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Charge induced closing of *Dionaea muscipula* Ellis trap ☆

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ABSTRACT

In terms of bioelectrochemistry, Venus flytrap responses can be considered in three stages: stimulus perception, electrical signal transmission, and induction of mechanical and biochemical responses. When an insect touches the trigger hairs, these mechanosensors generate receptor potentials, which induce solitary waves activating the motor cells. We found that the electrical charge injected between a midrib and a lobe closes the Venus flytrap leaf by activating motor cells without mechanical stimulation of trigger hairs. The mean electrical charge required for the closure of the Venus flytrap leaf is 13.6 μC. To close the trap, electrical charge can be submitted as a single charge or applied cumulatively by small portions during a short period of time. Ion channel blocker such as Zn^{2+} as well as an uncoupler CCCP, dramatically decreases the speed of the trap closing a few hours after treatment of the soil. This effect is reversible. After soil washing by distilled water, the closing time of Venus flytrap treated by CCCP or $ZnCl_2$ decreases back from 2–5 s to 0.3 s, but higher electrical charge is needed for trap closure. The mechanism behind closing the upper leaf of Venus flytrap is discussed.

1. Introduction

Electrical potentials have been measured at the tissue and whole plant level [1–7]. At the cellular level, electrical potentials exist across membranes, and thus between cellular and specific compartments. Anions, K^+, Ca^{2+}, and H^+ are actively involved in the establishment and modulation of electrical potentials [3–5].

Plants can sense mechanical stimuli. This process involves mechanosensitive channels that were found in all types of cells from animal and plant cells to fungi and bacteria. The omnipresence of these channels underlines their important physiological function in the regulation of osmolarity, cell volume and growth. These channels are ideal transducers of physiologically relevant mechanical forces. Mechanosensory ion channels (MSC) in plants are activated by mechanical stress and then transduce this information into electrical signals. These channels are involved in the growth, development, and response to environmental stress in higher plants. Detailed analyses of the electrophysiology in higher plants are difficult because such plants are composed of complex tissues. Plant response to mechanical stimulation has long been known [8–15]. Perhaps all plants react in response to the mechanical stimuli, but only certain plants with rapid and highly noticeable touch-stimulus response have received much attention, such as the trap closure of Venus flytrap — *Dionaea muscipula* [16–18]. The small plant consists of 5–7 leaves of which a leaf is divided into two parts: upper part of the leaf has a pair of trapezoidal-shaped lobes held together by a blade (midrib). The center of each lobe contains three or more trigger hairs (sensitive hairs) with a red anthocynanin pigment that attracts insects. The edge of each lobe is engulfed with hair-like projections (cilia). Lower part of the leaf, sometimes referred to as the footstalk, has an expanded leaf-like structure. Each leaf reaches a maximum size of 3–7 cm.

Touching trigger hairs protruding from the upper leaf epidermis of Venus flytrap activates mechanosensitive ion channels and generates receptor potentials, which can induce action potentials [11–14,19,20]. Receptor potentials always precede the action potentials and couple the mechanical stimulation step to the action potential step of the preying sequence.

The cilia protruding from the edges of both lobes form an interlocking wall when the trap is shut, impenetrable to all except the smallest prey. The trap shuts when a prey touches trigger hairs arranged in a triangular pattern three to a lobe. Partial closure occurs so that the spines overlap, but the lobes are still held slightly ajar. This is normally accomplished in only a fraction of a second, but it may take several minutes for the lobes to come fully together. If an insect is successfully caught, the lobes seal tightly and remain so for about 5–7 days while digestion occurs.

The mechanism by which Venus flytrap snaps is not clearly understood and numerous conflicting models have been proposed.

Darwin [21] was the first to observe that the lobes of traps are convex when held open and concave when held shut. Brown [8] noted that the area of the underside of the lobes expands during closure, whereas the area of the inner sides of the lobes increases upon reopening. This model helps explain the flipping action “of the most

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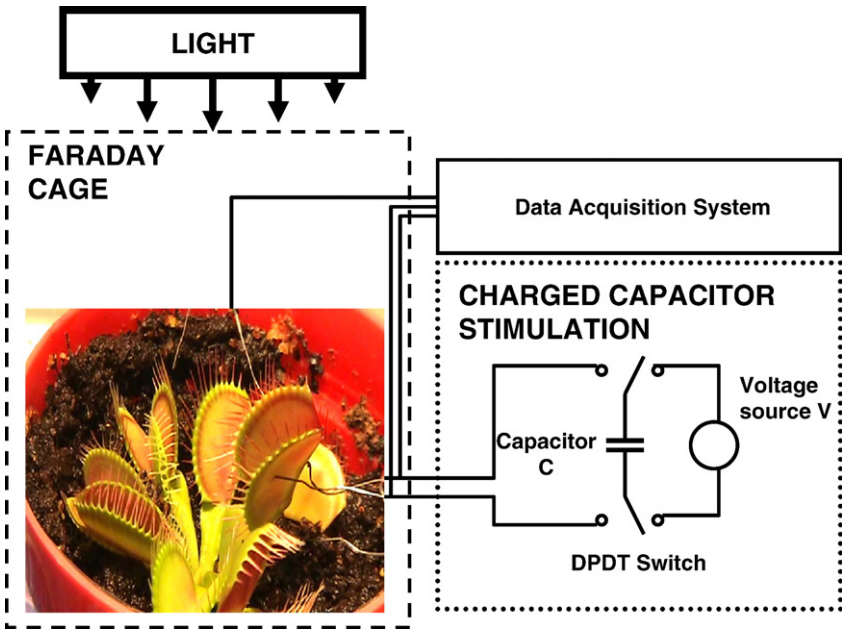

Fig. 1. Experimental setup.

wonderful plant" mentioned by Darwin [21]. By painting the surface with dots, Darwin [21] was able to prove that during the process of closing, the superficial layer of cells of the leaf contracted over the whole upper surface. Forterre et al. [22] suggested that the leaf's geometry plays a crucial role in a buckling instability and considered Venus flytrap as a bistable vibrator, which can open and close simultaneously.

The rapid trap closure of *Dionaea muscipula* Ellis has been explained by either a loss of turgor pressure of the upper epidermis or by a sudden acid-induced wall loosening of the motor cells. According to [18] experiments, both explanations are doubtful.

The traps probably do not move using only a rapid decrease in turgor because the changes in cell length have been observed to be irreversible. Several recent articles have linked trap closure with a rapid decrease in pH; traps have been shown to close when immersed in solutions with pH of 4.5 and below [23]. The low pH must activate the enzymes that expand lobe cell walls. Leaves infiltrated with neutral buffers that keep pH above 4.5 do not close in response to stimulation of their trigger hairs even though action potentials are generated. ATP is used by the motor cells for a fast transport of protons.

2. Materials and methods

2.1. Data acquisition

All measurements were conducted in the laboratory at constant room temperature 22 °C inside a Faraday cage mounted on a vibration-stabilized table. In order to estimate possible high frequency content of the responses evoked, a high performance *National Instruments* data acquisition system was used. High speed data acquisition of low-pass filtered signals was performed using microcomputers with simultaneous multifunction I/O plug-in data acquisition board NI-PXI-6115 or NI-PCI-6115 (*National Instruments*) interfaced through a NI SCB-68 shielded connector block to 0.1 mm thick nonpolarizable reversible Ag/AgCl electrodes (Fig. 1). The results were reproduced on a workstation with data acquisition board NI 6052E DAQ with input impedance of 100 GΩ interfaced through a NI SC-2040 Simultaneous Sample and Hold. The system integrates standard low-pass anti-aliasing filters at one half of the sampling frequency. The multifunction NI-PXI-6115 data acquisition board provides high resolution and a wide gain range. Any single channel can be sampled at any gain at up to 10 MSamples/s.

2.2. Electrodes

Ag/AgCl electrodes were prepared from Teflon coated silver wires (*World Precision Instruments*) with the diameter of 0.1 mm [13]. Following insertion of the electrodes into lobes and a midrib, the traps closed. We allowed plants to rest until the traps were completely open.

2.3. Plant electrostimulation

The Charge Injection Method (Fig. 1) has been used to precisely estimate the amount of electrical energy necessary to cause the closing of the leaves. Two critical parameters have been analyzed: *the amount of charge* and the *applied voltage*. Both parameters are tested

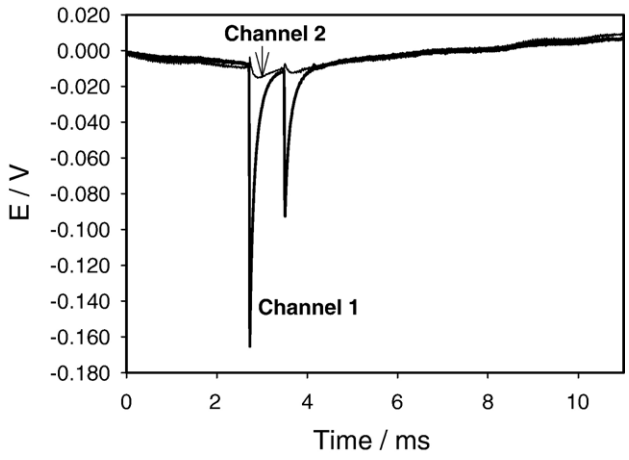

Fig. 2. Electrical signaling in Venus Flytrap induced by a peace of gelatin stimulating one trigger hair on each lobe. One Ag/AgCl electrode was located in the midrib and another Ag/AgCl electrode (channel 1) in the center of lobe and two Ag/AgCl electrodes (channel 2) were located in a lower leaf on the distance 1 cm between them. Channel 1 shows solitary waves between a lobe and the midrib, channel 2 shows electrical spikes in the lower part of the leaf (footstalk). The frequency of scanning was 250,000 samples per second.

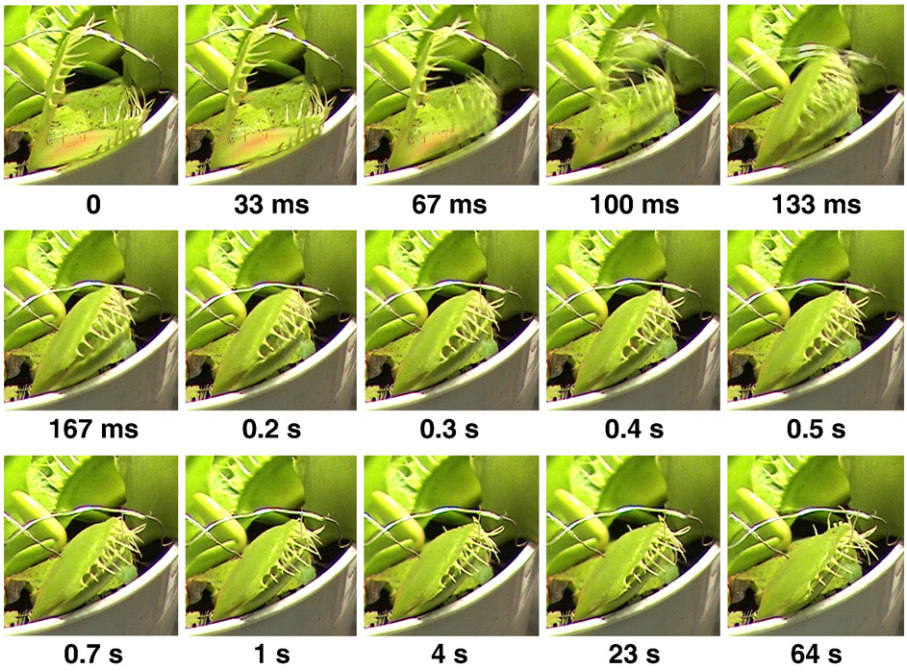

Fig. 3. Sequence of Venus flytrap photos during electrical stimulation (14 μC, 1.5 V) using two Ag/AgCl electrodes located in a midrib (+) and in one of the lobes (−).

to determine the minimum amount of charge and the minimum voltage sufficient to close the plant's trap. A double pole, double throw (DPDT) switch was used to connect the known capacitor to the voltage source during charging, and then to the plant during plant stimulation. Since the charge of capacitor Q connected to the voltage source V is $Q=CV$, we can precisely regulate the amount of charge using different capacitors and applying various voltages. By changing switch position, we can instantaneously connect the charged capacitor to the plant and induce an evoked response.

2.4. Images

Digital video camera recorders Sony DCR-HC36 and Canon ZR300 were used for the monitoring of Venus flytraps and to collect digital images, which were analyzed frame by frame.

2.5. Chemicals

Carbonyl cyanide 3-chlorophenylhydrazone (CCCP), gelatin, and $ZnCl_2$ were obtained from Fluka (New York, NY).

2.6. Plants

Two hundred bulbs of *Dionaea muscipula* (Venus flytrap) were purchased for this experimental work from Fly-Trap Farm (*Supply, North Carolina*) and grown in a well drained peat moss in plastic pots at 22 °C with a 12:12 h light:dark photoperiod. The soil was treated with distilled water. All experiments were performed on healthy adult specimens. Plants were fed a 6 mm×6 mm×2 mm cube of 4% (w/v) gelatin and induced to close by stimulating 2 of the 3 trigger hairs.

3. Results

We created a charge injection method, shown in Fig. 1. This method has been used to precisely estimate the amount of electrical charge necessary to cause the closing of the leaves.

Venus flytrap can be closed by mechanical stimulation of trigger hairs using a cotton thread or a small piece of gelatin.

We generated an electrical response by mechanically simulating the trigger hairs of Venus flytrap using a small piece of gelatin. Electrical signaling resembling an action potential propagates from the mechanosensitive trigger hairs in the upper part of the leaf from a lobe to a midrib as presented in Fig. 2. Action potentials between electrodes located in the footstalk have not been found (Fig. 2, channel 2). This indicates that fast electrical signaling is limited to the upper part of the leaf.

Venus flytrap was successfully closed when we applied an electrical pulse between the midrib (positive potential) and a lobe of the upper leaf (negative potential), without mechanical stimulation. Fig. 3 demonstrates the closing of the Venus flytrap in 0.3 s after electrical stimulation. Closing of Venus flytrap by electrical stimulation of motor cells is characterized by a slow initial phase, a rapid intermediate and slow final phases exactly depicted after mechanical stimulation of Venus flytrap (Fig. 4).

The inverted polarity pulse with negative voltage applied to the midrib was not able to close the plant, and we were not able to open the plant by applying the same voltage range and polarity for pulses up to 100 s.

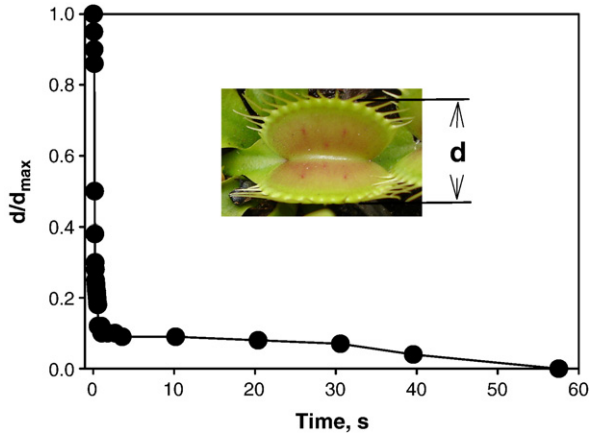

Fig. 4. Time dependence of the trap closing after electrical stimulation (14 μC, 1.5 V) using two Ag/AgCl electrodes located in a midrib (+) and in one of the lobes (−).

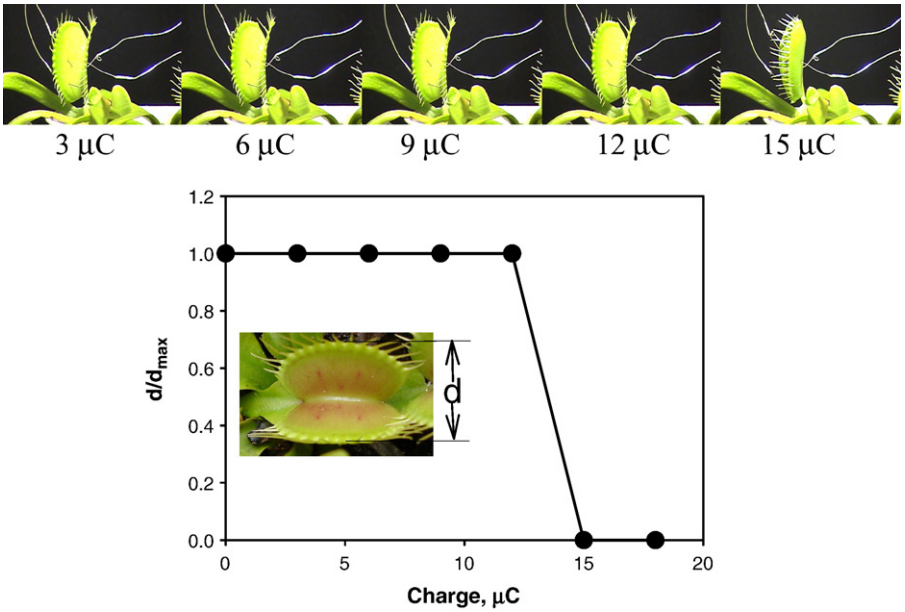

Fig. 5. Dependence of the distance between rims of lobes on injected charge using two Ag/AgCl electrodes located in a midrib (+) and in one of the lobes (−) and 3 μC charge was injected to the same plant every 7 s. Capacitor was charged 1 s from 1.5 V battery.

Transmission of a single electrical charge (mean 13.63 μC, median 14.00 μC, std. dev. 1.51 μC, $n=41$) causes closure of a trap and induces an electrical signal propagating between the lobes and the midrib. Fig. 5 illustrates that Venus flytrap can accumulate small charges and as soon as threshold value of accumulated charge is submitted, trap closes in 0.3 s. Repeated application of smaller charges demonstrates a summation of stimuli. If we apply two or more injections of electrical charges within a period of less then 20 s, the Venus flytrap upper leaf closes as soon as a total of 14 μC charge is transmitted.

Brown [8] indicated that electrical shock between lower and upper leaves can cause the Venus flytrap to close, however, the amplitude and polarity of applied voltage, charge, and electrical current were not reported. The trap did not close when we applied the same electrostimulation between the upper and lower leaves as we applied between a midrib and a lobe, even when the injected charge was increased from 14 μC to 1 mC. It is probable that the electroshock induced by Brown and Sharp [9] had a very high voltage or electrical current.

Fig. 6 shows that blocker of ion channel Zn^{2+} inhibit the closing process of a trap stimulated mechanically by a piece of gelatin (Fig. 6A) or by electrical charge injection (Fig. 6B). In the case of mechanostimulation, Zn^{2+} can block propagation of electrical signals and closing the trap. In the case of electrostimulation, Zn^{2+} directly inhibits closing the trap.

Ion channel blocker such as Zn^{2+}, as well as uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP), dramatically decreases the

Fig. 6. Sequence of Venus flytrap photos after stimulation of trigger hairs by a small piece of a gelatin (A) or by 14 μC electrical stimulation (B). 50 mL of 10 mM $ZnCl_2$ was added to soil 4.5 h before experiments.

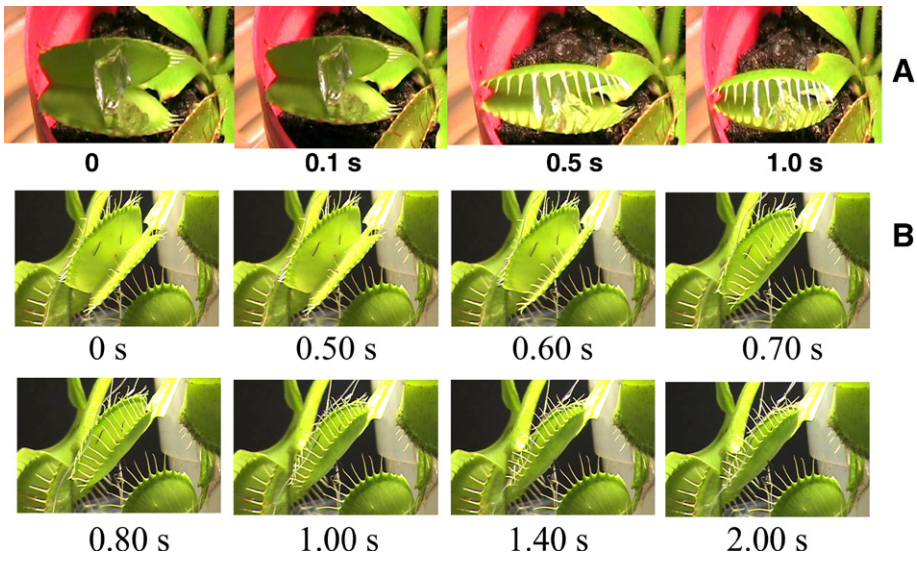

Fig. 7. Sequence of Venus flytrap photos after stimulation of trigger hairs by a small piece of a gelatin (A) or 70 μC electrical stimulation (B). 50 mL of 10 μM CCCP was added to soil 4.5 h before experiments.

speed of the trap closing a few hours after treatment of the soil. This effect is partially reversible. After soil washing by distilled water every day, the closing time of Venus flytrap treated by CCCP or $ZnCl_2$ decreases back to 0.3 s, but higher electrical charge is needed for trap closure.

CCCP, which are soluble in both water and lipids, permeate the lipid phase of a membrane by diffusion and transfer protons across the membrane, thus eliminating a proton concentration gradient and/or a membrane potential. Figs. 7 and 8 demonstrate the inhibitory effect of uncoupler CCCP on trap closure. It is known that uncouplers decrease and inhibit the action potential induced by mechanical stimulation (Fig. 7A), but we found also that uncoupler CCCP inhibits closing of Venus flytrap by electrical stimulation even with electrical charge injected to the midrib (Fig. 7B). Increasing the electrical charge from 14 μC to 70 μC does not accelerate the process of the trap closing. Inhibitory effects of ion channel blocker Zn^{2+} and uncoupler CCCP can be decreased by washing soil with distilled water (Fig. 8). Hodick and Sievers [17] reported an excitability inhibition of a *Dionaea* leaf mesophyll cells using uncoupler 2,4-dinitrophenol. Resting potential and excitability are completely restored after 30 min of washing a standard medium. This explains the results shown in Fig. 7A involving the inhibition of mechanically induced trap closure. Inhibition of electrically induced trap closure in the presence of protonophores (Fig. 7A), when electrical charge is submitted to a midrib, can be caused by depolarization of a membrane or dissipation of a proton gradient during ATP hydrolysis in the midrib.

4. Discussion

It is known from literature that the amplitude of action and resting potentials in the Venus flytrap depends on the concentration of K^+ and Ca^{2+} cations [16,19]. EGTA, $LaCl_3$ [17], ruthenium red, neomycin and anion channel inhibitor antrhracene-9-carboxylic acid [24], which inhibit the excitability of Venus flytrap, indicating that the calcium permeable anion channels and probably potassium channels are responsible for the propagation of action potentials.

Upon perception, electrical signals can be propagated via plasmodesmata to other cells of the symplast. As a first step, the plasma membrane is depolarized, a process known as formation of the receptor potential. The receptor potential is an electrical replica of the stimulus lasting for the period of time that the stimulus is present. An action potential is evoked when the stimulus is strong enough to depolarize the membrane. Subsequently, the action potential characterized by a large transient depolarization allows the rapid transmission of information via plasmodesmata. An action potential usually has an all-or-nothing character, and it travels with constant velocity and magnitude. Electrical coupling via plasmodesmata was

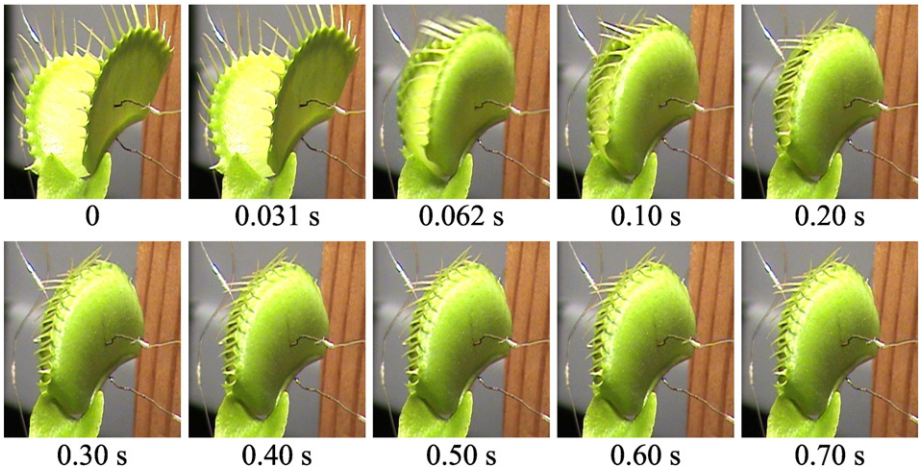

Fig. 8. Sequence of Venus flytrap photos after 28 μC electrical stimulation. 50 mL of 10 μM CCCP was added to soil 72 h before experiments. Soil around Venus flytrap was washed 24 h before experiment by distilled water to decrease CCCP concentration.

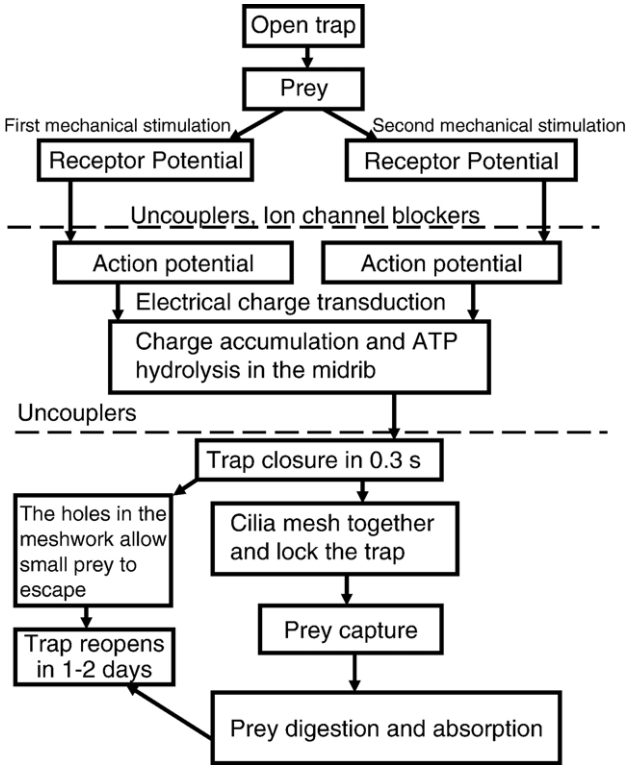

Fig. 9. How Venus flytrap snaps.

demonstrated in a variety of species such as *Nitella*, *Elodea* and *Avena* and *Lupinus*, indicating that plasmodesmata are relays in the signaling network between cells.

The upper leaf in the open position is convex and concave during closing of the trap. Forterre et al. [22] suggested that the leaves geometry plays a crucial role in a buckling instability and considered Venus flytrap as a bistable vibrator, which can be in open or closed states. This model contradicts with experimental facts: (a) the trap is stable and does not close spontaneously without stimuli; (b) two mechanical stimuli in interval of up to 35 s are required for the closing of the trap; (c) the trap does not close during rain or after blasts of air; (d) opening of the trap is a slow process and lobes change their shapes from flat to concave and finally to convex; e) Forterre et al. [22] observed no ringing in the process of closing; f) closing of the trap requires ATP hydrolysis in the midrib [25]. Opening of the trap is a slow process and lobes change their shapes from flat to concave and finally to convex shape. Forterre et al. [22] suggested that the measured speed at which the leaves closed depended on a dimensionless geometric parameter

$$\alpha = \frac{L^4 K^2}{h^2} \quad (1)$$

and the characteristic time for the trap movement

$$\tau \sim \mu L^2 / \kappa E$$

where L is the size of leaf, K its mean curvature, μ is viscosity in the porous plant tissue with κ hydraulic permeability, E is the elastic module of the tissue and h the thickness of the leaf. Our experiments on 200 Venus flytrap plants with different sizes of leave ranging from 1 cm to 5 cm do not show dependence of the closing time on size of the leaf L. This contradicts with Eq. (1) [22], which predicts a dramatic increase in the closing time for large Venus flytrap plants.

In terms of electrophysiology, Venus flytrap responses can be considered in three stages: (i) stimulus perception, (ii) signal transmission and (iii) induction of response (Fig. 9). In Venus Fly the first stage is due to the receptor potential, a transient depolarization with a critical threshold that triggers action potentials, which are responsible for stages (ii) and (iii). Receptor potentials are generated by MS ion channels. Action potentials involve a transient influx of Ca^{2+} to the cytoplasm, effluxes of K^+ and Cl^- and a temporary decrease of turgor pressure. Like the action potential, a critical threshold depolarization triggers Ca^{2+} influx, opening of Ca^{2+}-sensitive Cl^- channels and K^+ channels; effluxes that last over an hour and result in turgor regulation. However, since higher plants are composed of complex tissues, detailed analysis of electrical phenomena is rather difficult, and so the mechanism for generating the receptor potential has not yet been established.

Energy for trap closure is generated by ATP [23,25]. The amount of ATP drops from 950 μM per midrib before mechanical stimulation to 650 μM per midrib after stimulation and closure [25]. Electrical charge stimulation triggers closing process in the motor cells.

Acknowledgements

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