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.

In 1972, Bernard Katz and Ricardo Miledi, pioneers of the biology of the synaptic connections between cells, managed to infer from the opening of many channel proteins in the membrane that interpretations of macroscopic currents were directly related to channel activity, such as cell geometry and modulatory processes that regulate cell excitability. Also troublesome was the problem that the macroscopic current could be influenced by factors other than channel activity, such as changes in the membrane potential, which is mediated by currents of charged ions flowing through a neuron’s membrane. To achieve whole-cell recording, the first time. The recording confirmed key channel properties: when channels open, 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 other than channel activity, such as cell geometry and modulatory processes that regulate cell excitability. Also troublesome was the problem that interpretations of macroscopic-current features were based on unverified assumptions about the statistics of channel opening activity. Despite Katz and Miledi’s careful analyses, their work had been hampered by the large, ‘macroscopic’ current — the collective current mediated by many different types of channel — that flows through the tissue could be recorded.

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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 do ions pass through the insulated membrane of the neuron remain 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. 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 recorded.

Neuroscientists today.

Improvements in patch clamp made it feasible to study channels in various preparations to finally address long-standing questions. There was particular interest in verifying a model for action-potential generation 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 membrane structure of the channel is modified. 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 — the whole-cell configuration — found an audience with neuroscientists studying electrical phenomena in neurons beyond the channel level. To achieve whole-cell recording, the
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 provides 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. 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.

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 recordings 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). The mechanisms that underlie signalling between neurons were directly investigated by placing electrodes on either side of a synaptic connection. Moreover, triple recordings from neurons of different classes uncovered certain basic principles of network organization.

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 and pairs of neurons in anaesthetized rodents, and even from animals that are able to walk and run.

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 to translational researchers developing drugs for the treatment of certain brain conditions, including epilepsy and autism spectrum disorder.

Moreover, patch clamp complements modern ‘optogenetic’ techniques, which enable control and visualization of the activities of large populations of neurons using light. Emerging technologies, such as prostheses for vision, 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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