The Biophysicist

Using Plant Cells of *Nitellopsis obtusa* for Biophysical Education

Vilmantas Pupkis[1,*], **Rokas Buisas**[1], **Indre Lapeikaite**[1], **Vilma Kisnieriene**[1]

[1]Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Sauletekio Avenue 7, LT-10257 Vilnius, Lithuania

ABSTRACT Using giant characeaen algae *Nitellopsis obtusa* in laboratory exercises is proposed to familiarize students with basic concepts of electrophysiology and provide some simple hands-on practice. The described concept experiments present extracellular registration of action potentials (APs) and investigation of cytoplasmic streaming properties. Students are expected to register the propagation velocity of APs (found to be 3.4 ± 1.5 cm/s in *N. obtusa*), as well as the velocity of cytoplasmic streaming (66.7 ± 9 μm/s). Proposed exercises also concern recovery dynamics of cytoplasmic streaming after a stimulation (recovery time constant $\tau = 3.7 \pm 2.1$ min) as well as investigation of an effect of various chemicals (e.g., KCl) on all selected parameters. The experiments endorse characeaen algae as a model system to be routinely explored in education of biophysics and bioelectrical phenomena of the cell.

KEY WORDS cell signaling signal transduction and cellular networks; mechanobiology; cell mechanics, mechanosensing, and motility; laboratory exercises; learning goals; fundamental concepts and techniques

I. INTRODUCTION

Electrophysiology is a profound branch of biophysics, linking principles of physics—sometimes as basic as Ohm's law—to complex response patterns of excitable cells and their networks. Various imaging techniques have conditioned ever improving understanding of the structure of ion transporters, constantly breaching previously unassailable limits of resolution. Yet it should not be forgotten that structural information itself cannot substitute functional investigations of ion channels and pumps. It is electrophysiology that continues to obtain these answers, especially since tools of molecular biology have enabled diverse genetic manipulations of ion transporters.

Modern science has advanced far beyond what can be thought of as simple experiments. Even such routine electrophysiological techniques as patch clamping require skill and determination that can hardly be expected of students performing laboratory exercises. Therefore, there is a need for a middle ground where complicated electrophysiological techniques often can be implemented by relatively simple and cost-effective means.

Plant cell excitability (1) and transmission of electrical signals are directly linked with vital physiological functions such as regulation of photosynthetic activity, respiration, growth, phytohormone production, changes in gene expression, and, most evidently, rapid leaf closure in *Dionaea* and *Mimosa* (2). Because of their ability to generate distinct electrical signals, plants can be used as an alternative to

"*" corresponding author

Received: 20 December 2019
Accepted: 5 June 2020
Published: 24 November 2020

© 2021 Biophysical Society.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

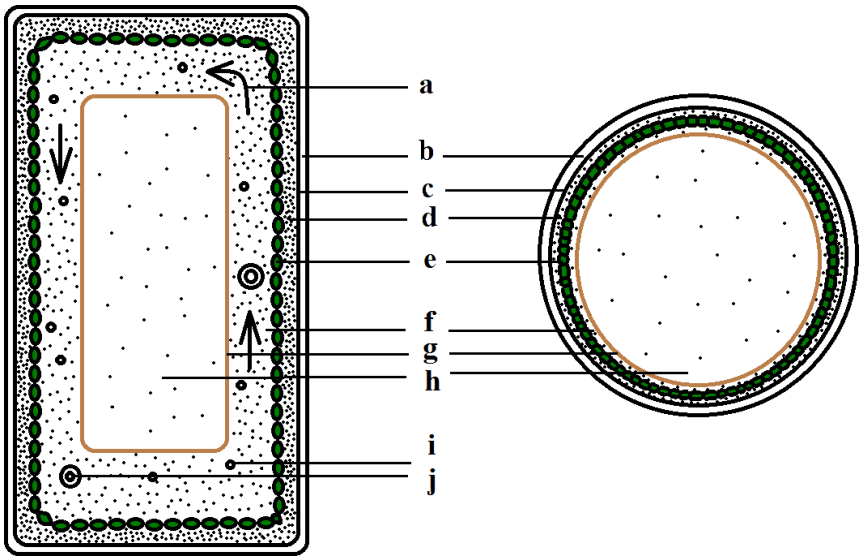

Fig 1. Schematic model of an internodal characeaen cell. (a) Direction of cytoplasmic streaming; (b) cell wall; (c) plasmalemma; (d) cytoplasm (exoplasm); (e) layer of chloroplasts; (f) cytoplasm (endoplasm); (g) vacuolar membrane (tonoplast); (h) vacuole; (i, j) nuclei, mitochondria, and various vesicles. The scheme to the right better represents proportions of cell compartments because the vacuole can fill up to 95% of cell volume.

animal cells for electrophysiological experiments. Giant algae of the Characeae family, whose internodal cells have often been called "green axons" (3), may provide the most simple, cost-effective, and robust model system for laboratory exercises (4, 5). Compared with animal-based model systems, characeaen algae inherently bypass bioethical obstacles and naturally cause less emotional discomfort. In this article we introduce several concept experiments on giant characeaen algae *Nitellopsis obtusa* (starry stonewort) that could be used to introduce students to electrophysiology, signal processing, and statistics education and provide some insights for further laboratory practices and discussions.

II. SCIENTIFIC AND PEDAGOGICAL BACKGROUND

A. Morphology of *Nitellopsis obtusa*

Nitellopsis obtusa (and other characeaen algae) is native to various shallow fresh or brackish waterbodies in temperate climate zones in Eurasia from Western Europe to Japan. In North America it is considered an invasive species (6). The thallus of *N. obtusa* consists of alternating nodes (made of multiple small cells) and huge internodal cells connected via plasmodesmata (7). Internodal cells of *N. obtusa* can exceed 30 cm in length and 1 mm in diameter. Separated from the thallus, internodal cells can survive for months and can be considered separate unicellular organisms. Storage of characeaen algae is exceptionally simple: once they are gathered from a waterbody, the cells can be stored for months in dechlorinated tap water under illumination with no supplement needed (other than occasionally changing the water in aquaria).

Characeaen cells have the structure of a typical plant cell (Fig 1): they possess a cell wall, an excitable plasmalemma, cytoplasm, a layer of chloroplasts, a vacuolar membrane (tonoplast, which is also excitable) (8, 9), multiple nuclei, and a vacuole that comprises up to 95% of the cell volume (7). During laboratory practice, students should become familiar with the morphology of characeaen cells and learn how their cellular structure has evolved to support specific functions. Characeaen algae may provide insights from a cell biology point of view: multiple nuclei and cytoplasmic streaming (see section II.C) enable more homogeneous nutrient distribution that would otherwise be impossible because of the cell size;

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

high turgor pressure compensates high external hydrostatic pressure caused by living underwater (7).

B. Excitability of characeaen cells

Electrical excitability of plant cells in its most mesmerizing form can be observed in nastic movements of Venus flytrap (*Dionaea muscipula*) or of *Mimosa pudica* (10). However, it was characeaen cells that have been used by leading scientists in search of the mechanism of plant action potentials (APs) since the early 20th century (11, 12). According to the widely accepted Thiel-Beilby model (9), an AP in a characeaen cell plasmalemma is generated when Ca^{2+} ions begin to flow into the cytoplasm from both the external medium and internal sources, thus causing membrane depolarization. Ca^{2+} ions in the cytoplasm activate Cl^- channels, allowing Cl^- efflux and further depolarization of the plasma membrane. Depolarization itself and Ca^{2+} ions then activate K^+ channels, thus allowing K^+ ion efflux and membrane repolarization to its resting potential, which is completed because of the activity of an H^+ pump, as well (13–15).

Conventional intracellular recordings enable thorough assessment of such electrophysiological parameters as membrane resistance, AP amplitude, peak and threshold values, dynamics of membrane potential, or ion fluxes during excitation. Various bioactive compounds or physical factors may influence these electrophysiological parameters (15). However, intracellular impalement might be too complicated for students not experienced in electrophysiology, especially if multiple impalements are required, as in the case of researching velocity of propagation of APs. Also, complex experiments require specific electrophysiological equipment (such as an electrode puller or a micromanipulator), which may not be available to every laboratory. High amplitude (up to 150 mV) and long duration (tens of seconds) of characeaen APs (16) make them suitable to be registered extracellularly (17). Although extracellular recordings can provide few main electrophysiological parameters to be examined (usually AP duration, amplitude, frequency, number, and conduction velocity), they can be much more easily and quickly mastered by students and do not require any sophisticated equipment compared with intracellular recordings. During laboratory practice, students should learn about plant excitability, assemble the system for extracellular recordings of APs in *N. obtusa* (or other characeaen), and register and analyze the signal. Students should be encouraged to try to compare registered signals to animal APs and try to explain the differences (e.g., longer duration of Characeaen AP) by different mechanisms and try to link that to their function (e.g., why is Characeaen AP longer?). After control measurements, students should apply some chemical of the supervisor's choosing to see its effects on APs in *Nitellopsis*, thus enabling analysis of the mechanism of AP generation.

C. Cytoplasmic streaming

Another fascinating phenomenon that can be studied in Characeaen cells is cytoplasmic streaming, which can be observed in various forms of life from protozoa to fungi, animals, and plants (18). In *Characeae*, even a low magnification (10×) is enough to distinguish vesicles and various other organelles being transported by the cytoplasm helically around the cell. Looking at a cross-section of a cell, half of the cytoplasm streams in an upward direction, and the other half streams downward. Both streams circle helically around the cell and connect near the nodes. The streams are separated by 2 indifferent zones that can be observed as a line of missing chloroplasts (7) (Fig 2). The layer of cytoplasm is very thin (∼10 μm), and in it, cytoplasm streams with an approximately constant velocity. Part of the movement is transferred to the vacuole, but streaming velocity there has a gradient from the highest in the vicinity of the tonoplast to the unmoving center of the vacuole (19).

Research on the mechanism of cytoplasmic streaming revealed that parallel to helical chloroplast rows, actin fibrils (20) form, along which myosin XI proteins (21, 22) slide, carrying their cargo. The generation of APs and dynamics of cytoplasmic streaming in Characeaen

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

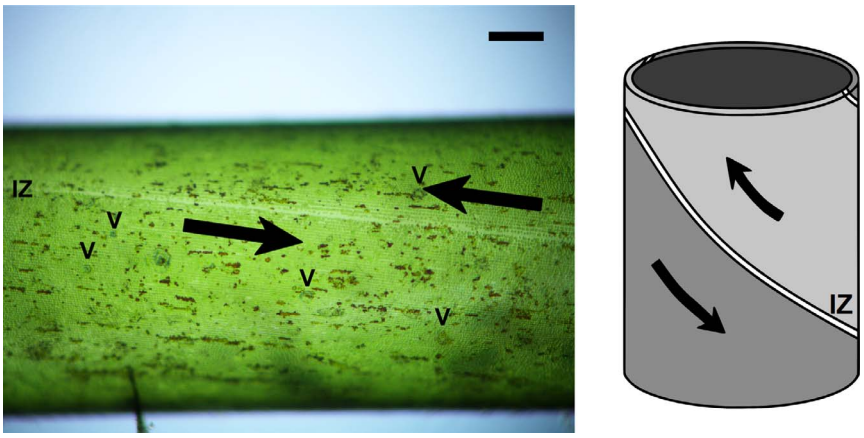

Fig 2. Cytoplasmic streaming in *Nitellopsis obtusa*. Left: a cell under 40× magnification (black bar represents 200 μm). Right: schematic model of a part of a cell. Indifferent zone (IZ) and moving vesicles (V, pointed at by arrowheads) can be seen. Arrows indicate direction of cytoplasmic streaming. Dark aggregates on the cell wall are caused by biomineralization (27).

algae have been found to be intimately connected through the dynamics of cytoplasmic Ca^{2+} content. The increase of Ca^{2+} ions in the cytoplasm during the depolarization phase of AP inhibits myosin movement, thus stopping cytoplasmic streaming [the mechanism is reviewed in Ref. (23)] It has been proposed that the velocity of cytoplasmic streaming could be used as an indicator of cytoplasmic Ca^{2+} concentration (24). Thus, it is possible to observe intracellular Ca^{2+} ion dynamics and cell excitation indirectly with a basic light microscope.

The function of cytoplasmic streaming in *Characeae* is thought to be associated with homeostasis and distribution of organelles and macromolecules because passive diffusion is not effective in such large cells. Cytoplasmic streaming also enhances nutrient transport between cells through plasmodesmata (4, 18).

Streaming sensitivity to increased intracellular Ca^{2+} levels can be thought of as a response to a possible membrane breach. The transmembrane concentration gradient of Ca^{2+} ions is higher compared with other ion species; thus, Ca^{2+} influx can serve as a signal of breached membrane integrity (25). When Ca^{2+} concentration increases, cytoplasmic streaming stops, preventing cell content leakage through putative membrane lesion (4).

Students should learn about the mechanism of cytoplasmic streaming and its function for the cell, as well as the whole organism. Video recordings of cytoplasmic streaming (preferably as a control and after application of some chemical of the supervisor's choosing) can be made by students themselves or be provided by the supervisor if time for laboratory exercises is limited. Students then should analyze videos and quantify parameters describing the dynamics of cytoplasmic streaming. Cytoplasmic streaming provides an excellent and visually attractive way to link cell excitation to increased cytoplasmic Ca^{2+} levels, a process that is also crucial to the excitation of animal cells.

III. MATERIALS AND METHODS

Nitellopsis obtusa (N.A. Desvaux) J. Groves algae were collected from Lithuanian lakes during autumn months and maintained at room temperature (21 ± 1 °C) in glass aquaria under daylight conditions (9.5 ± 0.19 μmol m^{-2} s^{-1}) with a light/dark photo regime of 12/12 h. Experiments were carried out on internodal cells that were separated from thalli and kept overnight in buffered artificial pond water (APW) under daylight conditions. The control solution APW contained 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM $CaCl_2$, 3 mM HEPES, and 1.5 mM Tris (pH 7.2). All chemicals were of analytical grade and were purchased from Sigma-Aldrich

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

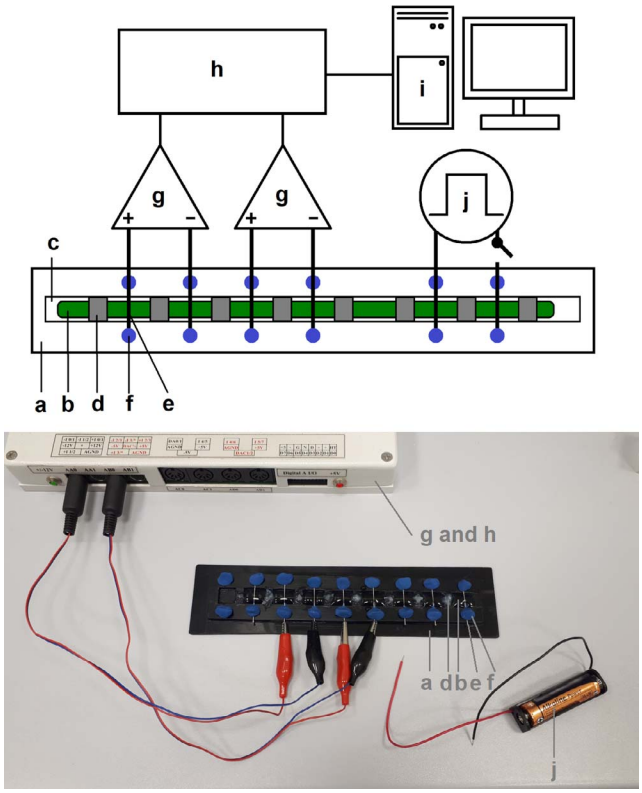

Fig 3. Extracellular recording system. A complete block scheme (top) and a partial photo (bottom). A cell (b) is put along the chamber (a). The gaps between the chamber's compartments (c) are isolated with petroleum jelly (d). The electrodes are made from sewing pins (e) and fixed to the chamber with plasticine (f). The e-Biol universal data acquisition and control system (26) is used for signal amplification (g) and digitization (h). The data acquisition system is controlled by a personal computer (i). The conventional 1.5V battery (j) is used for manual electrical stimulation.

(St. Louis, MO). Experiments were conducted at room temperature (21 ± 1 °C).

A. Extracellular recordings of action potentials

An internodal cell was placed in a custom-made plexiglass chamber (dimensions 205 × 50 × 6 mm) consisting of 9 compartments filled with APW. The chamber can be easily constructed out of plexiglass pieces glued together with superglue. Alternatively, a large Petri dish might be used with compartments formed out of any type of pure petroleum jelly. In any arrangement it is crucial to ensure proper electrical isolation between the compartments (whose dimensions in the described chamber were 15 × 10 × 3 mm). It is recommended to apply petroleum jelly in gaps between the compartments, place a cell, and top it with more petroleum jelly coating. Once a cell was placed into the chamber, each compartment was filled with experimental solution (usually APW). Sewing pins were used as electrodes, which were fixed on the chamber with plasticine pieces so that each electrode could contact the solution of its compartment. The distance between 2 adjacent pins was 2 cm. To avoid the effects of cell ends, pins were placed in such a way so that a pin closest to the end of a cell would be at least 2 cm away (17). Two pairs of sewing pins served as measuring electrodes. The e-Biol (ComLab project, Vilnius, Lithuania) universal data acquisition and control system (5, 26) was used for signal amplification ($10^5\times$) and digitization (analog to digital conversion [ADC] 12 bits, sampling rate 10 Hz). Data acquisition system was controlled by a standard personal computer. The principal scheme of the registration system is not sophisticated (Fig 3): any differential amplifiers and any ADC system might be used (e.g., PowerLab [Colorado Springs, CO] or Biopac [Goleta, CA]). An electrical stimulus was elicited by connecting a conventional 1.5V AA battery (any type of current generator can be used) to 2 adjacent pins. The electrical stimulation was applied (usually up to a few seconds) until the initiation of AP was observed. To apply a thermal stimulus, some APW was cooled to 0 °C; then, with the use of a pipette, a drop of this solution was applied directly on a cell portion in one compartment of the chamber. A change in temperature is known to alter membrane properties, activate mechanosensitive Ca^{2+} channels, and depolarize the membrane. If the stimulus is sufficient, an AP is generated (7).

In extracellular recordings with 2 electrodes, APs are registered as biphasic signals (Fig 4): the recorded signal is the potential difference between these 2 electrodes. Once an AP (local depolarization) is generated and reaches the first electrode, the second electrode acts as the reference and a deflection (rapid depolarization and subsequent repolarization) is registered. The

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

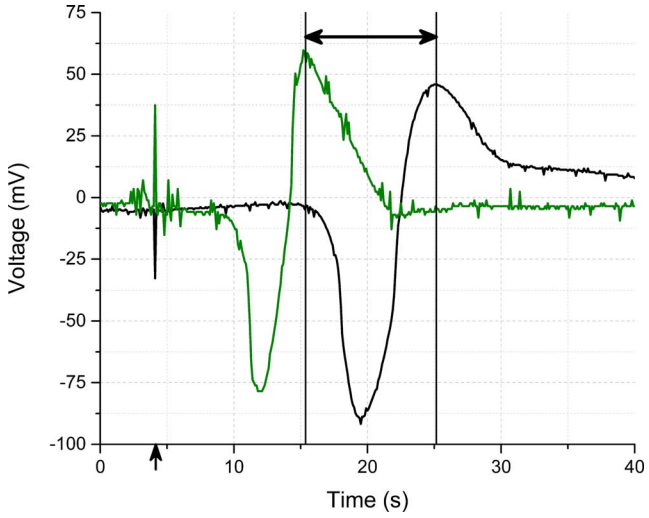

Fig 4. Typical extracellular recordings of an action potential of *Nitellopsis obtusa* made with 2 pairs of electrodes. The double-headed arrow indicates the time lag between 2 positive peaks of a conducted action potential. The single-headed arrow indicates the moment of electrical stimulation.

AP then propagates further, reaches the second electrode in the pair, and is registered by it (now the first electrode acts as a reference). With differential amplifiers and bipolar registration, the first deflection is positive (in this setup), and the second is negative. Conduction velocity was calculated from the time lag between 2 positive peaks of conducted AP and the distance between corresponding recording electrodes (Fig 4). It is possible to measure the velocity of propagation of AP with only 2 measuring electrodes if the distance between them is long enough, so that the deflections measured by each do not overlap.

B. Cytoplasmic streaming

For observations of cytoplasmic streaming, younger *N. obtusa* cells (closer to apex of the thallus) are preferred because, as cells age, their cell wall tends to become more mineralized (27), thus obstructing a clear view of cell insides. Cytoplasmic streaming might also be observed in *N. obtusa* rhizoids. In a natural habitat, rhizoids are hidden in the soil; thus, they do not possess fully developed plastids, allowing a very clear view of the cytoplasm. For the experiments, a cell was placed in a similar chamber as described above (Fig 3), except it was made from transparent plexiglass and had only one compartment composing the whole chamber. The cytoplasmic streaming was filmed with a DS-Fi1c (Nikon, Tokyo, Japan) camera at 100× magnification of the calibrated Eclipse FN1 (Nikon) microscope. Later videos were analyzed; the velocity of cytoplasmic streaming was determined by measuring the time a cytoplasmic particle needed to travel a certain distance. Attention should be paid to the area where the streaming is observed: if a particle comes too close to the indifferent zone, it can sometimes start to rotate or even cross the zone and reverse direction of movement. Also, it is best to observe vesicles of uniform smaller size, because large vesicles may temporarily get stuck.

Recovery of cytoplasmic streaming can be approximated with a simple exponential function (Eq. 1),

$$v(t) = v_0 + v_{\max} \times \left(1 - e^{\frac{-(t-t_0)}{\tau}}\right) \quad (1)$$

where $v(t)$ is the velocity of cytoplasmic streaming, v_0 indicates the velocity of cytoplasmic streaming at its minimum (when AP is generated), $v_{\max}$ indicates the maximal velocity of cytoplasmic streaming, t is time, t_0 indicates the moment of stimulation, and τ is the time constant of recovery of cytoplasmic streaming. Some of the parameters can be determined beforehand. In this research, a cell was stimulated 1.3 min after the start of recording ($t_0 =$ 1.3 min) and streaming usually stopped completely ($v_0 = 0$ μm/s). Maximal velocity of cytoplasmic streaming $v_{\max}$ can also be determined before the approximation.

C. Data analysis

Data analysis was performed by MicroCal OriginPro 2016 (OriginLab, Northampton, MA) software. All the data are expressed as a mean ± SD, and n indicates the number of cells measured. The Shapiro–Wilk test was used to confirm sample normality. If a sample was distributed normally, the Student's paired t-test was applied; otherwise, a nonparametric Mann–Whitney test was used. In both cases, the significance level was set to $P = 0.05$.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

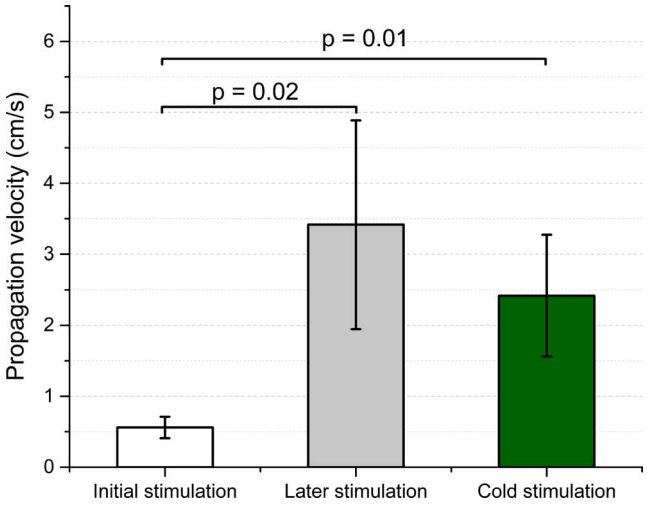

Fig 5. Velocity of the propagation of action potentials in *Nitellopsis obtusa*. Repeated electrical stimulation significantly increased the velocity; cold stimulation–induced action potentials also propagated significantly faster compared with the initial electrical stimulation–induced action potentials, but not to repeated electrical stimulation–induced action potentials.

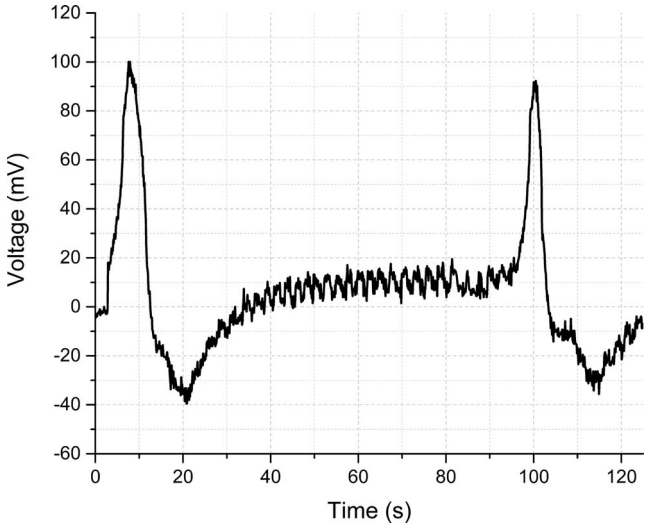

Fig 6. Investigation of the absolute refractory period in *Nitellopsis obtusa*. Once the first action potential was generated, a cell was repeatedly electrically stimulated (frequency ∼0.5 Hz) until another action potential was generated. Increased noise during the refractory period shows the electrical stimulus being repeatedly turned on and off.

IV. RESULTS

A. Extracellular recordings of action potentials

Typical extracellular recordings of an AP of *N. obtusa* are shown in Figure 4. Note the similar amplitude and biphasic shape. Any major discrepancies between recorded signals with different electrode pairs would indicate problems with the recording system, especially the electrodes.

Initially, the AP velocity of propagation was found to be comparatively slow (0.6 ± 0.2 cm/s, $n = 5$). However, after a further electrical stimulation (at least 3 APs with a rest period of at least 3 min), the velocity increased significantly ($P = 0.02$) to 3.4 ± 1.5 cm/s ($n = 6$). Although cold stimulation did not cause a significant change in the propagation velocity (2.4 ± 0.9 cm/s, $n = 6$), it showed a trend ($P = 0.09$) of being lower. The velocity after cold stimulation was significantly ($P = 0.01$) greater than after initial electrical stimulation (Fig 5).

The absolute refractory period was investigated by periodically (on/off, frequency ∼0.5 Hz) electrically stimulating a cell after it generated the first AP (Fig 6). The refractory period was found to be 51.5 ± 27 s ($n = 6$).

After the application of a drop of 3 M KCl solution on a cell (in one compartment), spontaneous APs were registered (Fig 7). Because of the different time scale, APs appear as high-amplitude spikes and deflections from the baseline; the apparent increase in noise (compared with Fig 4) is also explained by the same reasoning.

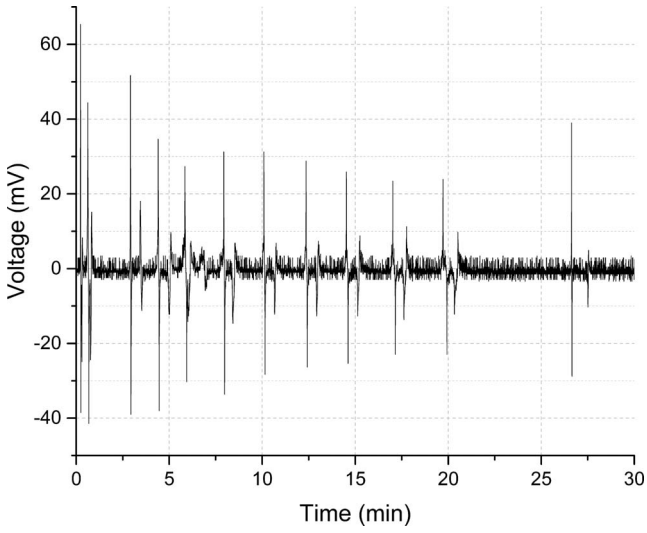

Fig 7. A recording of a spontaneous action potential train in *Nitellopsis obtusa* that was elicited by stimulating the cell with a drop of 3 M KCl solution.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

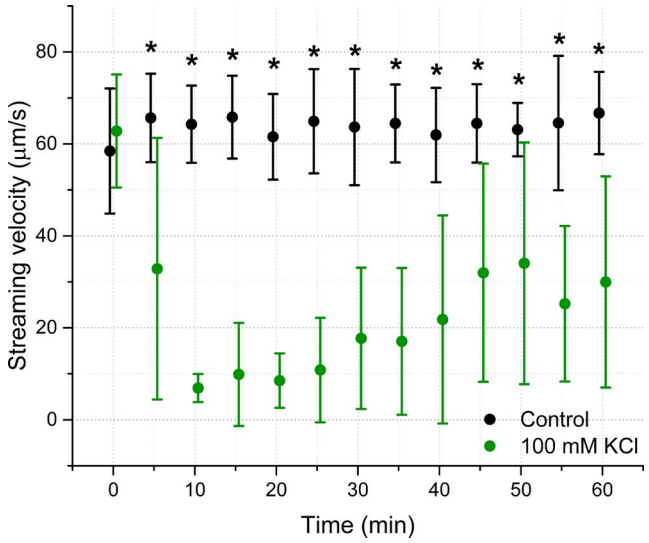

Fig 8. Dynamics of velocity of cytoplasmic streaming in *Nitellopsis obtusa*. In the control condition, the cell was immersed in artificial pond water solution; later, it was changed to 100 mM KCl solution. The change induced a significant decrease of the velocity marked by asterisks ($P < 0.05$, $n = 7$).

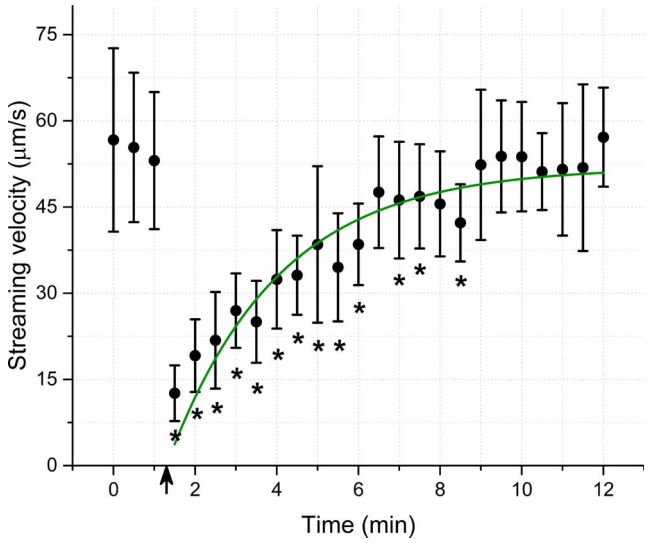

Fig 9. Recovery of cytoplasmic streaming after the generation of 1 cold-induced action potential. The arrow represents time of stimulation. Recovery dynamics are approximated by an exponential function from Eq. 1. Asterisks mark instances when the velocity of cytoplasmic streaming was significantly lower than its initial ($t = 0$) value ($P < 0.05$, $n = 6$).

B. Cytoplasmic streaming

Cytoplasmic streaming in *N. obtusa* retains a mostly constant value (60 min after the start of recording $v = 66.7 \pm 9$ μm/s, $n = 7$) for at least an hour if conditions are constant (Fig 8). Changing external solution from APW to 100 mM KCl invariably stopped cytoplasmic streaming. In 3 of 7 cells (43%) streaming stopped before the fifth minute after the application, and after 10 min it had ceased in all the cells ($n = 7$). After the initial stoppage, the recovery of cytoplasmic streaming was variable: in 3 of 7 cells (43%), the velocity of cytoplasmic streaming even after 60 min did not exceed 20% of its initial value (before the application of 100 mM KCl). The rest of the cells (4 of 7, 57%) showed considerably better signs of recovery. However, their recovery was not completely gradual: in these cells, cytoplasm stopped once again at least once during the time of recording. Overall, the velocity of cytoplasmic streaming was significantly decreased ($P < 0.05$, $n = 7$) from the fifth minute of the recording to its end at the 60th minute.

The recovery of cytoplasmic streaming was investigated after the generation of 1 AP (Fig 9): 1.3 min after the start of recording, a cold stimulus was applied. Cytoplasmic streaming stopped immediately and was gradually restored. The dynamics of recovery could be approximated by a simple exponential function (Eq. 1). From the approximation, the steady rate of cytoplasmic streaming is 58.2 ± 13.1 μm/s ($n = 6$) on average, which is consistent with the first experiment (Fig 8), and the recovery time constant is $\tau = 3.7 \pm 2.1$ min ($n = 6$). Compared with the first recorded value of cytoplasmic streaming ($t = 0$), the velocity of cytoplasmic streaming was significantly ($P < 0.05$) lower from 1.5 to 8.5 min after the start of the recording (except points $t = 6.5$ min and $t = 8$ min).

V. DISCUSSION

Recently, the *Chara braunii* genome was published (28), expanding possible scientific approaches (29, 30). Thus, Characeaen algae are not only complex organisms constantly providing new insights into plant electrophysiology, but also simple enough systems to be routinely used for student laboratory exercises.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

A. Extracellular recordings of action potentials

In this research, APs were registered extracellularly as biphasic signals. AP amplitude remained relatively constant as the AP propagated between the 2 electrode pairs (Fig 4). Some AP shape variability should be expected because of the nature of the extracellular recording itself; also, plant APs exhibit some intrinsic variation (7, 14). Students can best observe this variability by comparing APs of different cells. However, it should be stressed that characteristics of a propagating AP remain unchanged.

The small size of plant cells and plasmodesmata usually limits research on propagation of APs. Although there are data about AP propagation in plant tissues, very few species of plants are suitable for single-cell experiments. Tabata and Sibaoka (17) found that *C. braunii* AP propagation velocity differs in moist air (0.21 ± 0.05 cm/s) and in APW (1.5 ± 0.9 cm/s). APs propagated at a constant velocity through a cell, except for its ends (0.3 cm from an end in moist air and 1.8 cm in APW). In APW the conduction velocity near the ends increased to 5.7 ± 2.3 cm/s. We purposefully avoided this cell geometry–inflicted effect by placing the furthermost electrode at least 2 cm from the end of the cell. Action potential propagation velocity in *Nitellopsis* (3.4 ± 1.5 cm/s after repeated electrical stimulation) unsurprisingly is similar to that of *C. braunii* and other Characeaen algae [mentioned in Ref. (14)]. In tissues, propagation of plant APs is usually of a similar velocity or slower: 3–5 cm/s in maize sieve tubes and 0.2–2 cm/s in leaf mesophyll (31) and between 0.1 and 0.4 cm/s in tomato seedlings (32) and 0.3 and 0.5 cm/s in barley leaves (33). Interestingly, extremely fast propagating APs (up to a few meters per second) have been registered in Venus flytrap (34).

In our research, the value of the first stimulation-induced AP was significantly lower (0.6 ± 0.2 cm/s) compared with subsequent APs. The result might be explained by the fact that, before any stimulation, a cell was in its resting state with a resting potential of usually at least −200 mV. After further stimulation (especially with a period between stimuli that usually was shorter than the time needed for a cell to return completely to its resting state) (Fig 9), a cell persisted in a slightly depolarized state (35). If membrane resistance remains the same, for such a cell, less current is needed to reach the excitation threshold; thus, an AP is elicited quicker and propagates faster. Even though cold stimulus did not elicit a significantly slower propagation of AP compared with an electrical stimulus, the visible trend might be explained by the fact that the activity of ion channels responsible for excitation is temperature dependent (36).

Spontaneous APs are usually related to salt stress (7, 9). Application of a 3 M KCl solution elicited an extreme change in the extracellular environment, immediately changing the transmembrane Nernst potential for K^+ ions, depolarizing the plasmalemma, and bringing it to a level more positive than its normal excitation threshold. Consequently, a train of spontaneous APs was generated. The effect may also be connected to an extreme change in external osmolarity; however, it is known that the effect of salt stress is more prominent than the effect of osmotic stress (9).

B. Cytoplasmic streaming

Characeaen myosin is the fastest motor protein in the world (23, 37): its ATP- and Mg^{2+}-dependent "walking" velocity in Characeae can exceed 100 μm/s (38). The value of streaming velocity is at least 50 μm/s (20) and usually is observed in the range of 60–70 μm/s (39, 40). We found that the streaming velocity in *N. obtusa* is similar (66.7 ± 9 μm/s). A KCl solution of 100 mM markedly reduced the velocity of cytoplasmic streaming. As described above, KCl depolarized a cell, and it generated at least 1 AP. The exact response to the stimulus (either a very slow recovery of streaming velocity or a more rapid recovery with more spontaneous APs generated) indicates variability in electrophysiological parameters between cells of *N. obtusa* in their resting state.

After a thermal (cold) stimulus, an AP was always generated, and cytoplasmic streaming

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

stopped. On average, at least 7.5 min was needed for streaming to fully recover. According to Tazawa and Kishimoto, after an electrical stimulus, cytoplasmic streaming is fully restored within 5–10 min (39). Dynamics of the recovery of cytoplasmic streaming velocity could be approximated with a simple exponential function (time constant $\tau = 3.7 \pm 2.1$ min). As an easier way of quantifying the dynamics of the restoration of cytoplasmic streaming, a half-time of recovery (the amount of time needed for the velocity of cytoplasmic streaming to regain half of its initial value) can be used (41). In Hayama *et al*. (41), its value was about 2 min, a value very similar to our data (Fig 9). If the dynamics of cytoplasmic streaming indicate intracellular Ca^{2+} dynamics (24), then it can be thought that the exponential recovery of cytoplasmic streaming indicates that Ca^{2+} pump dynamics are responsible for lowering Ca^{2+} concentration in the cytoplasm (9).

C. Ideas for further experiments

Our concept experiments can be thought of as a starting point, because countless stimuli could affect both the velocity of propagation of APs and the velocity of cytoplasmic streaming. As described above, students could investigate the influence of pH, temperature, and illumination. For example, the students can fill the compartment with a more acidic (pH $\leq$ 6) or basic (pH $\geq$ 8) APW and observe cytoplasmic streaming slowing or even stopping (20, 42). Changing temperature should linearly affect velocity of the streaming (43, 44). Illumination affects streaming velocity by changing intracellular Ca^{2+} concentration: it is thought that under illumination, photosynthesis causes Ca^{2+} ions to be taken into the chloroplasts. Thus, in the dark, Ca^{2+} levels are higher, determining a slightly lower velocity of cytoplasmic streaming (45, 46).

If the budget allows, various chemicals (15, 47) could be used, especially those known to modulate cytoskeleton integrity or affect the enzymatic activity of myosin (e.g., cytochalasin and *N*-ethylmaleimide) (23), as well as a variety of ion channel blockers (e.g., La^{3+} ions) (13). If duration of an AP as a new parameter is introduced, the effect of K^+ channel blockers (e.g., Cs^+ or tetraethylammonium ions [TEA^+]), observed as prolongation of APs (48), should be easy to quantify. Naturally, all the ion species known to be involved in Characeaen AP generation (Ca^{2+}, Cl^-, K^+) also should have a clear effect on Characeaen electrogenesis, as shown by our research involving elevated K^+ concentration. (Figs 7, 8). A very easy experiment would be to increase or decrease (using a chelator) extracellular Ca^{2+} concentration and wait for it to affect intracellular Ca^{2+} concentration: streaming velocity is not affected when Ca^{2+} concentration does not exceed 10^{-7} M (20), but once it reaches about 0.8 mM, the streaming is completely inhibited (49). Salt stress (elevated Na^+ concentration) (9) could be investigated, as well.

If a current generator is available, it would be beneficial to examine the all-or-none quality of APs by starting stimulation from a subthreshold value and continuously increasing the stimulus until an AP is generated. We did not find any significant differences between the velocities of propagation of APs when cells were stimulated electrically or thermally (with a drop of cold solution). However, a bigger sample size might reverse such a claim. AP can also be elicited with mechanical stimulation (touching a cell). It would be interesting to compare mechanically elicited to electrically or thermally elicited APs. However, mechanical stimulation requires utmost precision and care, because if a cell is moved too much when touched, the integrity of the petroleum jelly seal between compartments can be compromised. Stimuli of different modality could also be applied when researching the dynamics of recovery of cytoplasmic streaming. Students could also investigate whether stimulating the opposite ends of a cell evokes any differences between APs. Exchanging the positive with a negative measuring electrode could also provide some insights for the students.

Described experiments could become a starting point of discussions about cytoskeleton and motor proteins in general, as well as the mechanochemical basis of cellular traffick-

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

ing, differences between electrogenesis in plant and animal cells, mechanism and functions of electrical signaling, and natural variability of biological systems. Basics of data analysis (normality of data, statistical hypothesis testing) can also be introduced as a part of laboratory exercises.

VI. CONCLUSION

In our research we present several simple experiments that could help familiarize students with basic electrophysiological concepts and provide some hands-on practice. *Nitellopsis obtusa* and other Characeaen algae are especially suitable for such purposes because of their excitability and specific morphological characteristics. Concept experiments measuring velocity of AP propagation and velocity of cytoplasmic streaming were introduced. The experiments could be expanded to suit the particular needs of a curriculum. Discussions of various topics from statistical analysis to the electrochemical basis of APs naturally arise from the experiments. In conclusion, Characeaen *N. obtusa* algae can be harnessed as a convenient experimental system for student laboratory practice.

AUTHOR CONTRIBUTIONS

VP and VK conceived the research; VP performed the experiments, analyzed the data, and wrote the manuscript; RB provided technical assistance; and RB, IL, and VK reviewed the manuscript. All authors have read and approved the final version of the manuscript.

REFERENCES

1. Fromm, J., and S. Lautner. 2007. Electrical signals and their physiological significance in plants. *Plant Cell Environ* 30(3):249–257.
2. Sukhov, V., E. Sukhova, and V. Vodeneev. 2019. Long-distance electrical signals as a link between the local action of stressors and the systemic physiological responses in higher plants. *Prog Biophys Mol Biol* 146:63–84.
3. Andjus, P. R. 1998. Harvesting the biophysical field of the "Green Axon." *Iugosl Physiol Pharmacol Acta* 34:1–9.
4. Johnson, B. R., R. A. Wyttenbach, R. Wayne, and R. R. Hoy. 2002. Action potentials in a giant algal cell: a comparative approach to mechanisms and evolution of excitability. *J Undergrad Neurosci Educ* 1(1):A23–A27.
5. Kisnieriene, V., K. Beitas, V. Sakalauskas, and A. Daktariunas. 2008. Information technologies for biology education: computerized electrophysiology of plant cells. *Inf Educ* 7(1):91–104.
6. Larkin, D. J., A. K. Monfils, A. Boissezon, R. S. Sleith, P. M. Skawinski, C. H. Welling, B. C. Cahill, and K. G. Karol. 2018. Biology, ecology, and management of starry stonewort (*Nitellopsis obtusa*; Characeae): a red-listed Eurasian green alga invasive in North America. *Aquat Bot* 148:15–24.
7. Beilby, M. J., and M. T. Casanova. 2014. The Physiology of Characean Cells. Springer, Berlin, Heidelberg.
8. Kikuyama, M. 1986. Tonoplast action potential of Characeae. *Plant Cell Physiol* 27(8):1461–1468.
9. Kisnieriene, V., I. Lapeikaite, V. Pupkis, and M. J. Beilby. 2019. Modeling the action potential in Characeae *Nitellopsis obtusa*: effect of saline stress. *Front Plant Sci* 10:1–15.
10. Król, E., H. Dziubińska, and K. Trębacz. 2010. What do plants need action potentials for? In: Action Potential. M. L. DuBois, editor. Nova Science Publishers, Hauppauge, NY, pp. 1–26.
11. Umrath, K. 1930. Untersuchungen über plasma und plasmaströmung an Characeen. *Protoplasma* 9:576–597.
12. Cole, K. S., and H. J. Curtis. 1938. Electric impedance of *Nitella* during activity. *J Gen Physiol* 22(1):37–64.
13. Lunevsky, V. Z., O. M. Zherelova, I. Y. Vostrikov, and G. N. Berestovsky. 1983. Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels. *J Membr Biol* 72:43–58.
14. Beilby, M. J. 2007. Action potential in charophytes. *Int Rev Cytol* 257:43–82.
15. Kisnierienė, V., I. Lapeikaitė, and V. Pupkis. 2018. Electrical signalling in *Nitellopsis obtusa*: potential biomarkers of biologically active compounds. *Funct Plant Biol* 45(2):132–142.
16. Lapeikaitė, I., U. Dragūnaitė, V. Pupkis, O. Rukšėnas, and V. Kisnierienė. 2019. Asparagine alters action potential parameters in single plant cell. *Protoplasma* 256(2):511–519.
17. Tabata, T., and T. Sibaoka. 1987. Conduction velocity and blockage of action potential in *Chara* internodal cells. *Plant Cell Physiol* 28(7):1187–1194.
18. Goldstein, R. E., and J.-W. van de Meent. 2015. A physical perspective on cytoplasmic streaming. *Interface Focus* 5(4):20150030.
19. Kamiya, N., and K. Kuroda. 1956. Velocity distribution of the protoplasmic streaming in *Nitella* cells. *Bot Mag Tokyo* 69(822):544–554.
20. Williamson, R. E. 1975. Cytoplasmic streaming in *Chara*: a cell model activated by ATP and inhibited by cytochalasin B. *J Cell Sci* 17:655–668.
21. Kato, T., and Y. Tonomura. 1977. Identification of myosin in *Nitella flexilis*. *J Biochem* 82(3):777–782.
22. Morimatsu, M., A. Nakamura, H. Sumiyoshi, N. Sakaba, H. Taniguchi, K. Kohama, and S. Higashi-Fujime. 2000. The molecular structure of the fastest myosin from green algae, *Chara*. *Biochem Biophys Res Commun* 270(1):147–152.
23. Shimmen, T. 2007. The sliding theory of cytoplasmic streaming: fifty years of progress. *J Plant Res* 120(1):31–43.
24. Okazaki, Y., and N. Iwasaki. 1991. Injection of a Ca^{2+}-chelating agent into the cytoplasm retards the progress of turgor regulation upon hypotonic treatment in the alga, *Lamprothamnium*. *Plant Cell Physiol* 32(2):185–194.
25. Brunet, T., and D. Arendt. 2016. From damage response to action potentials: early evolution of neural and contractile modules in stem eukaryotes. *Philos Trans R Soc Lond B Biol Sci* 371(1685):20150043.
26. eBiol: data acquisition and control system dedicated for biology and chemistry. 2004. Accessed 26 December 2019. http://www.pef.uni-lj.si/eprolab/comlab/ebioldaq/ebioldaq.htm.
27. Schöler, A., I. Zaharieva, S. Zimmermann, M. Wiechen, A. M. Manke, P. Kurz, C. Plieth, and H. Dau. 2014. Biogenic manganese-calcium oxides on the cell walls of the algae *Chara corallina*: elemental composition, atomic structure, and water-oxidation catalysis. *Eur J Inorg Chem* 2014(4):780–790.
28. Nishiyama, T., H. Sakayama, J. de Vries, H. Buschmann, D. Saint-Marcoux, K. K. Ullrich, F. B. Haas, L. Vanderstraeten, D. Becker, D. Lang, S. Vosolsobě, S. Rombauts, P. K. I. Wilhelmsson, P. Janitza, R. Kern, A. Heyl, F. Rümpler, L. I. A. C. Villalobos, J. M. Clay, R. Skokan, A. Toyoda, Y. Suzuki, H. Kagoshima, E. Schijlen, N. Tajeshwar, B. Catarino, A.J. Hetherington, A. Saltykova, C. Bonnot, H. Breuninger, A. Symeonidi, G. V. Radhakrishnan, F. Van Nieuwerburgh, D. Deforce, C. Chang, K. G. Karol, R. Hedrich, P. Ulvskov, G. Glöckner, C. F. Delwiche, J. Petrášek, Y. Van de Peer, J. Friml, M. Beilby, L. Dolan, Y. Kohara, S. Sugano, A. Fujiyama, P. M. Delaux, M. Quint, G. Theißen, M. Hagemann, J. Harholt, C. Dunand, S. Zachgo, J. Langdale, F. Maumus, D. Van Der Straeten, S. B. Gould, and S. A. Rensing. 2018. The *Chara* genome: secondary complexity and implications for plant terrestrialization. *Cell* 174(2):448–464.e24.
29. Cuin, T. A., I. Dreyer, and E. Michard. 2018. The role of potassium channels in *Arabidopsis thaliana* long distance electrical signalling: AKT2 modulates tissue excitability while GORK shapes action potentials. *Int J Mol Sci* 19(4):1–17.
30. Beilby, M. J. 2019. *Chara braunii* genome: a new resource for plant electrophysiology. *Biophys Rev Lett* 11:235–239.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15

31. Fromm, J., and T. Bauer. 1994. Action potentials in maize sieve tubes change phloem translocation. *J Exp Bot* 45(4):463–469.
32. Wildon, D. C., J. F. Thain, P. E. H. Minchin, I. R. Gubb, A. J. Reilly, Y. D. Skipper, H. M. Doherty, P. J. O'Donnel, and D. J. Bowles. 1992. Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. *Nature* 360:62–65.
33. Felle, H. H., and M. R. Zimmermann. 2007. Systemic signalling in barley through action potentials. *Planta* 226(1):203–214.
34. Volkov, A. G. 2019. Signaling in electrical networks of the *Venus flytrap* (*Dionaea muscipula* Ellis). *Bioelectrochemistry* 125:25–32.
35. Kisnierienė, V., V. Sakalauskas, A. Pleskačiauskas, V. Yurin, and O. Rukšėnas. 2009. The combined effect of Cd^{2+} and ACh on action potentials of *Nitellopsis obtusa* cells. *Cent Eur J Biol* 4(3):343–350.
36. Beilby, M. J., and H. G. L. Coster. 1976. The action potential in *Chara corallina*: effect of temperature. *Aust J Plant Physiol* 3(3):275–289.
37. Ito, K., M. Ikebe, T. Kashiyama, T. Mogami, T. Kon, and K. Yamamoto. 2007. Kinetic mechanism of the fastest motor protein, *Chara* myosin. *J Biol Chem* 282:19534–19545.
38. Shimmen, T. 1978. Dependency of cytoplasmic streaming on intracellular ATP and Mg^{2+} concentrations. *Cell Struct Funct* 3:113–121.
39. Tazawa, M., and U. Kishimoto. 1968. Cessation of cytoplasmic streaming of *Chara* internodes during action potential. *Plant Cell Physiol* 9(2):361–368.
40. Okazaki, Y., M. Ishigami, and N. Iwasaki. 2002. Temporal relationship between cytosolic free Ca^{2+} and membrane potential during hypotonic turgor regulation in a brackish water charophyte *Lamprothamnium succinctum*. *Plant Cell Physiol* 43(9):1027–1035.
41. Hayama, T., T. Shimmen, and M. Tazawa. 1979. Participation of Ca^{2+} in cessation of cytoplasmic streaming induced by membrane excitation in Characeae internodal cells. *Protoplasma* 99:305–321.
42. Tazawa, M., and T. Shimmen. 1982. Artificial control of cytoplasmic pH and its bearing on cytoplasmic streaming, electrogenesis and excitability of Characeae cells. *Bot Mag Tokyo* 95:147–154.
43. Pickard, W. F. 1974. Hydrodynamic aspects of protoplasmic streaming in *Chara braunii*. *Protoplasma* 82:321–339.
44. Shimmen, T., and S. Yoshida. 1993. Analysis of temperature dependence of cytoplasmic streaming using tonoplast-free cells of Characeae. *Protoplasma* 176:174–177.
45. Miller, A. J., and D. Sanders. 1987. Depletion of cytosolic free calcium induced by photosynthesis. *Nature* 326:397–400.
46. Plieth, C., and U.-P. Hansen. 1992. Light dependence of protoplasmic streaming in *Nitella flexilis* L. as measured by means of laser-velocimetry. *Planta* 188(3):332–339.
47. Beilby, M. J. 2016. Multi-scale Characean experimental system: from electrophysiology of membrane transporters to cell-to-cell connectivity, cytoplasmic streaming and auxin metabolism. *Front Plant Sci* 7:1–20.
48. Shimmen, T., and M. Tazawa. 1983. Activation of K^{+}-channel in membrane excitation of *Nitella axilliformis*. *Plant Cell Physiol* 24:1511–1524.
49. Williamson, R., and C. C. Ashley. 1982. Free Ca^{2+} and cytoplasmic streaming in the alga *Chara*. Nature 296(5858):647–651.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-15