symmetry breaking in B mesons (mesons that contain a bottom quark)[17,18], the theoretical model was confirmed, and helped to establish the standard model of particle physics, which is the current explanation of the Universe's particles and forces.

However, the standard model is not complete. For instance, it cannot explain why the Universe contains so little antimatter, nor what the mysterious substance called dark matter is. Researchers are therefore trying to search for a hint of particle physics beyond that of the standard model. For example, experiments in Japan[19] and Europe[20] are using extremely rare kaon decays to search for such a hint.

In retrospect, Rochester and Butler's V-shaped particle tracks are thought to have been caused by a K_S, produced in the lead plate, decaying into the $\pi^+\pi^-$ state. Since their work, kaons have been used to discover strangeness and the breaking of parity and CP symmetries, to build the quark model and the standard model, and now to search for previously unseen particle physics. Could Rochester and Butler have ever imagined that they had opened such a treasure chest?

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Neuroscience

Neuronal signals thoroughly recorded

Alexander D. Reyes

Originally developed to record currents of ions flowing through channel proteins in the membranes of cells, the patch-clamp technique has become a true stalwart of the neuroscience toolbox.

Information in the brain is thought to be encoded as complex patterns of electrical impulses generated by thousands of neuronal cells. Each impulse, known as an action potential, is mediated by currents of charged ions flowing through a neuron's membrane. But how the ions pass through the insulated membrane of the neuron remained a puzzle for many years. In 1976, Erwin Neher and Bert Sakmann developed the patch-clamp technique, which showed definitively that currents result from the opening of many channel proteins in the membrane[1]. Although the technique was originally designed to record tiny currents, it has since become one of the most important tools in neuroscience for studying electrical signals — from those at the molecular scale to the level of networks of neurons.

By the 1970s, current flowing through the cell was generally accepted to result from the opening of many channels in the membrane, although the underlying mechanism was unknown. At that time, current was commonly recorded by impaling tissue with a sharp electrode — a pipette with a very fine point. Unfortunately, however, the signal recorded in this way was excessively noisy, and so only the large, 'macroscopic' current — the collective current mediated by many different types of channel — that flows through the tissue could be resolved.

In 1972, Bernard Katz and Ricardo Miledi[2], pioneers of the biology of the synaptic connections between cells, managed to infer from the macroscopic current certain properties of the membrane channels, but only after a heroic effort to exclude all possible confounding factors. The problem was that the macroscopic current could be influenced by factors not directly related to channel activity, such as cell geometry and modulatory processes that regulate cell excitability. Also troublesome was that interpretations of macroscopic-current features were based on unverified assumptions about the statistics of individual channel activity[2,3]. Despite Katz and Miledi's careful analyses, there was a lingering doubt about whether their conclusions were correct. The crucial data were obtained by Neher and Sakmann using patch clamp.

The patch-clamp technique is conceptually rather simple. Instead of impaling the cells, a pipette with a relatively large diameter is pressed against the cell membrane. Under the right conditions, the pipette tip 'bonds' with the membrane, forming a tight seal. This substantially reduces the noise compared with that encountered using sharp electrodes, because the small patch of membrane encompassed by the pipette tip is electrically isolated from the rest of the cell's membrane and from the environment surrounding the cell (Fig. 1).

The tiny currents passing through the few channels in the patch were thus observed for the first time. The recording confirmed key channel properties: when channels open, there is a step-like jump in the current trace and, when they close, a step-like drop back to baseline. It was now possible to determine details such as the statistics of the opening and closing of channels, the amplitude of the currents they mediate and the optimal stimuli that trigger their opening. For this work, Neher and Sakmann were awarded the 1991 Nobel Prize in Physiology or Medicine.

Improvements in patch clamp made it feasible to study channels in various preparations[4] to finally address long-standing questions. There was particular interest in verifying a model for action-potential generation[5] proposed by Nobel laureates Alan Hodgkin and Andrew Huxley in the 1950s. Specific predictions of the model could now be tested directly by examining the current through individual channels and by observing the changes in current that occur when the molecular structure of the channel is modified[6]. Ultimately, the model was shown to be mostly correct and remains the gold standard for computational neuroscientists today.

One of the several variants of patch clamp[4] — the whole-cell configuration — found an audience with neuroscientists studying electrical phenomena in neurons beyond the channel level. To achieve whole-cell recording, the

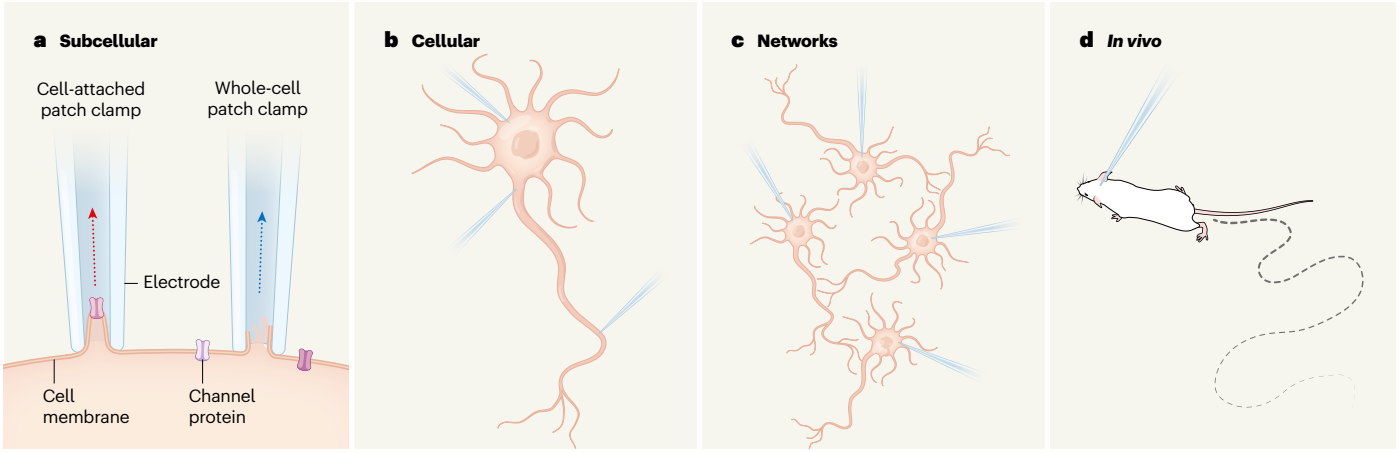

Figure 1 | The patch-clamp technique used at different scales. **a**, Neher and Sakmann[1] developed the cell-attached patch-clamp technique. An electrode (a fine pipette) is pressed against a 'patch' of the cell membrane so that ion currents (red dotted arrow) passing through channel proteins in the patch under the electrode can be recorded. In the whole-cell configuration, the patch is ruptured so that the whole-cell macroscopic current (blue dotted arrow), which represents the summed currents from the entire cell, can be recorded. **b**, Simultaneous whole-cell recordings from different parts of a neuron can determine, for example, the direction of travelling signals. **c**, Whole-cell recordings can be made from a small network of connected neurons. **d**, Whole-cell recording can even be made in the brains of animals performing a task or walking around freely.

patch of membrane under the electrode is ruptured, enabling electrical access to the cell. Compared with the use of sharp electrodes, whole-cell patch clamp allows much more accurate recordings and, crucially, is less damaging to the cell. This allowed systematic investigation of synergistic processes at the cellular level, such as the regulation of macroscopic currents by modulatory molecules, and interactions between the different types of channel in the neuron.

The relatively large opening created in the cell in the whole-cell configuration also provided access to the cell by chemicals, enabling dyes to be delivered for visualizing intricate cell structures, and RNA to be extracted for gene-expression analysis[7]. Neher's group examined the sequence of events that underlie the transfer of information between cells by introducing chemicals into the cell and simultaneously tracking the resulting changes in the electrical properties of the cell's membrane[8].

Whole-cell patch clamp proved ideal for studying the collective properties of neurons and neuronal networks in brain slices maintained *in vitro*. A challenge in working with more-complex systems such as neuronal networks is that the number of possible confounding factors increases. Sakmann's solution in the 1990s was to carry out simultaneous whole-cell recording using two or three electrodes, which to some seemed excessive because comparable data could be obtained by sequential recordings using fewer electrodes. However, the rationale was that taking time to design the near-perfect experiment mitigated later difficulties in data interpretation analogous to those faced by Katz and Miledi.

Hence, simultaneous recordings from different parts of the neuron definitively confirmed that action potentials are initiated at one part of the main long neuronal protrusion (the axon) and propagate back to the dendrites (clustered protrusions that receive inputs from other neurons)[9]. The mechanisms that underlie signalling between neurons were directly investigated by placing electrodes on either side of a synaptic connection[10]. Moreover, triple recordings from neurons of different classes uncovered certain basic principles of network organization[11].

The patch-clamp technique is also used to examine cell activities under more natural conditions. To study how sensory stimuli and movements are represented in the brain, experiments must be carried out in living animals. The challenge with this approach, however, is that the slightest movement can dislodge an electrode from the neuron. Whole-cell patch-clamping turns out to be remarkably stable because of the tight seal between the electrode and the membrane. Thus, this technique has permitted recording from dendrites[12] and pairs of neurons[13] in anaesthetized rodents, and even from animals that are able to walk and run[14].

"Patch-clamp recording is arguably still the most effective way of studying electrical signals in the brain."

Patch-clamp recording is arguably still the most direct and effective way of studying electrical signals in the brain. The data obtained with this technique essentially represent the ground truth for investigators in many branches of neuroscience, from theorists[15] to translational researchers developing drugs for the treatment of certain brain conditions, including epilepsy[16] and autism spectrum disorder[17].

Moreover, patch clamp complements modern 'optogenetic' techniques, which enable control and visualization of the activities of large populations of neurons using light[18]. Emerging technologies, such as prostheses for vision[19], will probably rely heavily on patch-clamp recording to establish the optimal conditions for converting external stimuli into electrical signals. Patch-clamping will clearly remain a vital tool for the neuroscientist in the foreseeable future.

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