From Twitching Frogs to Brain Implants: 5 Key Advances in Electrophysiology

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Understanding how the brain works is one of the most ambitious scientific challenges of our times. Early morphological studies of the brain revealed the presence of highly interconnected and complicated networks of neurons.

In recent decades, functional investigation of neuronal physiology became the gold standard for decoding brain function. Electrophysiology therefore garnered a lot of public attention because of its seminal discoveries into healthy brain function. This list will look at the main differences between electrophysiological approaches, the most recent innovations in the field and opportunities that recent technological advances have given us.

1. From Galvani to Frankenstein: the discovery of bioelectricity

In the 18th century, research into electricity had captured the imaginations of many of Europe's top scientists. The laboratory of Luigi Galvani, an Italian surgeon at the University of Bologna, demonstrated how a dead frog's legs would twitch when exposed to an electric shock, therefore laying the base for what became one of the most exciting concepts in modern physiology. In subsequent years the discovery, which Galvani called “animal electricity”, was noted in other animal studies as well as grisly human experiments that involved jolting corpses and making decapitated criminals sit upright. Eliciting movements in deceased bodies inspired Shelley's gothic novel *Frankenstein* as well as a series of follow-up studies designed to discover the origin of biopotentials. Nevertheless, the world had to wait until the late 19th century and the experiments of Matteucci and du Bois-Reymond for the first recording of electrical potential generated by biological systems.

But how are biological potentials generated? Neuronal membranes are populated by a variety of pumps to segregate ionic species across the membrane, and channels that allow the same species to cross the membrane. For example, sodium and calcium ions accumulate outside neurons and their concentration gradient across the neuronal membrane acts to pull them inside the neurons when their respective ion channels open. Synaptic activation triggers the opening of transmembrane channels, allowing the transit of electrically charged ionic species and causing a rapid and transient change in the distribution of positive and negative charges across the neuronal membrane (i.e. membrane potential). The rapid increase of positively charged ionic species inside the neuron results in a depolarization of the membrane and is called an action
potential. The continuous transit of charges from the extracellular space into the cytoplasm (and vice versa) generates fluctuating electric potentials in the brain: the biological basis of our brain waves.

2. Single-unit recordings

The adult human brain is believed to contain 85-100 billion neurons, each having around 10,000 synaptic connections to other neurons. In the 1950s, Alan Hodgkin and Andrew Huxley designed an experimental set-up called voltage-clamp, which allowed them to record macroscopic currents in the squid axon, therefore demonstrating the physiological importance of ion fluxes through ion channels. Years later, thanks to the innovative work of Neher and Sackman, who developed the patch-clamp technique in 1976, scientists were able to measure (and therefore model) the behavior of single neurons in the brain. Today, this approach (and its further evolution, the whole-cell patch techniques) are widespread in electrophysiological laboratories around the world and were central to the discovery of neuroactive substances and other foundational papers in functional neuroscience.

For example, by studying single-unit neuronal recordings, scientists worked out that brain cells encoded information by modulating the timing and the frequency at which they fire action potentials. Furthermore, it was possible to cluster neurons in different subpopulations according to their firing patterns and morphology (i.e. excitatory pyramidal neurons and inhibitory interneurons), and to study the relative impact of these subpopulations in health and disease. Nevertheless, these individual recordings were, in some cases, not enough. In light of the vast number of neurons and synapses, and the unknown relative weight of single synapses in driving cognitive and/or motor processes, scientists developed approaches that could give them information on the activity of a greater number of neurons: multi-unit recordings.

3. Multi-unit recordings

Systems neuroscience, which looks at high-level neural circuits and systems, relies on the acquisition and analysis of signals that integrate the activity of large neuronal populations. Extracellular recording is a
broad term that describes the technique of recording neuronal activity with an electrode placed outside the neuron (as opposed to the whole cell patch-clamp technique) therefore integrating the activity of an unknown number of neurons, called the field potential. Extracellular electrodes record changes in the frequencies of field potential, recording everything between direct current (DC) up to over 1 kHz. This bandwidth is then subdivided into local field potentials (LFPs, for the frequency components between DC and 300 Hz) and multi-unit activity (MUA, for frequencies between roughly 300 Hz and 1 kHz).

While LFPs are thought to report on the integrated activity of excitatory pyramidal neurons over a radius of hundreds of micrometers from the recording site (depending on the characteristics of the electrode), the MUA allows electrophysiologists to discriminate individual action potentials from an unknown number of neurons in the close proximity (i.e. a few dozens of micrometers) of the electrode tip. The MUAs are commonly used to relate changes in neuronal activity in different areas of the brain to behavioral tasks (e.g. this technique allowed the identification of “place cells”, which are the subpopulation of hippocampal neurons responsible for navigation in known environments).

4. EEGs and scalp recordings

The main shortcomings of intra- and extracellular recordings is that they require an invasive surgical procedure to gain direct access to the brain tissue. While this can be implemented in preclinical research, it represents a major setback for human studies. For this reason, electroencephalograms (EEGs) are routinely used in clinical studies. EEGs are recorded by electrodes placed on the scalp and reflect the synaptic activation of cortical neurons orthogonal to the surface of the brain (complementary to EEG is magnetoencephalography or MEG, which is sensitive to the small changes in magnetic fields generated by currents flowing in the brain). The main limitation of this approach is poor spatial resolution (i.e. it is only possible to record the integrated activity of a large volume of brain tissue, containing millions of neurons). Furthermore, EEG can only record cortical, not deep-brain activity.

On the other hand, EEGs are generally acquired using a standardized procedure and setting, which has facilitated the creation of large datasets across multiple centers. These large datasets, in combination with recent advancements in signal processing and data analysis, have allowed scientists to advance our understanding of the functional meaning of EEG recordings. As in the case of LFPs, neuronal activity is clustered into “frequency bands” to separate out the neural activity recorded during acquisition.

Lower frequency bands (i.e. delta/theta) report on the activity of neurons engaged in long range communication, whereas high frequency bands (i.e. gammas) supposedly report on the activity of small local networks. Shifts in neuronal activity between frequency bands within a brain area, or synchronization/desynchronization of bands across different areas of the brain, are used to detect engagement in a tasks
and/or changes in brain states (e.g. alertness, wakefulness, sleep). In light of the non-invasive nature of this approach, scalp-EEG recordings have been used in patient populations to study and diagnose epileptic seizures and neurodegenerative disorders (e.g. Alzheimer's Disease).

5. High-density probes and intracranial implants for humans

Electrophysiology allows us to map neuronal activity with unparalleled spatiotemporal resolution and promises to bridge the gap between brains and machines. For this reason, over the last few decades, a number of companies have invested copious resources to develop bio-compatible high-density probes. These devices are designed to be safely implanted into patients' brain for years on end, recording the activity of millions of neurons simultaneously. Once brain activity is registered it can be used to train software algorithms to control artificial devices (from moving the mouse pointer on a computer screen to controlling prosthetic limbs). BrainGate's implant successfully helped patients affected by central and/or peripheral nervous system damage to control prosthetics. More recently, Elon Musk's Neuralink implants have claimed unprecedented channel count (>3000), low impact on the human brain and superior flexibility, although there is currently limited evidence in support of the biocompatibility and the stimulation capability of the system.