Firing and Resetting Characteristics of Carnivorous *Utricularia reflexa* Traps: Physiological or only Physical Regulation of Trap Triggering?

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Received December 12, 2011

Accepted May 29, 2012

Key words: Aquatic carnivorous plant, Lentibulariaceae, *Utricularia reflexa*, trap firing and resetting, water flow, NaN₃, ether, low temperature, mechanical stimulation.

Summary


Firing and resetting of traps in aquatic *Utricularia* (*Lentibulariaceae*) species are associated with water flow and trap volume changes. Using an electronic position sensor, trap thickness as a measure of water flow was monitored in large *Utricularia reflexa* traps and the effects of a narcotic (diethyl ether), an aerobic respiration inhibitor (NaN₃), and low temperature (2 °C) on trap firing and resetting were investigated. Ether, known to inhibit membrane ion channels in animals, gradually and significantly decreased the trap resetting rates, while the mechanical trap stimulation to fire was not influenced. NaN₃ (0.5 mM) added to partly reset traps did not influence the magnitude of the next firing but very markedly reduced the resetting rates to ca. 30 % of the controls. Reset traps refrigerated at 2 °C could normally fire but their resetting rates were only around 6–9 % of the controls at room temperature. Due to such low resetting rates, another firing was not possible. Wet traps kept in humid air were able to normally fire and reset once with a small air bubble inside, but after the 2nd firing, they contained ca. 40–50 % of air and their resetting rates were significantly lower. Generally, when all these treatments allow the traps to reset the subsequent firing of reset traps is not inhibited. Our data indirectly support the

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purely physical (mechanical) concept on the regulation of *Utricularia* trap triggering as all treatments, which should inhibit the hypothetic electrophysiological signalling pathway from trigger hairs down to the trap door, led to normal trap stimulation and firing.

**Zusammenfassung**


**Introduction**

The rootless carnivorous genus *Utricularia* L. (Lentibulariaceae) contains around 50 species of aquatic or amphibious plants which usually grow in standing, nutrient-poor humic waters (TAYLOR 1989, GISANDE & al. 2007). The plants are able to capture small animal prey, typically fine zooplankton, by their traps of foliar origin (e.g., HARMS 1999, RICHARDS 2001, GORDON & PACHECO 2007) and to utilise mineral nutrients (N, P and K) from prey carcasses (FRIDAY & QUARMBY 1994, ADAMEC 1997, 2008).
These discoid traps are hollow bladders, usually 1–5 mm long with a wall thickness of two cells, and are filled with trap fluid. They contain a variety of glands and trichomes on both the inner and outer surfaces, the function of which is still partially unresolved (Sydenham & Findlay 1973, 1975, Sasago & Sibaoka 1985a,b, Juniper & al. 1989). In a set state, when the trap is prepared for firing, a negative pressure of ≈16 kPa relative to the ambient water is maintained inside the trap (Sydenham & Findlay 1973, Sasago & Sibaoka 1985a, Singh & al. 2011). When trigger (sensory) hairs situated on the trap door are touched by a prey species the door opens, the prey is aspirated into the trap lumen and the watertight door closes again. A high-speed camera reveals that this process is complete within 5 ms and is caused by the reversible buckling/unbuckling of the door associated with a convex/concave door inversion (Joeyux & al. 2011, Singh & al. 2011, Vincent & Marmottant 2011, Vincent & al. 2011a,b). The negative pressure is restored by removal of ca. 40% of the water from the trap lumen within 25–30 min, after which the trap is ready to fire again (Sydenham & Findlay 1973, 1975, Sasago & Sibaoka 1985a). However, the complete process of trap resetting (water pumping) lasts at least 6–10 h (Adamec 2011a,b). In studies of water pumping from Utricularia traps (Sydenham & Findlay 1973, 1975, Sasago & Sibaoka 1985a,b), the trap thickness (i.e., the distance between two parallel walls) as an easily and accurately measured parameter, correlated closely with the magnitude of the negative pressure during trap resetting. Yet the stable negative pressure inside the traps was reached in only 30 min after firing, while trap thickness decreased and water removal continued for several hours (Sydenham & Findlay 1973, 1975, Sasago & Sibaoka 1985a). On the basis of a possible regulatory role of the negative pressure for water pumping, Sasago & Sibaoka 1985a suggested the concept of a physiological negative pressure sensor in the traps. However, Adamec 2011b has recently found a strict linear resetting rate of trap thickness changes, without any lag-period, during the first 3 min after firing in U. reflexa traps and has suggested that water is pumped out of the trap continuously and probably recirculates through some leaks in the reset state.

Moreover, the classic concept that Utricularia traps fire only after mechanical stimulation has recently been overturned by the finding that the traps – both cut off and intact – can also fire spontaneously in the course of time, i.e., without any mechanical stimulation (Adamec 2011a,b, Vincent & Marmottant 2011, Vincent & al. 2011a,b). There was no quantitative difference between spontaneous and mechanically stimulated firings and subsequent resetting rates (Adamec 2011a,b). In response, Vincent & al. 2011b suggest that spontaneous firing occurs when the internal negative pressure equals a critical value for the buckling of the trap door. Yet the crucial question on the trap functioning remains unanswered: are Utricularia traps stimulated to fire via an electrophysiological signalling
pathway (including a rise in action potential in the trigger hairs) or purely mechanically, by the force acting on the trigger hairs as a lever (see Sydenham & Findlay 1973, Adamec 2011c, Vincent & al. 2011a,b)? As reviewed e.g. by Juniper & al. 1989 all other genera of carnivorous plants with rapid trap movements exhibit the electrophysiological way of regulation. However, the integration of all the above recent findings on Utricularia trap functioning indirectly supports the mechanical concept of trap triggering.

Due to demanding physiological functions (water pumping, enzyme secretion, nutrient uptake), energy consumption of traps and their aerobic respiration rate are very high, yet sufficient to cover these functions in isolated traps for at least 24 h (Sasago & Sibaoka 1985b, Adamec 2006, 2007, 2011a,b). Dark respiration of all inner trap structures runs in the (almost) anoxic trap fluid (Adamec 2007). This implies that the aerobic respiration of the bifid glands responsible for the water pumping under these conditions is extremely efficient (Laakonen & al. 2006). Application of inhibitors of aerobic respiration (1 mM: KCN, NaN3, dinitrophenol) to the trap fluid markedly decreased the resetting rate or water outflow, while their external application usually has a much lesser effect (Sasago & Sibaoka 1985b). The 1 mM solution of NaN3 applied externally to Utricularia sp. traps just after firing decreased the resetting rate by 40% but the application of the same solution or low temperatures (2 °C) to fully reset traps did not change the trap thickness for long time (Sydenham & Findlay 1975). Thus, once a trap is reset it can keep its status even under unfavourable conditions.

The aim of this study was to automatically monitor trap thickness as a measure of water flow in Utricularia reflexa traps and to investigate the effect of diethylether as a narcotic, NaN3 as a potent aerobic respiration inhibitor, and low temperature on the basic processes of trap functioning: trap firing and resetting. Thus, the aim was to use the indirect data to decide whether trap triggering is caused by the electrophysiological mechanism or purely mechanically. Moreover, quantitative data on trap functioning in humid air are also provided.

Materials and Methods

Adult plants of Utricularia reflexa Oliver (from Okavango Delta, Botswana) were grown indoors under natural light in a 3 l aquarium in tap water with a litter of robust Carex species used as a substrate (see Adamec 2007, 2011a,b). The water in this culture was considered oligotrophic, slightly humic, and neutral (pH ~7.0). Small zooplankton species were added weekly to the culture to promote plant growth. U. reflexa plants were convenient for this study as they have large traps (4–6 mm long) of a relatively homogeneous size and were used commonly in our previous studies (Adamec 2007, 2011a,b). In this study, greenish young traps, 5.0–6.0 mm long, from the 3rd–4th mature leaf node (denoted as ‘young’ in our previous papers; Adamec
2007, 2011a,b) as counted from leaf apex and without any captured macroscopic animal prey were used.

A freshly excised trap without any air bubbles at the initial stage of resetting (2–5 min) was carefully inserted into the holder of an electronic position sensor to monitor trap thickness changes as a measure of water flows (for all technical details, see Adamec 2011a). The position sensor had a 1 μm resolution and pressed against the laterally fixed trap by a force lesser than the weight of 0.4 g. Trap thickness was monitored at 30 s intervals and the chamber was partly covered by a perspex lid to reduce water evaporation and ether volatilization. Water temperature was relatively stable during the measurements; mean values for single types of experiments were within 21.5–24.0 °C, with the total temperature range <2 °C for each type of experiment. During the measurements, the temperature change was usually <1 °C and the traps were in natural dim daylight. When needed, with the aid of a loupe, the fixed traps were mechanically triggered to fire using a very fine narrow brush which gently touched the trigger hairs but the trap position in the holder remained unchanged (Adamec 2011a).

A fixed trap in the holder was submerged in a 10 ml perspex chamber in a solution composed of 0.1 mM KCl, 0.05 mM CaCl₂ and 0.2 mM NaHCO₃ of pH 7.3–7.4 (Adamec 2011a,b). Measurements of trap thickness were usually made according to the following schedule: 60 min after the insertion of a fired trap into the holder (resetting period), the trap was re-fired mechanically (denoted as 1st firing) and this step was repeated in total up to four times, each after a further 60 min. Thus, up to four sets of firing-resetting cycles were recorded for each trap. In the experiment on ether addition as a narcotic, the solution in the chamber was exchanged for the same solution with 4 % (v/v) diethylether within 20 s. Ether was added immediately after the 2nd firing or before it, without causing the fixed trap to fire. In another set of experiments, the solution with 0.5 mM NaN₃ was applied to the chamber 1 h after the 1st firing, i.e., in the reset state, but the 2nd firing occurred after another 1 h. Thus, at the time of the 2nd firing, only the external trap structures including trigger hairs were directly treated by NaN₃ for 1 h. To learn if wet traps occurring in humid air can normally fire and reset (analogy with resetting the traps in liquid paraffine, see Sydenham & Findlay 1975, Sasago & Sibaoka 1985a), a freshly excised fired trap was carefully blotted dry using a soft paper tissue and inserted into the holder without the water. Having no air bubbles inside, the trap was sufficiently wet so as not to dry out for at least 4 h. High air humidity was kept in the chamber during this experiment. As a result of the 1st and 2nd firing in the air, air bubbles of an increasing size were aspirated in the traps and, thus, any other exact trap thickness changes were excluded due to air expansion. To learn whether low temperature equally affects both the firing and resetting processes, a freshly
excised fired trap was inserted into the holder in a 25 ml glass chamber and was reset at a room temperature for 60 min. Afterwards, the chamber was cooled using a flow-through cryostat. After 15 min of cooling, the temperature in the chamber decreased to between 2.1–2.3 °C and remained stable. The trap was then mechanically fired so that trap resetting occurred at the low temperature. The temperature was measured by a miniature electronic thermometer.

The following parameters were evaluated from the measurements (after Adamec 2011a,b): change of trap thickness due to mechanically stimulated firings and initial resetting rate after firing over 10 min (expressed as trap thickness decrease between 1 min and 11 min) and over 30 min (0–30 min). Each type of experiment was repeated on 6–17 traps from different shoots. As the trap size (trap length and thickness) of the used material was rather homogeneous the data are not corrected for trap size (see Adamec 2011b) and are presented as such. Where possible, the data for the 1st firing-resetting cycle served as controls. The same data were also used as controls for the experiments on wet traps in air and at low temperature. Significant differences in the parameters between the controls and treatments were evaluated by 1-way ANOVA (Tukey HSD test for unequal n). Mean ± S.E. values are shown where possible. For better comparability we indicated mean and standard error of mean in all our experiments, although some of them had less than eight samples.

**Results**

The addition of ether to the working solution (4 % v/v) as a narcotic following the 2nd firing did not cause a statistically significant decline of the immediate trap resetting rates but both the magnitude of trap thickness changes within the 3rd and 4th firing and the subsequent resetting rates were significantly lower (P < 0.01) than the controls (Table 1). The effect graduated in the course of time. A very similar effect was attained after the ether solution had been applied immediately before the 2nd firing with the exception that even the 30-min resetting rate following the 2nd firing was significantly decreased at P < 0.05. NaN₃ added to partly reset traps 1 h before the 2nd firing did not influence the magnitude of the 2nd firing (326 ± 28 μm) but very markedly reduced both resetting rates to ca. 30 % of the controls (Table 1). Moreover, these traps were able to be stimulated mechanically to the 3rd firing but its magnitude was greatly reduced (data not shown). Wet traps kept in humid air were able to normally fire and reset once with a small air bubble inside (ca. 20–30 % of the trap volume), but after the 2nd firing, they contained ca. 40–50 % of air and their resetting rates were significantly lower. Usually, they could not be stimulated to fire within the 3rd firing cycle (data not shown). Reset traps refrigerated at 2 °C could normally fire but their resetting rates were only
around 6–9 % of the controls at room temperature. Due to such low resetting rates, another firing was not possible. However, when the traps were transferred to room temperature again they could be stimulated mechanically to fire (data not shown). Generally, the results show that when all these treatments allow the traps to reset, the subsequent firing of reset traps is not inhibited.

Discussion

A set of experiments was conducted in this study to specify how Utricularia trap firing is regulated. Diethylether as a well-known medicinal narcotic (anaesthetic) was used to test Utricularia trap functioning. It is accepted that it inhibits neuronal receptors and membrane ion (Na⁺, K⁺, Ca²⁺) channels in humans or animals (Campagne & al. 2003). Ether applied either after trap firing or before it gradually inhibited resetting rates to around 35–55 % of the controls and, consequently, trap thickness changes due to firing also significantly decreased (Table 1). If 4 % ether solution was able to markedly reduce the water pumping rate from the trap, it should theoretically inhibit also the electrophysiological processes which could underlie trap stimulation. However, the traps were normally stimulated to fire even in the presence of ether. In line with literature data (Sydenham & Findlay 1975, Sasago & Sibaoka 1985b), 0.5 mM NaN₃ added externally to U. reflexa traps 1 h before the 2nd firing severely decreased (to ca. 30 %) both resetting rates but the firing itself ran normally and was unchanged (Table 1). In contrast, Sydenham & Findlay 1973 reported that Utricularia traps treated externally with 1 mM NaN₃ or old traps could not be stimulated mechanically by a hair to fire. Very low above zero temperature is considered to inhibit processes requiring the metabolic ATP energy (e.g., Sydenham & Findlay 1975). Though low temperature drastically decreased both resetting rates in U. reflexa traps after firing, the processes of stimulation to fire and firing itself were unchanged.

Our data on the Utricularia trap functioning indirectly support the purely mechanical concept on the regulation of trap triggering (Joeyux & al. 2011, Vincent & al. 2011a,b) as all treatments which should inhibit the hypothetic electrophysiological signalling pathway from trigger hairs down to the trap door, led to normal trap stimulation and firing. This view is also directly supported by Sydenham & Findlay 1973 who were not able to stimulate Utricularia sp. traps to fire by externally or internally applied electrical current. Older results by Diannelidis & Umbrath 1953 using a high-voltage stimulation from an inductive coil may be considered ambiguous and confused. If the mechanical concept seems to be valid on the basis of all recent data, it is the magnitude of the negative pressure inside the trap (relative to the external medium) that has the central regulatory role for trap firing (Joeyux & al. 2011, Singh & al. 2011, Vincent & Mar-
MOTTANT 2011, VINCENT & al. 2011a,b). Negative pressure inside the trap is then an indispensable integral component part of each trap stimulation to fire and a pre-requisite for it. In their mechanical model of Utricularia trap firing, JOEYUX & al. 2011 assume that trap door buckles as a flexible valve under the combined effect of the internal negative pressure and the mechanical stimulation of trigger hairs as a lever when a critical stability threshold of the trap door is slightly exceeded. It follows from time recordings of the internal negative pressure that trap can be stimulated mechanically to fire at different values of the negative pressure (cf. SYDENHAM & FINDLAY 1973, 1975, SASAGO & SIBAOKA 1985a) which correspond to different time period of trap resetting. In our study, when Utricularia traps

Table 1. Results of the inhibitory action of 4 % (v/v) ether or 0.5 mM NaN₃ or effect of humid air on firing and resetting characteristics of young Utricularia reflexa traps measured electronically as trap thickness changes. Traps were usually fired (f.) due to mechanical stimulation successively four times in 1 h intervals, with or without the inhibitory treatment in the ambient medium. The control values are shown in italics, while the values under the influence of the inhibitor are bold. A, treatment of ether added after the 2nd firing. B, treatment of ether added before the 2nd firing. C, treatment of NaN₃ added between the 1st and 2nd firing. D, measurements on wet traps kept in humid air; the 2nd firing included an air bubble inside the trap. E, firing and resetting of reset traps at 2 °C. Controls of A and B were taken as controls for D and E. Means ± SE are always shown. Statistically significant difference between the control value and the treatment was tested using a 1-way ANOVA and indicated by asterisks: ** – P < 0.01; * – P < 0.05; ns – P > 0.05.

<table>
<thead>
<tr>
<th>Trap thickness increase (µm) during firing</th>
<th>Resetting rate (µm) after firing during 10 min</th>
<th>Resetting rate (µm) after firing during 30 min</th>
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<tbody>
<tr>
<td>1st f.</td>
<td>2nd f.</td>
<td>3rd f.</td>
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<tr>
<td><strong>A. Ether added immediately after the 2nd firing (n = 8–9)</strong></td>
<td></td>
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<tr>
<td>309</td>
<td>281</td>
<td>234**</td>
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<tr>
<td>±16.5</td>
<td>±13</td>
<td>±10</td>
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<tr>
<td><strong>B. Ether added immediately before the 2nd firing (n = 8–9)</strong></td>
<td></td>
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</tr>
<tr>
<td>316</td>
<td>290ns</td>
<td>223**</td>
</tr>
<tr>
<td>±17</td>
<td>±15</td>
<td>±12</td>
</tr>
<tr>
<td><strong>C. NaN₃ added 1 h after the 1st firing, i.e., 1 h before the 2nd firing (n = 6)</strong></td>
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<tr>
<td>320</td>
<td>326ns</td>
<td>–</td>
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<tr>
<td>±30</td>
<td>±28</td>
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<tr>
<td><strong>D. Wet traps in humid air (n = 7–17)</strong></td>
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<tr>
<td>335ns</td>
<td>268ns</td>
<td>–</td>
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<tr>
<td>±18</td>
<td>±16</td>
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<tr>
<td><strong>E. Firing and resetting at 2 °C (n = 10)</strong></td>
<td></td>
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<tr>
<td>306ns</td>
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<td>–</td>
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were stimulated to fire continuously by introducing fine air bubbles close to the trap door or by addition of 50–100 zooplankton individuals to the chamber, the shortest period between two firings was 39 min in *U. reflexa* and 16 min in *U. vulgaris* (unpubl. results). These threshold periods of trap resetting mean the negative pressure inside the trap is already sufficiently great to fire the trap as a result of the mechanical stimulus. The fact that in our previous study, (Adamec 2011a) *U. reflexa* traps were mechanically stimulated (using a fine brush) to fire as early as every 30 min indicates that the sensitivity of traps to mechanical stimulation is not constant; it is rising in the course of resetting time leading ultimately even to spontaneous firing (Vincent & al. 2011b).

Sydenham & Findlay 1975 and Sasago & Sibaoka 1985a provided evidence that wet *Utricularia* traps normally reset in liquid paraffine and used this procedure for collecting the exudation solution. Wet *U. reflexa* traps fixed in humid air could also be mechanically stimulated to fire and reset at least twice (Table 1). Thus, the absence of water around the trap does not exclude the normal trap functioning but the presence of a larger air bubble inside the trap greatly reduces the apparent resetting rates due to air expansion. It is conceivable that this procedure might be used experimentally.

In conclusion, it is evident that only a direct recording of an electrophysiological activity in trigger hairs of *Utricularia* trap stimulated mechanically to fire can decide whether or not these hairs give a rise in action potential like in *Dionaea muscipula* and *Aldrovanda vesiculosa* (e.g., Iwima & Sibaoka 1981, see Juniper & al. 1989) which could serve as an electrical signal for the trap-door buckling.

Acknowledgements

This study was supported partly by the Research Project CSF No. P504/11/0783 and the Research Programme of the Academy of Sciences of the Czech Republic (No. AV0Z60050516). Sincere thanks are due to Dr. Brian G. McMillan for his linguistic correction of the paper.

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