Perspectives

The discovery of the action potential

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Beginning in the early eighteenth century, biologists and physicists alike strove to find a link between electricity and nervous function. Luigi Galvani took the first step by demonstrating the presence of electricity in animal tissues. Over the next half-century, others went on to show that nerve and muscle tissues generate electrical transients that accompany excitation, but the lack of sensitive instruments hampered these studies. Julius Bernstein, with the help of Emil du Bois-Reymond, found a way to overcome these technical limitations and in about 1865 made the first recordings of the time course of the action potential.

This paper reviews briefly how the first recordings of the action potential were made, focusing on work done in the mid-1860s by the pioneering physiologist, Julius Bernstein[1]. Despite severe technical limitations, Bernstein determined the time course of the action potential, measured its conduction velocity, and demonstrated its overshoot. In order to put these experiments into perspective, I will first outline what was known about animal electricity when Bernstein started his work.

The birth of electrophysiology took place during the eighteenth century as an offshoot of studies on electricity[3,5,13]. At that time, a spark was the only fully accepted assay for electricity. Not surprisingly, many empirical observations showed that electrical sparks elicit muscular contractions. Indeed, many therapists administered shocks to the infirm to make lame limbs move again[3,11].

These observations led to speculation that electricity might be important in the operation of the nervous system[3]. Most thinkers of the day, however, still believed that the nervous system operated via the movement of nervous fluids or ethers. In addition, some noted that the most striking similarity between electricity and the nervous principle was that very little was known about either. As a result, the connection between electricity and nervous function was not explored until the end of the century.

Luigi Galvani, a Bolognese physiologist, took up the problem around 1780 (Ref. 8). He developed the 'prepared frog', which consisted of the skinned lower limbs, the attached nerves, and occasionally also a portion of the spinal column. Doing countless experiments, Galvani tested every conceivable way of stimulating the preparation electrically. He found that both direct stimuli and sparks jumping over various distances were effective; discharges produced by both electrostatic machines and Leyden jars were effective; and stimuli delivered using all sorts of electrical conductors were effective. Having exhausted this line of experimentation based on 'artificial' (man-made) electricity, Galvani undertook to study the effects of 'natural' electricity.

Natural electricity in the atmosphere had been discovered not long before by Benjamin Franklin[7]. In 1750, Franklin wrote to the Royal Society proposing that buildings might be protected from electrical storms by putting sharp rods, connected to the earth, on the buildings' peaks. The rods would then conduct the atmospheric electricity away from the buildings before it did damage. The Royal Society refused to publish the letter, citing the lack of evidence showing that lightning was, in fact, electrical. This led to Franklin's famous kite-flying experiment. By flying a kite during a storm, Franklin showed that he could tap electricity from the clouds that was indistinguishable from artificial electricity.

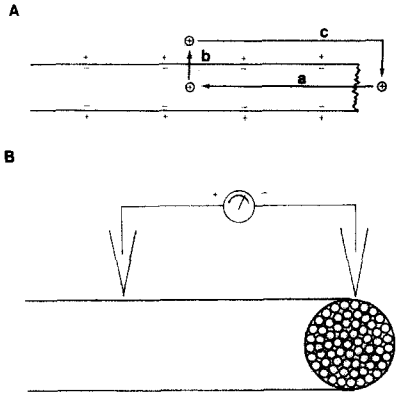

Fig. 1. *Origin of the injury current.* **(A)** *When an axon or muscle fiber is cut, the damaged end forms a leaky, non-specific seal. Because of the inside negative membrane potential, there is a net movement of positive charge through the damaged region into the cell (a). This partially depolarizes the cell, generating an outward current at distal points (b). The return path is along the outside of the fiber (c).* **(B)** *When a peripheral nerve or whole muscle is cut, the injury currents of the individual fibers sum. The net muscle or nerve current is large enough to be detected with a galvanometer.*

Franklin's discovery generated enormous excitement throughout the western world. The Royal Society not only published the work, but even awarded Franklin its Copley Medal. Galvani could hardly have escaped notice of Franklin's discovery, since an Italian translation was published in 1774 (Ref. 5). Given the combination of Galvani's desire to explore every means of stimulating muscle and the fame surrounding Franklin's discovery, Galvani's next experiment was inevitable. In the face of an approaching storm, Galvani hung a prepared frog from his railing outside, and connected it to a long wire set up to capture atmospheric electricity[8]. As one would expect, the experiment was a success: atmospheric electricity was as effective as artificial electricity in stimulating the muscle. Upon repeating the experiment, however, Galvani was astounded to find that he did not need atmospheric electricity. Suspended from an iron railing on brass hooks, the muscle twitched whenever the assembly was jostled. Furthermore, the same procedure worked indoors. Knowing of no other source of electricity that might have stimulated the muscle, Galvani concluded that the stimulus must have come from the tissue itself. He considered this a manifestation of animal electricity.

Alessandro Volta, a physicist working in Pavia, learned of Galvani's work in 1791 and was astonished by the findings[3,13,14]. Already a pre-eminent investigator of electrical phenomena, Volta began his experiments on animal electricity in 1792. Within weeks he confirmed Galvani's main results, and his initial scepticism became fanaticism.

A key finding, also reported by Galvani, was that a nerve–muscle preparation stimulated itself effectively only when contacted by two dissimilar metals. Although this observation did not concern Galvani, it troubled Volta. Like others before him, Volta had noted that placing two dissimilar metals on his tongue generated the same sourish taste as that elicited by a weak electrical stimulus delivered to the same spot. After exploring this further, Volta declared that Galvani's 'animal electricity' was in fact electricity generated by the wetting of dissimilar metals and not a force generated by the tissue itself[14].

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The debate between Galvani and Volta soon became acrimonious, with the physiologists of the day quickly taking sides[3]. By 1794, Galvani countered Volta's objections with an experiment, published anonymously, showing that a muscle can be stimulated by bringing its nerve into contact with a skinned part of the muscle[3,11]. In this case, the damaged muscle generated a sizeable injury current that acted as the stimulus (Fig. 1). Although Galvani did not understand injury currents, he correctly stated that this was a demonstration of animal electricity free of metallic electricity artefacts.

Volta was not moved: he argued that contact of dissimilar conductors (in this case, muscle and nerve) inevitably generated an electrical current. Though Galvani withdrew from the battle, 3 years later Alexander von Humboldt repeated both Galvani's and Volta's experiments, and confirmed Galvani's discovery of animal electricity[3,11,13]. Nevertheless, Volta still refused to believe that biological tissues are electrically active.

There was one exception: virtually everyone, including Volta, agreed that certain organs in the eel and *Torpedo* were electrical[3,11]. Volta, however, reversed the argument and claimed that the electric organ of *Torpedo* was the exception that proved the rule. Volta believed that the electric organ was electrical only because it contained alternate layers of dissimilar materials.

To illustrate his point, he constructed an apparatus consisting of multiple discs of zinc, copper, and moist pasteboard that were piled one on top of the other in an alternating series. This 'artificial electric organ', as Volta referred to it, was capable of generating sizeable electric currents. Volta presented his apparatus as a realistic model of the natural electric organ, and dismissed from further consideration the question of animal electricity in other tissues and organs[11].

Volta's discovery revolutionized physics by providing a useful, steady source of electricity, but it also effectively stopped further studies of animal electricity. It was not until about 40 years later that Carlo Matteucci, a physicist at Pisa, convincingly demonstrated the existence of animal electricity[3,13]. Using a 'galvanometer' devised about 20 years earlier by C. L. Nobili, Matteucci showed that there is an electrical current between the cut and intact surfaces of a damaged muscle (Fig. 1). Today this current is called an injury current; at that time, however, the role of injury was not understood and it was called simply 'muscle current'.

Matteucci also noted that the muscle current decreased during strychnine-induced tetany. This happens because the muscle current is fuelled by the resting membrane potential, and the volley of action potentials that accompanies tetany depolarizes the cells.

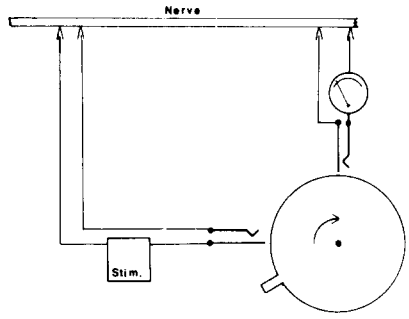

Fig. 2. *Schematic diagram of the differential rheotome. See text for details.*

Matteucci's results drew the attention of Johannes Mueller, a Berliner and the most prominent physiologist of the day[3]. In 1841, Mueller asked his Swiss student, Emil du Bois-Reymond, to repeat Matteucci's experiments. A brilliant experimentalist, du Bois-Reymond soon repeated Matteucci's findings and, with the aid of his superior instruments, went on to extend the results to nerves[6]. When he demonstrated that nerve current, like muscle current, decreases during stimulation, du Bois-Reymond declared: 'If I do not greatly deceive myself, I have succeeded in realizing . . . the hundred years' dream of physicists and physiologists, to wit, the identity of the nervous principle with electricity'[3].

Today we know that during the peak of the action potential, the nerve current does not fall to zero; rather, it overshoots zero and transiently reverses polarity. Because his galvanometer was too slow to follow the very brief reversal, Du Bois-Reymond could detect only a transient decrease in the injury current. He named this decrease the 'negative variation'. du Bois-Reymond was well aware of the limitations of his instruments, however, and he suggested that the negative variation might actually be stronger than the resting nerve current[1,6].

This was an important point, since not everyone agreed with du Bois-Reymond's claim that the negative variation was the signal that travelled along nerves and stimulated muscles. Du Bois-Reymond believed he could strengthen his case by showing that the negative variation was in fact a reversal of nerve current, since this would indicate that the negative variation was an active process. As further evidence, he wanted to show that the conduction velocity of the negative variation equalled the conduction velocity of the excitatory signal. Though not a critical test, an identity of conduction velocities was an obvious prediction of his theory.

The conduction velocity of the excitatory signal was already known. This measurement had been made by Hermann von Helmholtz[3], another student of Johannes Mueller. Von Helmholtz constructed a timing device that was turned on by the same switch that stimulated the nerve of a nerve–muscle preparation. One tendon of the muscle was attached to a second switch that stopped the clock. Thus, when the nerve was stimulated, the clock timed the interval between stimulus and twitch. By doing several trials with the stimulating electrodes placed at different points along the nerve, von Helmholtz determined the conduction velocity of the excitatory signal.

Du Bois-Reymond then set out to measure the conduction velocity of the negative variation. He built a machine that was supposed to determine the time course of the negative variation with sufficient resolution to detect any reversal of the injury current[1]. Such a machine could also yield the conduction velocity. Unfortunately his machine failed, so he passed the problem to his student, Julius Bernstein.

Bernstein built an improved version of the machine, called a differential rheotome[1,9]. Like its predecessor, it was designed to measure the time course of the negative variation. The principal design obstacle was due to the only measuring instrument available, the galvanometer: it was too slow to follow the time course of an event as rapid as an action potential. It was also relatively insensitive.

The solution lay in a clever approach: Bernstein circumvented the slow response time of the galvanometer with a mechanical timing and sampling circuit. Specifically, the machine connected the galvanometer to the recording electrodes for only a fraction of a millisecond, and then only after a certain delay following the stimulus. The delay was variable and could be set with sub-millisecond precision. Since the sampling interval was short compared to the action potential, he could examine any portion of the negative variation (i.e. the action potential) simply by readjusting the delay time. This is explained in more detail below.

Another difficulty was that the galvanometer was too insensitive to respond to a single, very brief sample of injury current. Bernstein solved this problem by stimulating the nerve rapidly. The galvanometer was connected to the nerve for the same interval after each stimulus (with a constant delay) so that the meter received a volley of identical samples. In this way, the samples were additive. The galvanometer reading

was proportional to the number of samples per second multiplied by the amplitude of the individual samples. By keeping a constant sampling rate, Bernstein could compare readings with different delay times. That is, he could compare the relative strengths of different portions of the action potential and thus reconstruct its time course. The resolution was limited by the length of the sampling interval, typically 0.5 ms.

A simplified schematic diagram of Bernstein's differential rheotome is shown in Fig. 2. Stimulating and recording electrodes were placed at opposite ends of a nerve. The stimulator was connected to a switch that was tripped once per revolution of a spinning wheel. The galvanometer and recording electrodes were connected to another switch that was also closed briefly during each revolution of the same wheel. When the machine was in operation, the stimulus rate was set by adjusting the speed of the wheel. The delay time between the stimulus and the sampling interval was set by adjusting the angle between the two switches. Although not illustrated in the diagram, the duration of the sampling interval was also adjustable. These features can be seen in Bernstein's drawings of his rheotome (Figs 3, 4).

One problem with the design of Fig. 2 is that the galvanometer, because of its low input resistance, draws so much current from the nerve that closing the recording circuit also stimulates the nerve. Bernstein overcame this problem with a simple nulling mechanism: he wired the galvanometer in series with a variable voltage source of opposing polarity. The variable voltage source was simply a battery (Voltaic pile) connected to a copper wire 1.5 meters long. By moving a slide along the wire, he could tap off any fractional part of the battery's voltage. In practice, the slide was set so that the battery exactly countered the injury current. As a result the galvanometer drew no current from the nerve whenever the preparation was at rest.

Both the galvanometer and the stimulator were conventional for the time. The galvanometer consisted of a bar magnet suspended from the ceiling by a thread and surrounded by a coil of wire mounted on the floor. The stimulator was simply an induction coil connected to a battery.

The machine was used as follows. First, two pairs of electrodes were connected to the nerve, one for stimulation and one for recording. These were connected to the rheotome, which was set spinning at a fixed, carefully measured rate. In addition, the compensating current was set to cancel exactly the resting nerve current. Then the rheotome was adjusted for a zero delay time between stimulation and recording, and the stimulator was activated. The delay time was gradually increased until the galvanometer responded.

Bernstein's first finding was that 'a measurable time elapses between stimulation at one point of the nerve and the begin-

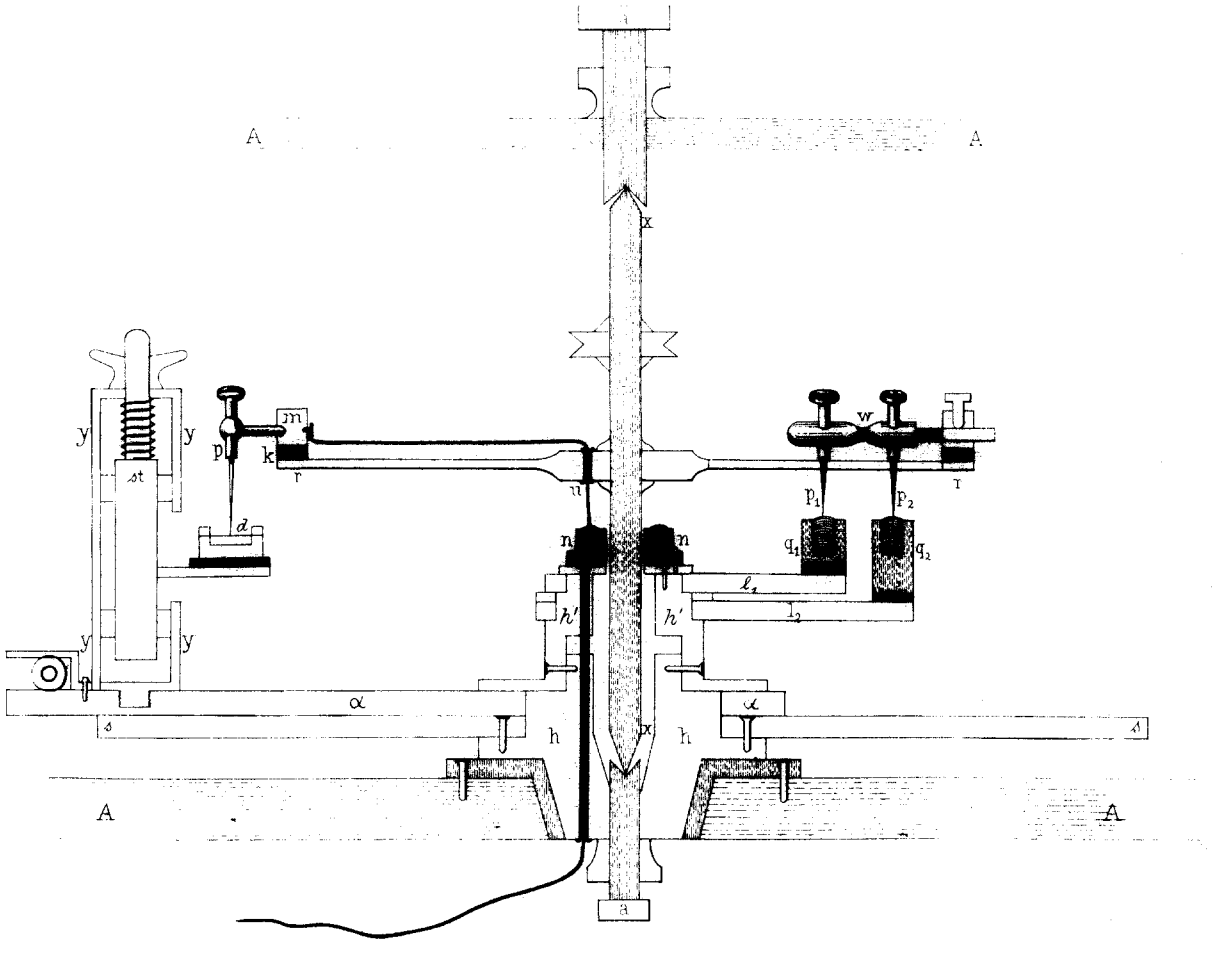

Fig. 3. *Cross-section of the differential rheotome. The shaft (X–X) is rotated via a pulley, causing pins p, p_1 and p_2 to turn with it. The other elements in the drawing are stationary. Pin p and wire d are connected to the stimulator; an annular pool of mercury (n–n) acts as a commutator. The recording circuit is connected to two rectangular pools of mercury (q_1, q_2). The pools are shorted by pins p_1 and p_2 as they pass through the menisci of the pools.*

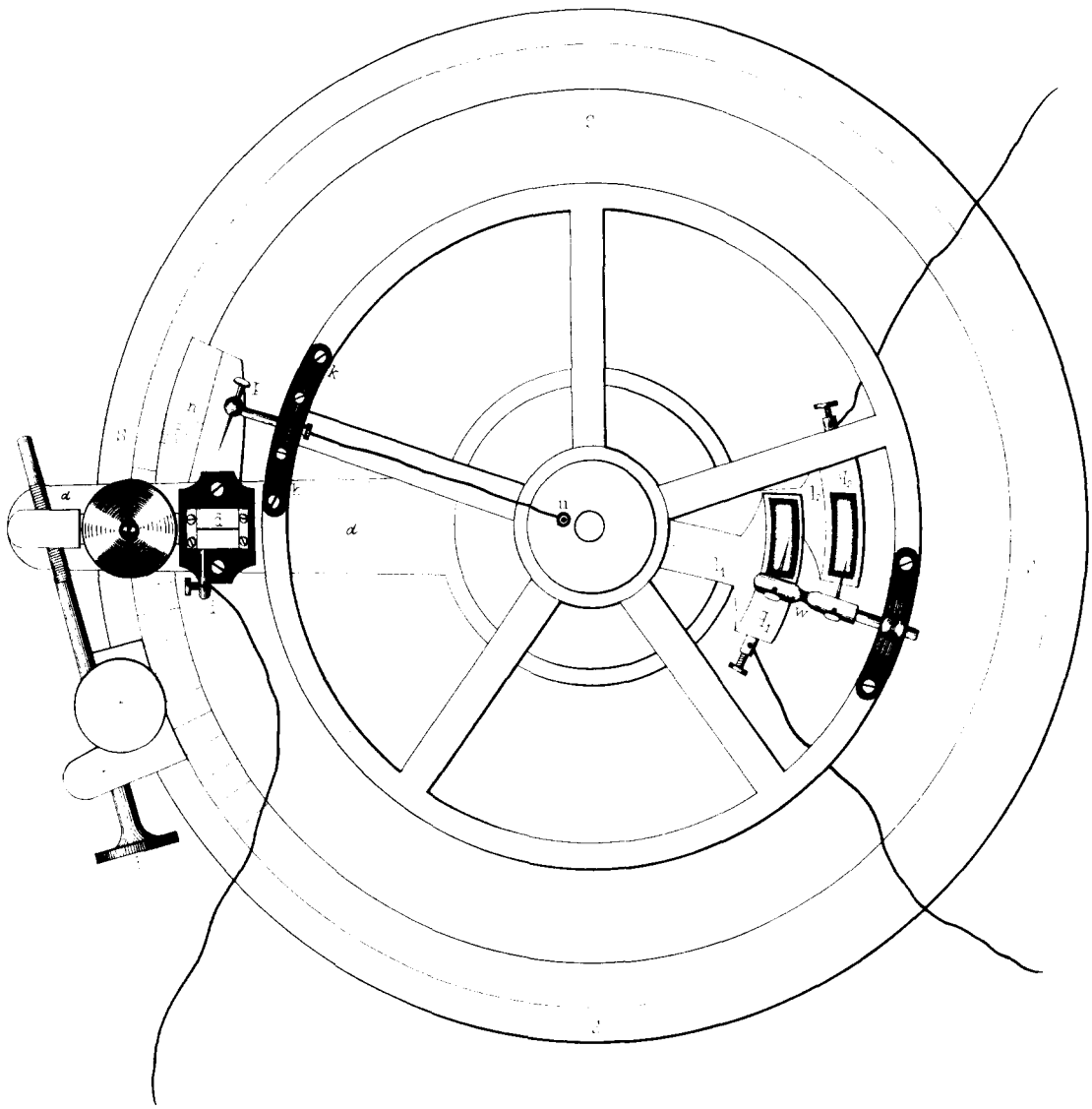

Fig. 4. *Top view of the rheotome. The rotating part consists of the wheel holding the three pins; most other parts are adjustable but do not normally rotate. The duration of the sampling interval is set by adjusting the degree of overlap of pools q_1 and q_2. The delay time between stimulus and recording is set coarsely by moving the arms l_1 and l_2 relative to arm d. Finer adjustment is made by turning the thumbscrew b.*

ning of the negative variation at a more distant point'. In other words, when the delay between stimulation and sampling was zero, the galvanometer did not respond. This was because the meter sampled before the arrival of the negative variation and it saw only the resting current, for which it was fully compensated. When an appropriate delay was set, however, the action potential reached the recording electrodes at the same time that the galvanometer sampled the nerve current. In this case, the nerve current was weaker than the compensating current; hence, the galvanometer moved.

By changing the delay, Bernstein was able to sample different parts of the negative variation and to recreate its entire time course. In order to get as accurate a record as possible, he wanted to make the sampling interval very short. If the intervals were too short, however, there was not enough charge movement to power the galvanometer. A sample time of 0.3 ms proved to be the practical limit, and this was true only with the expedient of recording from two nerves placed side by side in order to increase the signal. The results are shown in Fig. 5. The upper trace is a plot of magnitude versus time. This is, to my knowledge, the first plot of an action potential ever published, yet it is a remarkably faithful representation. The lower traces are plots of the magnitude of the negative variation versus distance along the nerve at a single point in time. This drawing was constructed by Bernstein to point out that the action potential is a wave that travels along the nerve in both directions away from the stimulus.

This brings us back to the original objectives of these experiments: to measure the conduction velocity and to see if the nerve current changes direction during excitation. To measure the conduction velocity of the negative variation, Bernstein delivered stimuli to either of two points at different distances from the recording electrodes. In each case, he measured the minimum delay time for a just-detectable deflection of the galvanometer. Knowing these numbers and the difference in distance, it was a simple matter to calculate the conduction velocity: '28.718 meters/second', a value in good agreement with von Helmholtz's value for the velocity of the excitatory influence.

To determine if the nerve current

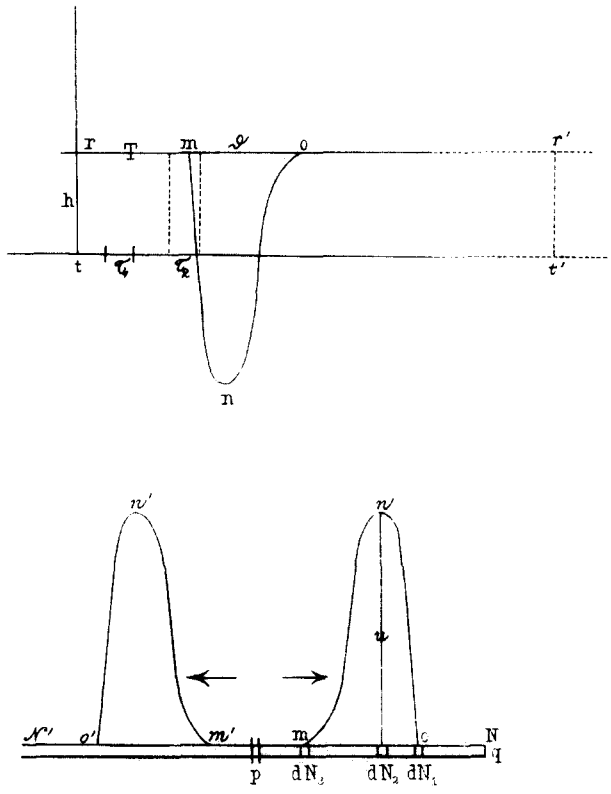

Fig. 5. *The first published drawings of an action potential. See text for details.*

changed its direction at the peak of the negative variation, Bernstein had to take into account the effect of stimulus strength. Since he was recording from a bundle of axons, and not a single fiber, the size of the response was roughly proportional to the number of fibers excited, which in turn varied with the stimulus strength. Bernstein noted that varying the stimulus strength altered only the size of the negative variation, and not its duration.

For the critical experiment, the rheotome was adjusted so that it sampled the peak of the negative fluctuation. The stimulus was made as large as possible, and the compensating current was removed. In this way, Bernstein only had to note in which direction the galvanometer moved – with or against the resting current. In almost every case, the answer was the same: with strong stimuli, the strength of the negative variation exceeds that of the resting current. This result did not depend on stimulus polarity, showing that the reversal was not an artefact created by a leakage current from the stimulator. In a few cases, the current approached zero but did not change direction at the peak of the signal. The most likely explanation is that, in these cases, not all of the fibers had been stimulated and so there was a significant background of resting current that obscured the reversal.

In sum, Bernstein determined the conduction velocity of the action potential and showed that it has an overshoot. In so doing, he met the goals laid out for him by du Bois-Reymond. In a parallel study, Bernstein used the differential rheotome to study the action currents of muscle, with similar results.

About 35 years later, in 1902, Bernstein proposed what, at the time, was a comprehensive theory of resting and action potentials[2]. Drawing on the latest studies of Nernst and others, Bernstein postulated that nerve cells are selectively permeable to potassium ions and that the resting potential is generated by a potassium gradient. This, of course, is widely accepted today. Furthermore, he proposed that action potentials result from a transient loss of selective permeability, so that the membrane potential falls to zero. The possibility of a sodium influx was discounted, despite Overton's finding[12] that excitability in muscle depends on the presence of extracellular sodium. Bernstein's hypothesis, which curiously ignored or discounted one of his own major results – the overshoot of the action potential – misled an entire generation of neurophysiologists. It was not until about 1940 (Refs 4, 10) that this error was corrected and the overshoot of the action potential became firmly established.

Acknowledgements

I thank the staff of the Biology Library at Columbia University for help in locating manuscripts; Drs Harry Grundfest, John Hildebrand and Roberta Pollock for helpful discussions; and Dr William Pickard for pointing out the existence of Bernstein's early papers. Stephen M. Schuetze is an Alfred P. Sloan Foundation Fellow.

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