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Signaling in electrical networks of the Venus flytrap (*Dionaea muscipula* Ellis)

Alexander G. Volkov *

Department of Chemistry and Biochemistry, Oakwood University, Huntsville, AL 35896, USA

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ABSTRACT

The Venus flytrap captures insects with one of the most rapid movements in the plant kingdom. There is a significant difference between properties of electrical signals generated in the Venus flytrap described in literature. Amplitudes of action potentials vary from 14 mV to 200 mV with duration of signals from 2 ms to 10 s. Here we present experimental study of potential differences between Ag/AgCl electrodes inserted to the trap, petiole, and into soil or external ECG electrodes attached to surfaces of the Venus flytrap. Diverse types of electrodes with various positions in a plant tissue or in soil show different amplitude and duration of electrical signals because potentials are measured in different electrochemical circuits. Electrical signals in the Venus flytrap were induced by mechanical stimulation of the trigger hairs or by chemical stimulation of a midrib using small drops of H_2O_2 or HNO_3. Here we found that action potentials can propagate with speed up to 10 m/s in the trap of *D. muscipula*. Results are compared with equivalent electrical circuits.

1. Introduction

The history of studying the Venus flytrap spans a few centuries [1–10]. Touching trigger hairs activates mechanosensitive ion channels and generates receptor potentials which can be amplified by voltage gated ion channels to action potentials propagated through the trap's tissue with constant amplitude, duration, and speed [11–13]. This process depends on the transmembrane flow of protons, calcium, chloride, and potassium ions. It was found that two action potentials are required to trigger the closing of the trap at room temperature, and only one action potential is required at higher temperatures. It has been found that uncouplers and blockers of aquaporins and ion channels can reduce or completely inhibit the response of the Venus flytrap to physical, chemical or electrical stimuli [14,15]. Action potentials can be also generated when epidermis or mesophyll cells in the lobe of the trap are stimulated by applied pressure or mechanical damage.

Darwin demonstrated that the basic catching movement of the Venus flytrap involves the transformation of the leaf curvature from concave to convex resulting in the closing of the trap [5]. The upper leaf of the Venus flytrap includes two distinct layers of cells at upper and lower surfaces that behave quite differently in the process of trap closure. The finding of these two independent layers and their role was related to the turgor pressure was later confirmed by many authors. It is well known that some functions in plants and fungi can be driven by exploiting hydrodynamic flow, such as the stomata guard cell opening and closing, leaf pulvini motor organ, mechanical traps of carnivorous plants, and fungal appressed penetration.

It is common knowledge that the leaves of the Venus flytrap actively employ turgor pressure and hydrodynamic flow for fast movement and catching insects. In these processes the upper and lower surfaces of the leaf behave quite differently [16]. During the trap closing, the loss of turgor by parenchyma, lying beneath the upper epidermis, is accompanied by the active expansion of the tissues of the lower layers of parenchyma near the under epidermis. The cells on the inner face of the trap jettison their cargo of water, shrink, and allow the trap lobe to fold over. The cells of the lower epidermis expand rapidly, folding the trap lobes over.

Different environmental stimuli evoke specific responses in living cells which have the capacity to transmit a signal to the responding region. A variety of electrical signaling in the Venus flytrap includes receptor, electrotonic, and action potentials [7,17–25]. A receptor potential always precedes an action potential and couples the mechanical stimulation step to the action potential step of the preying sequence [21,26]. A possible pathway of action potential propagation includes vascular bundles and plasmodesmata in the trap [27–35].

Generation of electrical signals which looks like action potentials in the Venus flytrap [1–4,15,19,22,23,33–37,39–50] has been registered by plant physiologists (Table 1) in the last 150 years usually with slow registration systems without low pass filters. Due to the electronic effects of aliasing, many publications show different amplitude from a

Abbreviations: *C*, capacitance; *ECG*, electrocardiogram; *R*, resistance; $\tau = RC$, time constant; *V*, voltage; V_{in}, input voltage.

* Corresponding author.
E-mail address: gvolkov@oakwood.edu.

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Table 1
Action potentials in *D. muscipula*.

	Amplitude, [mV]	Duration, [s]	Electrodes: Contact Method and Location	Stimuli	Reference
1	130	1	Inserted in a trap	Mechanical stimulation of trigger hairs	[33]
2	100	0.6–3	Inserted in a lobe and midrib	Mechanical stimulation of trigger hairs	[34–36]
3	140–200	2–10	Inserted in the trap	Mechanical stimulation of trigger hairs	[19]
4	150–200	2–5	Inserted in mesophyll cells	Mechanical stimulation of trigger hairs	[37]
5	50	5	Trigger hair	Mechanical stimulation of trigger hairs	[40]
6	14	6	Both lobes	Mechanical stimulation of trigger hairs	[46]
7	160	2	In a lobe with disconnected trap	Mechanical stimulation of trigger hairs	[8]
8	100	2	On a trap surface with conductive gel, reference electrode in soil	Mechanical stimulation of trigger hairs	[48]
9	20–50	2–5	On a trap surface with conductive gel, reference electrode in soil	Mechanical stimulation of trigger hairs by a live pillbug (Isodopa)	[49]

few 7mV to 200 mV, duration from 0.2 s to 10 s, and estimated ***without direct measurements*** [35] speed of propagation of action potentials from 0.05 m/s to 0.2 m/s. These results were criticized by plant physiologists 130 years ago because such slow action potentials can be the result of the Venus flytrap closure, but not its direct cause [10]. Recently, it was shown that the fast closure of the Venus flytrap starts 0.1 s after mechanical stimulation and finishes in 1 s [5,51–53].

When electrochemical signals are measured, it is extremely important to take into consideration the *sampling rate* which determines how often the measurement device samples an incoming analog signal. According to the sampling theorem, the original signal must be adequately sampled in order to be properly represented by the sampled signal. If the sampling rate is too slow, the rapid changes in the original signal between any two consecutive samples cannot be accurately recorded. As a result, higher frequency components of the original signal will be misrepresented as lower frequencies. In signal processing, this problem is known as *aliasing*. According to the Nyquist Criterion, the sampling frequency must be at least twice the bandwidth of the signal to avoid aliasing. Undersampling may result in the mispresentation of the measured signal and increasing the duration of electrical signals such as action or electrotonic potentials. This is a major reason for irreproducibility of electrical signal duration shown in the Table 1.

Action potential in the trap can induce electrotonic potential in the petiole, which decreases exponentially with distance from the trap [17]. Another reason for the distinction of action potentials is the different location of electrodes inside lobes, inside the midrib, on the surface of lobes or midrib attached by conductive glue, in lower leaf, and in soil. Electrical potentials in different electrochemical cells can vary and results can differ significantly [18,54]. There is a difference in duration and amplitude of electrical potentials measured by electrodes inserted in a leaf and those attached to a leaf's surface [54]. If the external reference electrode is located in the soil near the root it changes the amplitude and duration of electrical potentials due to existence of additional resistance, capacitance, ion channels, and ion pumps in the root. The arrangement of electrodes in plant tissue is very important for the understanding of plant electrical circuit organization and communication in the electrical networks of plants. Many authors also used different types of electrodes with diverse locations in plant tissue, outside plant tissue, in soil and as a result their diverse electrochemical circuits produced various results.

This paper presents experimental investigation of electrical networks in the Venus flytrap and generation of electrical signals by different stimuli. Reversible Ag/AgCl electrodes were located in lobes, in the midrib, in a petiole and in soil or ECG electrodes on the surface of traps were attached by conductive hydrogel.

2. Materials and methods

2.1. Plants

One hundred bulbs of *Dionaea muscipula* (Venus flytrap) were purchased for this experimental work from Fly-Trap Farm (*Supply, North Carolina*) and grown in well drained peat moss in 250 mL plastic pots at 22 °C with a 12:12 h light:dark photoperiod. ***All plants were cultivated from seeds.*** The soil was treated with distilled water. Irradiance was 800–900 μmol photons m^{-2} s^{-1} PAR at plant level. All experiments were performed on healthy adult specimens.

2.2. Chemicals

Hydrogen peroxide solution and HNO_3 was purchased from *Sigma-Aldrich* (USA).

2.3. Extracellular Ag/AgCl electrodes

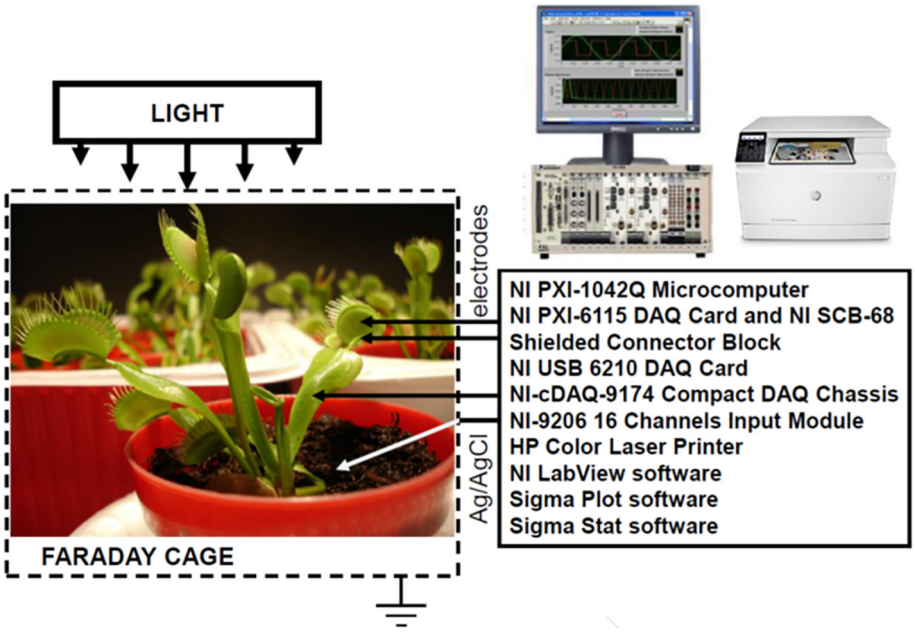
Teflon coated silver wires (*A-M Systems, Inc., Sequim, WA, USA*) with a diameter of 0.2 mm were used for preparation of non-polarizable electrodes. Reversible Ag/AgCl electrodes were prepared in the dark by electrodeposition of AgCl on 5 mm long silver wire tips without Teflon coating in a 0.1 M KCl aqueous solution. The anode was a high-purity silver wire and the cathode was a platinum plate. Electrical current in the electrolytic cell was limited to 1 mA/cm^2 of the anode's surface. Stabilization of electrodes was accomplished by placing two Ag/AgCl electrodes in a 0.1 M KCl solution for 24 h and connecting a short circuit between them. The response time of Ag/AgCl electrodes was less than 0.1 μs. Identical Ag/AgCl electrodes were used as working and reference electrodes for measurements of potential differences in the plants. Following insertion of the electrodes into lobes, the traps closed. We allowed plants to rest until the traps were completely open after transition from the convex to concave leaf curvature.

2.4. External "surface" ECG electrodes

McKesson ECG Tab electrodes (https://mms.mckesson.com) with Ag/AgCl sensing system covered by a conductive hydrogel and low impedance were used as working and reference electrodes at the surface of plants. Since the surface of ECG electrodes is sufficiently large, only small piece of electrode was attached to a plant tissue. Electrodes for ECG have a number of drawbacks, such as diffusing of hydrogel into the bio-tissue and the requirement of cleansing to remove gel residues after data recording. ECG electrodes are usually operated at frequencies between 1 Hz and 100 Hz for detection of slow electrical signals with small amplitude [46].

2.5. Data acquisition

All measurements were conducted in the laboratory at 21 °C inside a Faraday cage mounted on a vibration-stabilized table. Experimental setup is shown in Schema 1. High speed data acquisition was performed using NI-PXI-1042Q microcomputers with simultaneous multifunction I/O plug-in data acquisition board NI-PXI-6115 (*National Instruments, Austin, TX, USA*) interfaced through a NI-SCB-68 shielded connector block to Ag/AgCl electrodes. The system



Schema 1. The diagram of the experimental setup. Two types of Ag/AgCl electrodes were used for the measurements of the plant electrical responses: a silver wire with a tip covered by Ag/AgCl or electrodes for ECG.

integrates standard low-pass anti-aliasing filters at one half of the sampling frequency.

The NI-9206 16-Bit Analog Input Module was also used with a NI-cDAQ-9174 CompactDAQ chassis for data acquisition interfaced to personal computer. The NI-9206 features 16 differential analog inputs, 16-bit resolution, and a maximum sampling rate of 250 kS/s. The power supply for the data acquisition system was Energizer XP-1800 external battery.

The NI USB-6210 data acquisition card interfaced to personal computer was used in some experiments. All experimental results obtained with NI PXI-6115, NI-9206, and USB-6210 data acquisition cards coincide.

2.6. Mechanical stimulus

The mechanical stimulation was performed by using a cotton thread to gently touch two of the six trigger hairs inside the upper leaf of the Venus flytrap. The tip of the cotton thread was between the lobes after closing the trap. Gentle moving of a cotton thread after the trap closing generates additional electrical signaling.

2.7. Images

A photo camera Nikon D3x with AF-S Micro Nikkor 105 mm 1:2.8 G ED VR lens was used for the photography of plants. Digital video recorders, Sony DCR-HC36 and Sony HDR-XR500, were used to monitor the Venus flytraps and to collect digital images, which were analyzed frame by frame. The NTSC format consists of 30 interlaced frames of video per second, which represents the maximum sampling frequency of parameters extracted from the video stream.

2.8. Statistics

All experimental results were reproduced at least 16 times using different plants. Software SigmaPlot 12 (*Systat Software, Inc., San Jose, CA, USA*) was used for statistical analysis of experimental data.

3. Results

3.1. Mechanical stimulation: Effects of extracellular and external electrodes arrangement

Extracellular recording is a bioelectrochemical technique that uses an electrode inserted into bio-tissue to measure electrical activity coming from adjacent cells. Various electrode locations in a leaf, stem, root, and soil can be used to study multiple electrical circuits in the electrical networks of plants. An ideal reference electrode should be reversible and reproducible. Ag/AgCl electrodes can be prepared by electrodeposition of AgCl on a silver wire or plate [55].

Action potentials in the Venus flytrap propagates inside the trap and do not penetrate to the petiole (Fig. 1 A, B). Action potentials in the trap can induce small electrotonic potentials in the petiole (Fig. 1 B2). Multiple electrical signals can be generated by a continuous mechanical stimulation of trigger hairs using a cotton soft string inside the closed trap (Fig. 1B).

Electrical networks in plants consist of different electrical circuits. The arrangement of electrodes in plant tissue is very important for understanding plant electrical circuits, their organization and communication in electrical networks of plants. Experiments shown in Fig. 1 can be modified using external McKesson ECG Tab electrodes with conductive film attached to the midrib (Fig. 2) or to a lobe (Fig. 3) instead of extracellular Ag/AgCl electrodes shown in Fig. 1. Results on Figs. 1 and 2 look similar, but amplitude of electrical signals decreases twice in the case of ECG electrodes attached to the surface of a midrib. It can be caused by the additional *RC* circuit with a hydrogel between a flat Ag/AgCl electrode and the trap. If potential difference is measured between external ECG electrode at a lobe and a reference Ag/AgCl electrode in soil (Fig. 3), the amplitude of electrical response is higher than between a midrib and soil (Fig. 2).

We can cut one of the lobes above the midrib. Electrical signaling between a lobe and the midrib in the Venus flytrap with amplitude up to 40 mV induced by mechanical stimulation of trigger hairs can be measured even if one of the lobes is removed from the plant as it is shown in Fig. 4. Such experiment can be repeated continuously many times. Action potentials between Ag/AgCl electrodes inserted in lobes and midribs with amplitude up to 0.15 V were presented in our previous works [17,31,32].

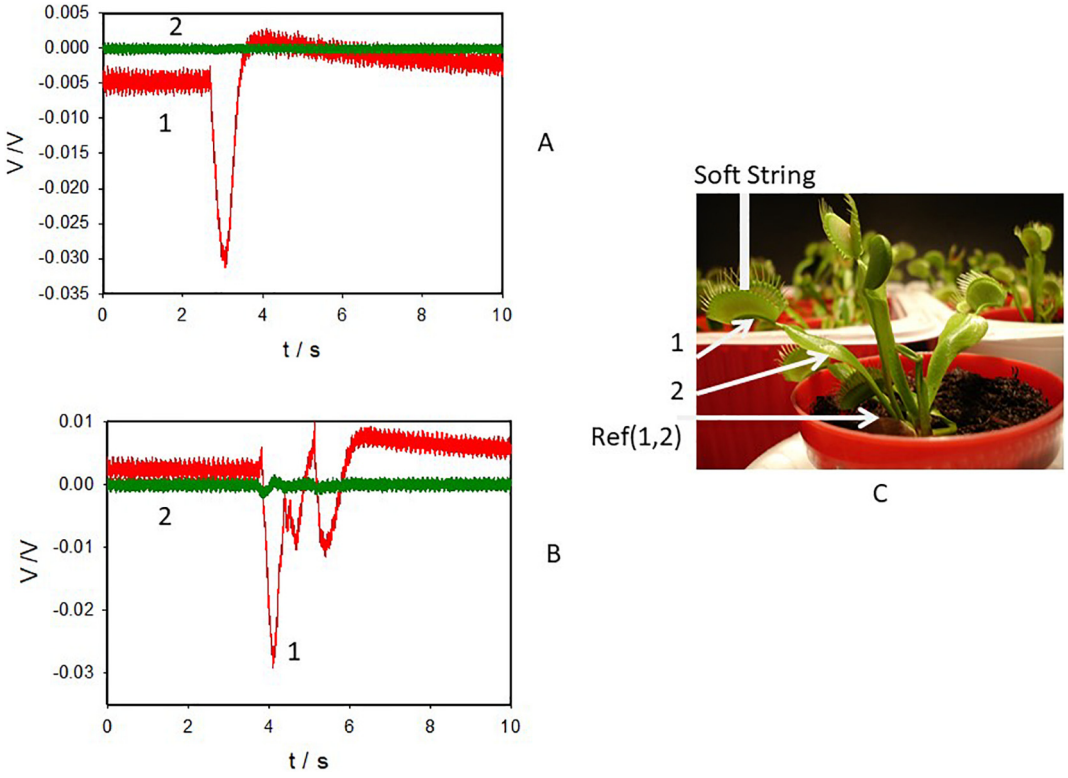


Fig. 1. Electrical signals in the Venus flytrap (A, B) were induced by mechanical stimulation using a cotton soft string for gently touching of trigger hairs when the trap was open (A) or a few seconds after closing it (B). The tip of the cotton thread was between lobes after closing the trap. Working Ag/AgCl wire electrodes were inserted in a midrib (1) and petiole (2) as it is shown in Fig. 1C. Reference electrodes were located in soil. Measurements were performed at 50,000 scans/s with low pass filter at 25,000 scans/s.

3.2. Electrical signaling induced by chemicals deposited on a midrib

Generation of action potentials in the Venus flytrap can be also stimulated by deposition of a small drop of HNO_3 (Figs. 5, 6), H_2O_2 (Fig. 7) or anesthetic agents [38–44] on the surface of a midrib without touching of mechanosensitive hairs. Very small drop of chemicals and electrodes were located on sufficiently long distances and did not have any direct interactions. These experiments were produced in a short time up to 10 s. Since amplitude, speed and duration of these electrical signals are the same in all experiments, they are action potentials. Action potentials can be measured between a midrib and any point in a lobe (Figs. 5, 6). Sibaoka [34–36] estimated without direct measurements that speed of action potentials in lobes of the Venus flytrap can reach up to 20 cm/s or 0.2 mm/ms. If Sibaoka's estimations were correct, the maxima of action potentials at the Fig. 5 should be separated by 10 ms, but this difference in experiments is about 0.2 ms. This means that the real speed of action potentials in the Venus flytrap can be much faster than it was predicted by Sibaoka [34–36]. The same conclusion follows from the Fig. 6. The speed of action potential propagation in the trap is equal to distance (2 mm) between electrodes divided by time difference between two peaks (0.2 ms) which is about 10 m/s (mean 8.10 m/s, median 9.50 m/s, stg. Dev. 5.49 m/s, std. err. 1.73 m/s, 95% conf. 3.92 m/s, 99% conf. 5.64 m/s, $n = 10$). Exact speed is unknown because we do not know the length of electrical pathway between electrodes inside the trap. Plasmodesmata and phloem can be involved in the transmission of action potentials along plasma membranes.

Generation of action potentials induced by a 10 μL drop of 3% aqueous solution of H_2O_2 is shown in Fig. 7. A drop has very low amount of H_2O_2(34 μg) which corresponds to a very low concentration of hydrogen peroxide in the trap if it will penetrate to the midrib and lobes. The speed of penetration is extremely low and a drop of H_2O_2 will stay on the midrib far away from electrodes for the duration of the experiment Amplitude of this electrical signal is two times less and duration of action potentials is much longer than in previous results with a drop of HNO_3. Different chemical stimuli can induce different electrical responses in the Venus flytrap.

4. Discussion

The Venus flytrap is a marvel of plant mechanical, electrical, and chemical engineering. The trap of *Dionaea muscipula* can be closed by mechanical [5–7,53], electrical [7,29–32], and chemical [38,56] stimulation, by 7.35 μm infrared laser with 50 μW power [57] or by an atmospheric pressure argon or helium plasma jet [56].

The electrode arrangement while monitoring the transmission of electrical signals in plants is of great importance [58]. Various electrode locations in a leaf, stem, root, and soil can be used to study multiple electrical circuits in the electrical networks of plants (Schema 2). Electrical signals can propagate along the plasma membrane on long distances in vascular bundles and on short distances in plasmodesmata and protoxylem.

There are a few different experimental methods to measure electrical signal propagation in plants. Reversible Ag/AgCl electrodes can be inserted into plant tissue or attached to the surface of a plant for measuring a combination of extracellular and external potentials (Schema 2). Amplitude and duration of electrical signals along the surface of a leaf or a stem depends on location and the type of contact between the attached electrodes and the plant's surface. Propagation of electrical signals inside plants can induce electrical signals between external electrodes attached to their surface. In many publications, the external reference Ag/AgCl electrode was inserted in the soil, compost or aqueous buffing solution. Amplitude, duration, and speed of electrical signal transmission are obtained using Ag/AgCl extracellular or external electrodes attached to a leaf surface or inserted in a soil can be different.

There are three mayor types of electrical signaling in plants and animals: action potentials, electrotonic potentials and graded potentials.

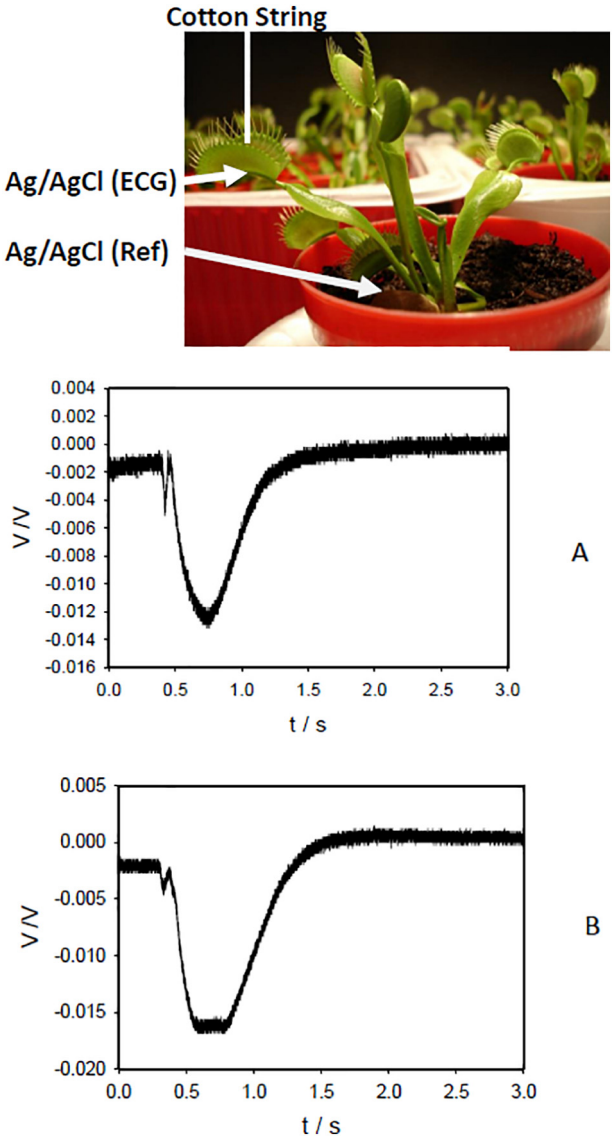


Fig. 2. Electrical signals in the Venus flytrap (A, B) were induced by mechanical stimulation using a cotton soft string for gentle touching of trigger hairs inside the trap of the *D. muscipula* when the trap was open (A) or during additional mechanical stimulation a few seconds after closing it (B). The tip of the cotton thread was between lobes after closing the trap. McKesson ECG Tab electrode with a conductive film and Ag/AgCl sensing system was attached to the midrib. Reference Ag/AgCl wire electrode was in the soil. Measurements were performed at 50,000 scans/s with low pass filter at 25,000 scans/s.

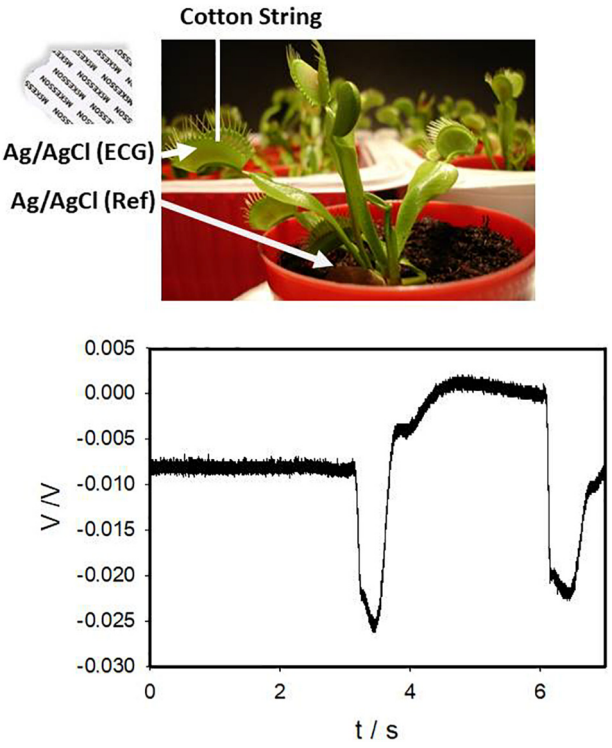


Fig. 3. Electrical signals in the Venus flytrap were induced by mechanical stimulation using a cotton soft string for gently touching of trigger hairs between lobes of the Venus flytrap when the trap was open. McKesson ECG Tab electrode with a conductive film and Ag/AgCl sensing system was attached to a lobe. Reference Ag/AgCl wire electrode was in soil. Measurements were performed at 50,000 scans/s with low pass filter at 25,000 scans/s.

The action potential can propagate over the entire length of the cell membrane and along the conductive bundles of tissue with constant amplitude, duration, and speed. Electrotonic potentials in plants exponentially decrease with distance. An intermediate place take graded potentials that involve the process of electrical excitation but do not evolve into full fledge action potential. A graded potential is a wave of electrical excitation that corresponds to the size of the stimulus. Electrical signals can propagate along the plasma membrane on short distances in plasmodesmata, and on long distances in a phloem.

Action potentials can propagate in the trap and do not penetrate to a petiole to protect other empty traps in the *Dionaea muscipula* from closing. Mechanism of this phenomenon is unknown. Electrostimulation of a petiole induces propagation of electrotonic potentials along a petiole to the trap [17]. Electrostimulation of the trap induces propagation of electrotonic potentials to a petiole [17]. It means that there is electrical conductivity between the trap and a petiole for electrotonic potentials but not for action potentials.

Action potentials in the Venus flytrap can be measured between electrodes 1 and 2 inserted to the midrib and lobes (Schema 2). External electrodes 3 and 4 can measure sum action potentials and passive electrical signals outside the trap. Results of such measurements can give results with different amplitude and duration of electrical signals due to additional passive cables with different RC constants. Due to additional RC circuits and constants in a petiole, root, soil, or between an ECG electrode and a surface of a lobe, the duration of electrical signals can increase and amplitude can decrease (Figs. 2, 3). Not all electric responses in plants are actions potentials; some of them could be electrotonic potentials, others arise from the disturbance to the electric circuits between electrodes and bio tissue.

Electrical signals can propagate to adjacent excitable cells due to the electrical coupling between plant cells and plasmodesmata, which is the major path for cell-to-cell electrical coupling [17,18,58]. The arrangement of electrodes in plant tissue is very important for the understanding of plant electrical circuit organization and communication in the electrical networks of plants. Electrical circuits in the roots and at the root/soil interface are very complicated and many authors proposed different active and passive equivalent electrical schemes [18,54,60,61]. Different arrangement of electrodes in lobes, midrib, petiole, roots and soil gives possibilities to study electrochemical circuits in electrical networks of the Venus flytrap.

Despite the vast amounts of accumulated information concerning the electrical effects in the plants, the organization and structure of their electrical networks remain poorly understood. These reasons provide significant support for the importance of further profound investigations of electrochemical circuits in the plant kingdom. The knowledge gained from studying electrically controlled morphing structures in plants are key input for designing adaptive structures and intelligent materials. History shows that engineers can learn a great deal from studying electrical, mechanical and biochemical properties of phytomaterials [59]. Time-dependent maps of electrical activities in

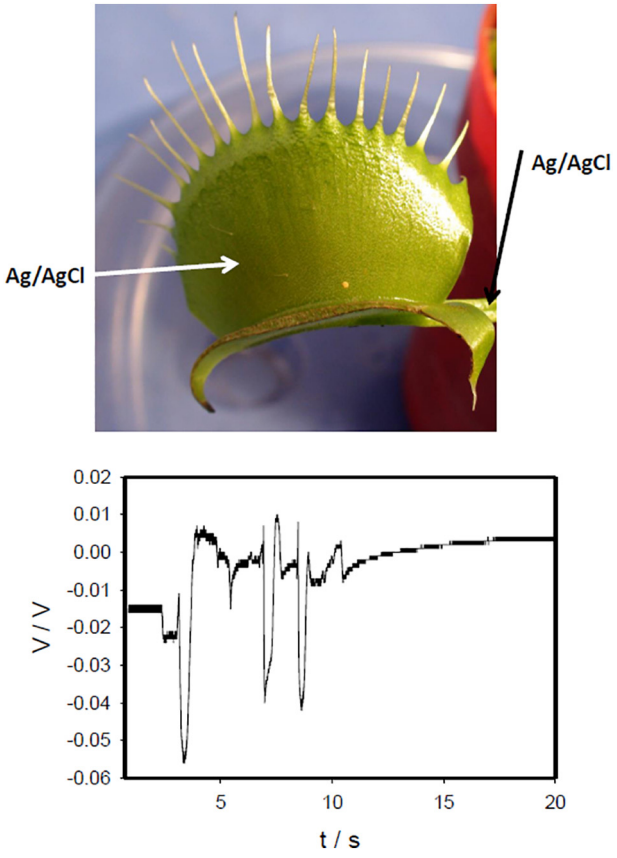


Fig. 4. Electrical signals in a single lobe of the Venus flytrap induced by a mechanical stimulation using a cotton soft string for gentle touching of trigger hairs. Measurements were performed at 50,000 scans/s with low pass filter at 25,000 scans/s.

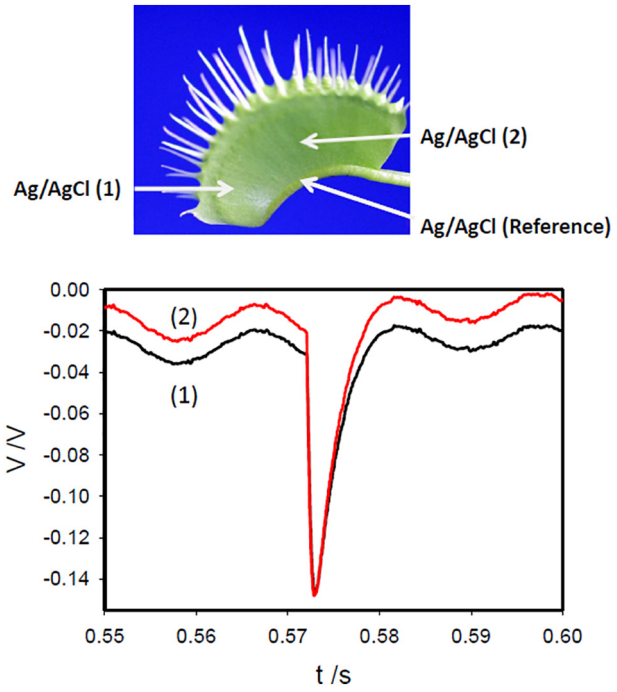


Fig. 5. The potential difference between Ag/AgCl wire electrodes inserted to a lobe and midrib after deposition of 10 μL drop of 0.01 M HNO_3 to a midrib of the Venus flytrap. The distance between electrode 1 and reference electrode was 6 mm, between electrode 2 and the reference electrode was 4 mm. Measurements were performed at 100,000 scans/s with low pass filter at 50,000 scans/s.

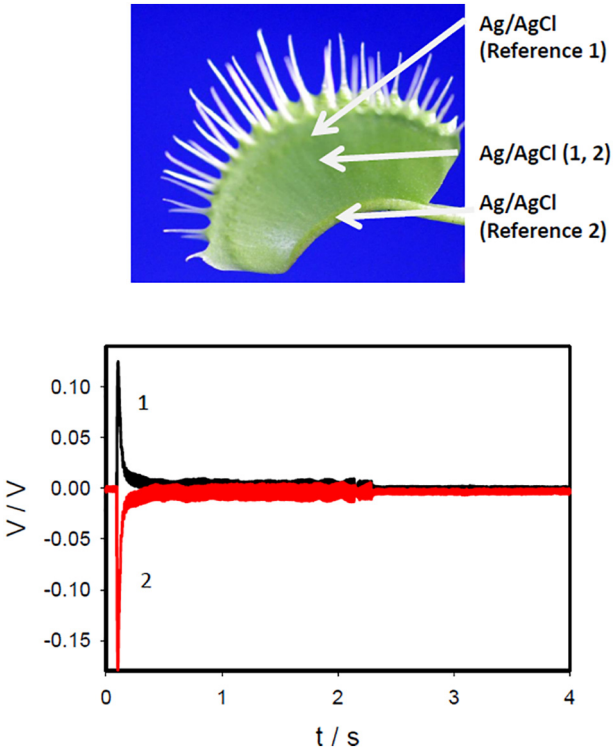


Fig. 6. The potential difference between Ag/AgCl electrodes inserted to a lobe and midrib after deposition of 10 μL drop of 0.01 M HNO_3 to a midrib of the Venus flytrap. Measurements were performed at 100,000 scans/s with low pass filter at 50,000 scans/s.

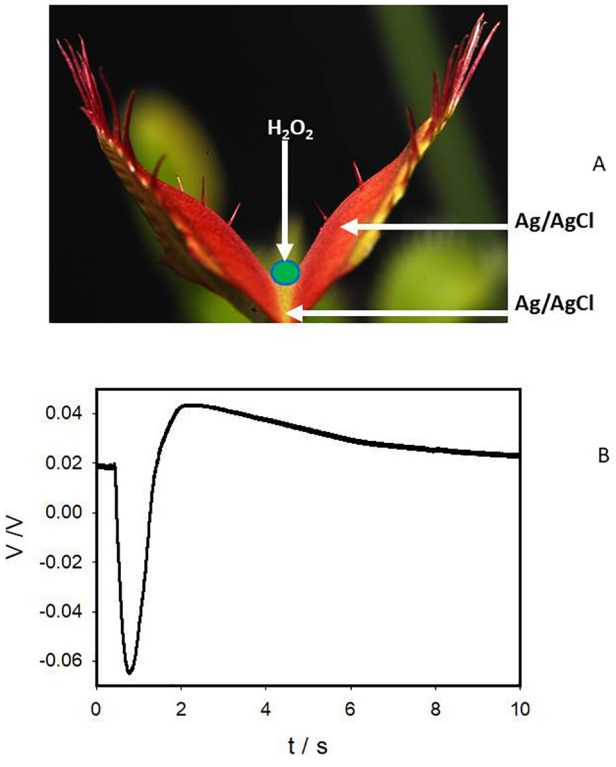
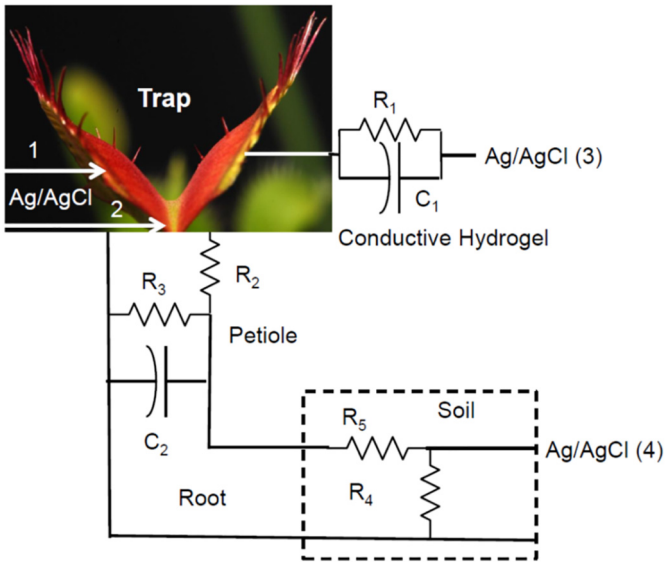


Fig. 7. The potential difference between Ag/AgCl electrodes inserted to a lobe and midrib after deposition of 10 μL drop of 3% H_2O_2 to a midrib of the Venus flytrap. Measurements were performed at 100,000 scans/s with low pass filter at 50,000 scans/s.



Schema 2. Passive electrical circuits between Ag/AgCl electrodes located outside the trap in a lower leaf, in soil or on the surface of a lobe covered by a conductive gel.

plants will give a key to understanding reversible morphing processes in plants and mechanisms of electrical management of these phytomaterials. These results will lead to the enhancement and improvements of nano-scale bioinspired technology including new electronic/ionic devices and artificial materials with applications in modern engineering and information technology.

5. Conclusions

We have measured potential differences between Ag/AgCl electrodes inserted to the trap, petiole, and into soil or external ECG electrodes attached to surfaces of the Venus flytrap. Diverse types of electrodes with various positions in plant tissue or soil show different amplitude and duration of electrical signals because potentials are measured in different electrochemical circuits. We found that action potentials propagate with speed up to 10 m/s in the trap of *D. muscipula* using direct measurements with multi-electrode data acquisition at high speed of scanning (up to 100,000 measurements per second). We have observed that action potentials in the trap induce electrotonic potentials in the petiole and amplitude and duration of signals obtained by extracellular or external electrodes can be different.

Disclosure of potential conflicts of interest

The author declares no competing financial interest.

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