Receptor Response in Venus's Fly-Trap

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ABSTRACT The insect-trapping movement of the plant *Dionaea muscipula* (Venus's fly-trap) is mediated by the stimulation of mechanosensory hairs located on the surface of the trap. It is known that stimulation of the hairs is followed by action potentials which are propagated over the surface of the trap. It has been reported that action potentials always precede trap closure. The occurrence of non-propagated receptor potentials is reported here. Receptor potentials always precede the action potentials. The receptor potential appears to couple the mechanical stimulation step to the action potential step of the preying sequence. Receptor potentials elicited by mechanical stimulation of a sensory hair were measured by using the hair as an integral part of the current-measuring path. The tip of the hair was cut off exposing the medullary tissue; this provided a natural extension of the measuring electrode into the receptor region at the base of the hair. A measuring pipette electrode was slipped over the cut tip of the hair. Positive and negative receptor potentials were measured. Evidence is presented which supports the hypothesis that the positive and negative receptor potentials originate from independent sources. An analysis is made of (a) the relation of the parameters of mechanical stimuli to the magnitude of the receptor potential, and (b) the relation of the receptor potentials to the action potential. The hypothesis that the positive receptor potential is the generator of the action potential is consistent with these data.

Since its discovery over two centuries ago, the complex insect-preying behavior of the plant *Dionaea muscipula* (Venus's fly-trap) has received much attention. Events in the preying sequence have been studied by several investigators. Lloyd (1942) presented a thorough review of much of this work. The preying process is mediated by mechanosensory hairs (usually six in number) found on the inner surface of the trap. Each hair is a multicellular structure which appears to consist of two morphological regions: a distal lever region, and a basal podium region. Mechanical stimuli are transmitted to the podium region by the lever. Brown and Sharp (1910) showed that the podium was the locus of the receptor site. They found that, after removal of the sensory lever, mechanical stimuli delivered directly to the remaining part of the hair (the podium) resulted in closure of the trap.

Fig. 1 is a block diagram suggested by Benolken (1963) which summarizes

the known steps in the preying sequence. The order and functional relationship of steps indicated in the diagram are derived from data presented in the present study as well as from several other sources.

Normally, closure of the trap is brought about by a series of two or more mechanical stimuli delivered to one or more of the sensory hairs. Brown (1916) and Brown and Sharp (1910) have characterized the series of stimuli ade-
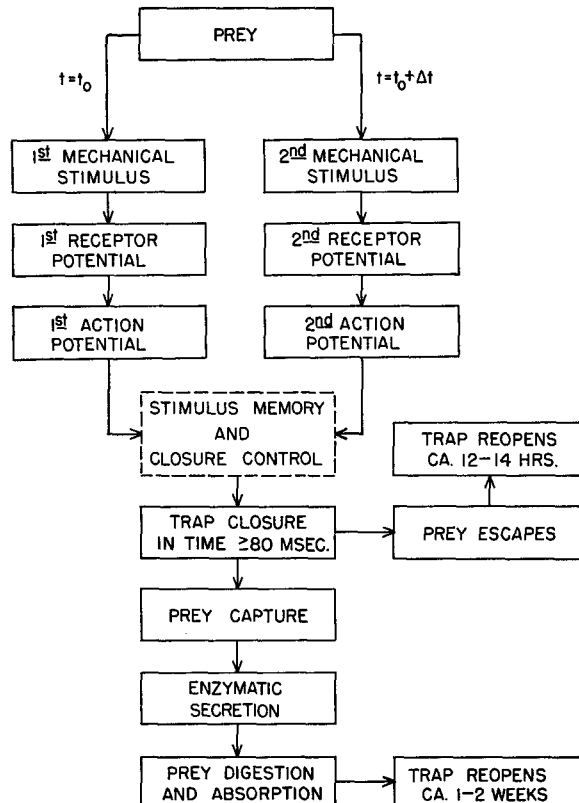

FIGURE 1. The preying sequence of the plant *Dionaea muscipula*. The sequence shown is one in which two stimuli are delivered within the critical time Δt to elicit closure. Dashed outline indicates hypothetical functions.

quate to bring about closure. Adequate series consist of n stimuli, $n \geq 2$, delivered within an interstimulus interval Δt. The number of stimuli, n, required for closure was found to be a monotonically increasing function of the interstimulus interval Δt. The total time, $n\Delta t$, of a stimulus series could vary from a fraction of a second for $n = 2$, to at least 5.5 hours for $n = 18$. That a series of stimuli delivered over several hours can elicit closure of a trap suggests strongly that a stimulus "memory" exists in the system.

Mechanical stimulation of the sensory hairs was shown to be followed by

propagated action potentials (see Bourdon-Sanderson, 1888; and Stuhlman and Darden, 1950). The action potentials were detected by electrodes placed in contact with the surface of the trap. DiPalma *et al.* (1961) observed that action potentials always preceded closure of the trap.

Schneider (1957) and Wolbarsht (1960), studying insect sensory hairs, measured non-propagated electrical responses by using the hair as an integral part of the current-measuring path. Data reported here were obtained using this technique in a study of coding of sensory information in Venus's fly-trap.
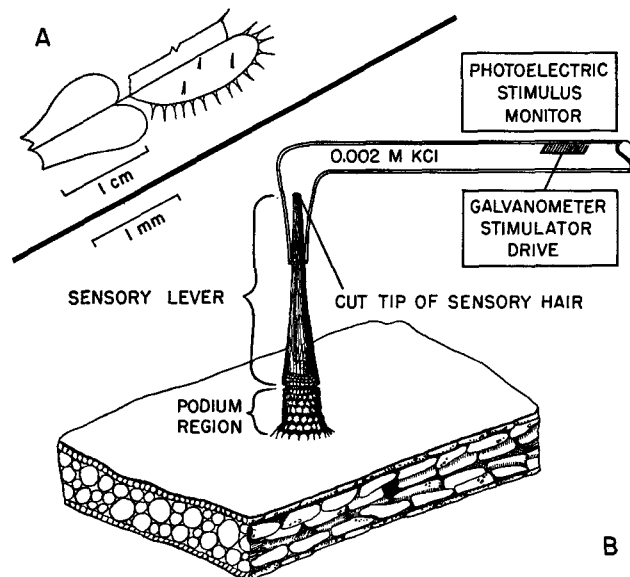

Figure 2.   The experimental preparation: A. Gross view of the trap preparation. One lobe was removed. The sensory hairs were accessible on the remaining lobe as shown. B. Detailed view of the pipette fitted over the cut tip of the sensory hair. Pipette served as measuring electrode. Sensory hair was stimulated by lateral displacement of the pipette.

METHODS AND MATERIALS

Dionaea plants were grown in a greenhouse in natural light augmented by 16 hours per day of incandescent light. Greenhouse temperature was maintained above 15°C during the winter months and was allowed to follow ambient temperatures during the remainder of the year.

When selected for study, traps with approximately 5 mm of petiole were cut from robust plants. To extend the preparation life, the cut end of the petiole was placed in a cotton pad soaked with distilled water. Fig. 2A is a sketch of the preparation. One lobe of the trap was cut off about 3 mm from the midrib, leaving the inner surface of the remaining lobe and its three sensory hairs readily accessible.

Approximately 0.5 mm of the tip of one sensory hair was cut off. The cut exposed

the medulla of the hair which provided a relatively low impedance current path to the receptor site. The preparation was immobilized in a clamp and was placed in a moist chamber for the duration of the experiment.

A single electrode served as a reference for all potential difference measurements. This reference electrode was a glass tube filled with 2 mM KCl which contacted the surface of the outer epidermis of the trap approximately in the center of the triangle defined by the three sensory hairs. Contact was made with the trap through a drop of 2 mM KCl. Two cotton wick electrodes saturated with 2 mM KCl were used to measure propagated action potentials on the surface of the inner epidermis of the trap relative to the reference electrode. One of the wick electrodes was located near the base of the cut sensory hair, and the other near the margin of the trap lobe.

A glass pipette filled with 2 mM KCl served as an electrode to measure potential differences between the cut tip of the sensory hair and the reference electrode (see Fig. 2B). The pipette was lowered over the hair until its fire-polished tip made a tight seal about the sensory lever. When enclosed in the pipette, the lever acted as an extension of the pipette-electrode into the basal region of the sensory hair.

The hair was mechanically stimulated by laterally displacing the pipette in which it was enclosed. The pipette was displaced by a lever attached to a D'Arsonval penmotor. The penmotor was driven by a waveform generator. The waveform of the stimulating motion of the pipette was monitored with a photocell whose illuminated area, and hence its output, was a function of the position of the pipette. The amplitude of mechanical stimuli used ranged from about 10 to 10^3 μ. The rise times of stimuli (time to attain maximum amplitude) ranged from about 10 to 10^4 msec. Limiting values of stimulus parameters were dictated by practical considerations. Ten micra was a lower limit for calibration of stimulus amplitude, and stimulus amplitudes greater than 10^3 μ frequently damaged the sensory hair or caused it to slip out of the pipette. Rise times of 10 msec. and 10^4 msec. represented, respectively, the fastest response of the D'Arsonval galvanometer and the longest rise time waveform attainable with the waveform generator.

Stimulus and response data were recorded by a four-channel recorder equipped with specially designed high impedance DC preamplifiers. On the records (e.g. Fig. 3), the first trace recorded the waveform of the stimulus. The second and third traces recorded propagated action potentials measured on the surface of the trap relative to the reference electrode. The fourth trace recorded the potential difference between the electrode on the cut sensory hair and the reference electrode.

All measurements were made with silver silver–chloride electrodes. A very gradual drift of the resting potential difference between the sensory hair and reference electrode was observed. Occasionally this amounted to as much as ±30 mv over a period of several hours. The drift was compensated for by a zero suppression control at the recorder input. Temperature was held at 23 ± 2°C throughout the experiments.

OBSERVATIONS

A propagated action potential (Fig. 3 b and c) occurred on the surface of the trap for stimuli of critical rise time and sufficient amplitude. A potential variation which was the correlate of the action potential on the trap surface was

measured at the tip of the sensory hair (Fig. 3 *d* to right of arrow). This potential variation was always preceded by a non-propagated positive potential. The polarity of the initial phase of the action potential correlate was the same as that of the non-propagated positive potential. The onset of the action potential correlate and of the action potential detected at separate electrodes on the trap surface did not occur simultaneously. The order of occurrence was (*a*) onset at the sensory hair, (*b*) onset at electrode at the base of the sensory hair, (*c*) onset at electrode on the trap margin.

For stimuli which were not adequate to elicit propagated action potentials, non-propagated potentials (receptor potentials) were detected at the tip of the
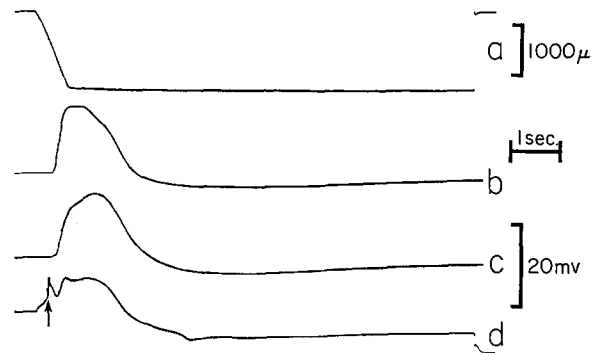

FIGURE 3.   Recording of potential differences occurring in response to superthreshold mechanical stimulus. Positive upward. *a*, stimulus waveform; *b*, propagated action potential at electrode on margin of trap surface; *c*, propagated action potential at electrode on trap surface at base of sensory hair; *d*, electrode at tip of sensory hair. In *d* the initial positive-going potential to the left of arrow is a positive receptor potential. The receptor potential is interrupted by the action potential invading the recording site. Retouched to remove grid lines from record.

sensory hair but were not detected on the surface of the trap. The receptor potentials (Fig. 4) were of graded amplitude and were of two classes. The first class (negative receptor potential) was characterized by negativity of the tip of the sensory hair. The second class (positive receptor potential) was characterized by positivity of the tip of the sensory hair. In general the amplitude and waveform of both classes of receptor potential depended upon stimulus amplitude and waveform.

The negative receptor potential occurred only in response to very fast-rise stimuli. A negative receptor potential was rarely followed by a propagated action potential. This was true even for negative receptor potentials elicited by stimuli of very large amplitude. The wave form of the negative receptor potential (Fig. 4A) was an initial positive transient followed by a rapidly developing negative phase and a gradual return to the base line (recovery).

The recovery period was observed to last for as long as 4 minutes after termination of the stimulus. The amplitude of the negative receptor potential showed a strong dependence upon recent history of stimulation of the preparation as well as upon stimulus amplitude. If two fast-rise stimuli were delivered in rapid succession (as in Fig. 4A), two negative receptor potentials were elicited. The second negative receptor potential originated from a base line determined by the extent of recovery of the preparation from the first stimulus. In other words, the negative receptor potentials showed temporal summation. The receptor sensitivity usually appeared to be temporarily reduced after the first of the two fast-rise stimuli. Hence for short enough interstimulus intervals, the response to the second stimulus was less than the response to the first. Due to this decreased response, the temporally summed response was usually less than the initial response and only rarely exceeded it by more than a few per cent.

The positive receptor potential (Fig. 4B) usually exhibited a positive transient, the maximum amplitude of which was coincident in time with the maximum amplitude of the stimulus. The positive transient was followed by a negative-going transient, a steady positive phase, and a decay or adapting phase. The degree to which the transient phases of the positive receptor potential appeared in the receptor potential varied with stimulus amplitude and rise time. The positive and negative transients were absent from the positive receptor potentials elicited by very slow-rise stimuli (Fig. 4C). When the positive receptor potential amplitude reached threshold, it was followed by a propagated action potential. The onset of the action potential was observed to occur either immediately before, during, or immediately after the positive receptor response reached maximum amplitude. The action potential was never observed to precede the receptor potential. Action potentials could also be elicited by two apparently subthreshold mechanical stimuli if these were delivered in rapid succession. When this was observed, two successive positive receptor potentials were elicited, and these usually exhibited temporal summation.

A compound response (Fig. 4D) was elicited by a compound stimulus. (A compound stimulus consisted of a slow-rise stimulus interrupted by a fast-rise stimulus.) The compound response was a positive receptor potential interrupted by a negative receptor potential which shifted the base line. This was followed by the remainder of the positive receptor potential.

ANALYSIS

The relation of the waveform of the receptor response to the stimulus parameters is a complicated one. Fig. 5 shows records of two sets of positive receptor potentials elicited by subthreshold stimuli. One set is for stimuli of different amplitude and constant rise time. The other set is for stimuli of different rise

time and constant amplitude. The maximum amplitude of the receptor response appeared to be a monotonically increasing function of the stimulus amplitude (for constant stimulus rise time). On the other hand, the maximum
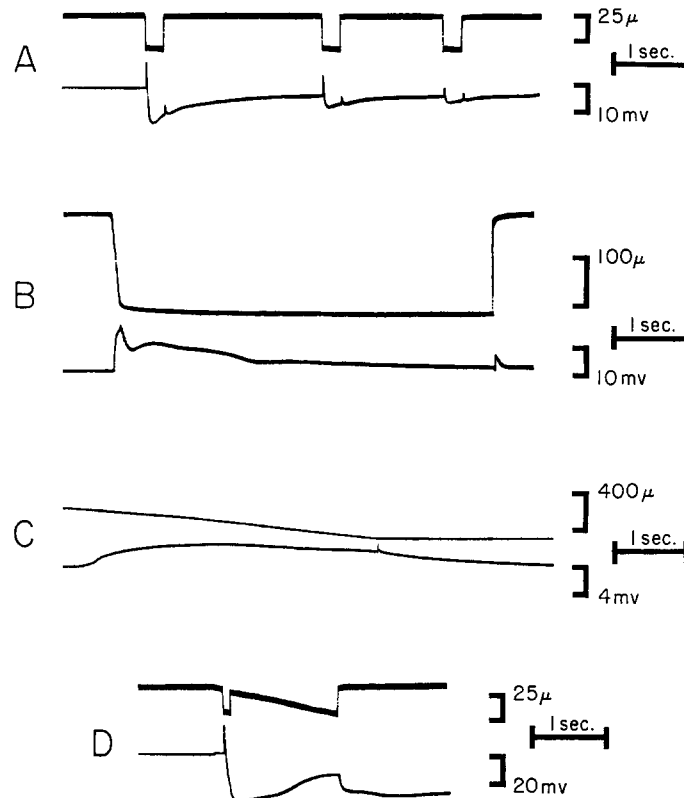

FIGURE 4. Recordings of typical receptor potentials in response to subthreshold stimuli. Positive upward. Upper traces are recordings of stimulus waveform; lower traces are recordings from electrode on tip of sensory hair. A. Negative receptor response to fast-rise stimulus. B. Response to medium-rise stimulus. C. Response to slow-rise stimulus. D. Response to compound stimulus. Small potentials in A and B coinciding with termination of stimulus are "off responses."

Positive pip on lower trace in C is an artifact due to imperfect matching of amplitudes of initial and final phases of the stimulus. Comparison of records B and C shows that the rate of onset of positive receptor potentials depends upon stimulus velocity. Retouched to remove grid lines from record.

amplitude of the receptor response was clearly not a monotonic function of the stimulus rise time (for constant stimulus amplitude).

One feature of the response waveform, *i.e.* its maximum positive amplitude, was studied as a function of the stimulus parameters. The functional relationship was displayed on log-log isoresponse contour plots. Fig. 6 is a typical

isoresponse plot. The x axis represents values of stimulus rise time. The y axis represents values of stimulus amplitude. Any point in the x-y plane of the plot, therefore, defines a unique stimulus. The isoresponse lines join points representing stimuli which would elicit receptor responses of equal *maximum positive amplitude*. The loci of points representing stimuli of constant velocity (stimulus amplitude/rise time) are lines of $+45°$ slope, and the loci
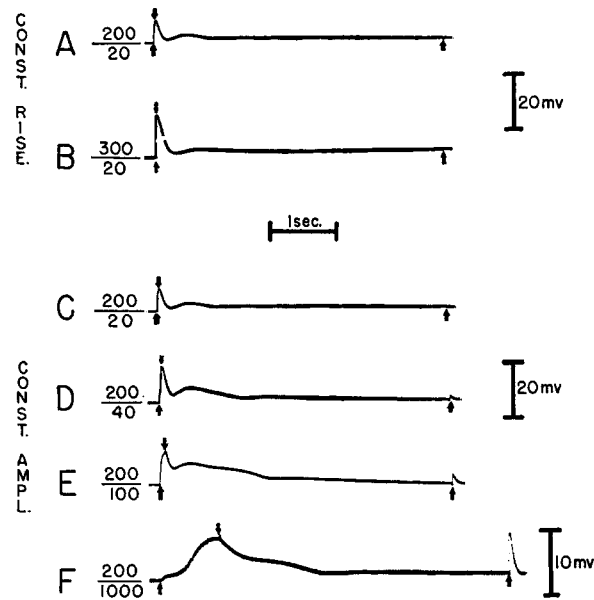

FIGURE 5. Recordings of positive receptor potentials elicited by subthreshold stimuli. Positive upward. Records A and B are for stimuli of constant rise time and of different amplitudes. Records C through F are for stimuli of constant amplitude and of different rise times. (Note scale change in F.) Stimulus waveforms are not shown but are similar to that in Fig. 4B. Arrows indicate onset, completion of rising phase, and termination, of stimulus. Fractions to left of traces designate stimulus amplitude in micra over stimulus rise time in milliseconds. Positive pip in trace F is an artifact due to imperfect matching of amplitudes of initial and final phases of the stimulus. Retouched to remove grid lines from record.

of points representing stimuli whose product (amplitude × rise time) is constant are lines of $-45°$ slope.

Data used in constructing a contour plot such as that shown in Fig. 6 were taken from measurements of the responses to a series of stimuli delivered to a single preparation over a continuous period of 6 to 8 hours. The maximum positive value of the response to each stimulus in the series was recorded next to the appropriate point defining each stimulus on the contour plot. Points of apparently equal response amplitude were then joined by isoresponse lines in the fashion of contours on a topographic map.

The waveform of stimuli delivered in the series consisted of an initial phase of variable rise time and amplitude followed by a 5 second phase during which the maximum amplitude of the stimulus was maintained. The abrupt termination of a stimulus usually resulted in a receptor "off response." The 5 second phase of maintained amplitude was used in the stimulus to delay the off response. This allowed the receptor response to the initial phase of the stimulus to develop completely, unmasked by the off response. Stimuli delivered at 5 minute intervals were found to give reproducible responses. This
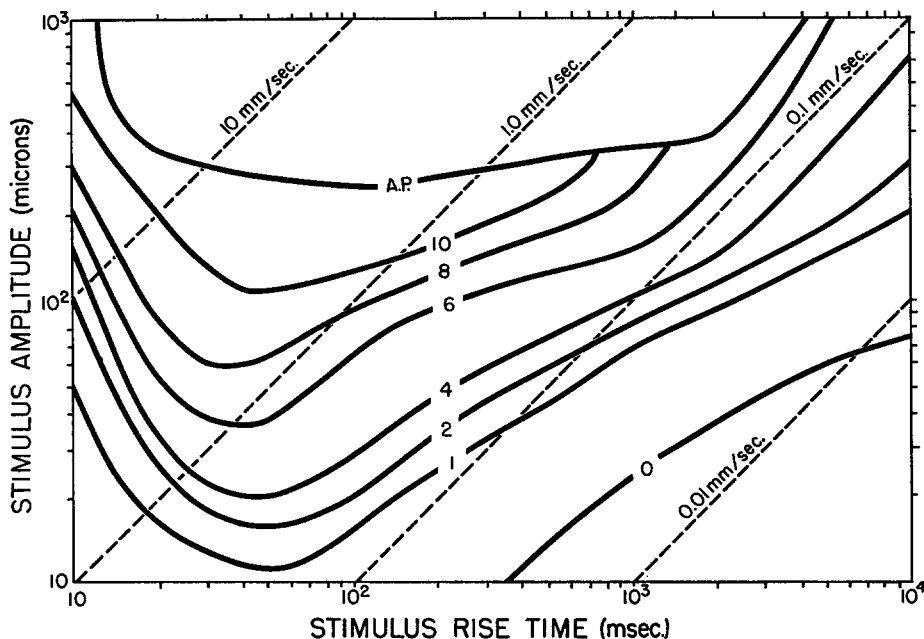

FIGURE 6. A typical isoresponse contour plot derived from data on one preparation. Values on isoresponse lines in millivolts. Isoresponse line marked A.P. indicates the boundary of the action potential response domain. 45° dashed lines are loci of constant velocity stimuli.

was taken to indicate that 5 minutes was sufficient time for the preparation to recover from previous stimuli to a state of relatively constant sensitivity. In order to minimize the effect of previous stimulation on the response to a given stimulus, stimuli were delivered at 5 minute intervals and the order of stimuli was varied from one preparation to the next. Reproducibility of response was checked by periodically repeating a control stimulus.

There was considerable variability in the amplitude of receptor responses to the same stimuli delivered to individual preparations as well as among different preparations. This variability notwithstanding, several characteristics of the stimulus-response relation were common to all preparations and are indi-

cated by the contour plots. (a) The magnitude of receptor responses elicited by slow-rise stimuli was a function of the velocity of sensory hair displacement. This is evident from the approximately +45° slope of the isoresponse lines on the right side of the plot. (b) The magnitude of receptor responses elicited by fast-rise stimuli was a function of the product of the amplitude of sensory hair displacement × the displacement rise time. This is evident from the approximately −45° slope of the isoresponse lines on the left side of the plot. (c) Positive receptor potentials which reached or exceeded threshold were elicited by a group of large amplitude, medium rise time stimuli which are defined for the upper central region of the plot. The value of the isoresponse lines which intersect the action potential response line indicates the approximate value of threshold. (d) Subthreshold receptor potentials were elicited by a group of stimuli of very fast-rise and apparently superthreshold amplitude. These stimuli are defined for the upper left region of the plot. Negative receptor potentials were elicited by these stimuli. Hence, contours in this region of the plot represent the maximum amplitude of the positive transient phase of these negative receptor potentials. (e) No measurable receptor potentials were elicited by a group of low amplitude, slow-rise stimuli defined for the lower right region of the plot.

DISCUSSION

Although the configuration of the recording path through the sensory hair was well defined, its electrical properties were unknown. It was important to investigate the effect of strains induced in the sensory hair electrode by mechanical stimulation. Preparations fixed in alcohol for approximately 12 hours were used for this purpose. When these preparations were stimulated in the usual way, potentials of very short duration with amplitudes of about 500 μv were detected. The receptor responses considered in this paper were of considerably longer duration and greater amplitude. It is possible that strains induced by stimulation in the *living* sensory hair could have caused changes in its electrical properties. These changes might account for some of the observed characteristics of the receptor potential. Despite these complications, some properties of the *Dionaea* preying system are suggested by the data.

Encoding of sensory information appears to proceed in at least two steps. In one step, sensory information is transformed into the analog form of the receptor potential. Stimulus parameters appear to be represented in terms of the amplitude, waveform, and polarity of the receptor potential. In another step, the analog form of information in the receptor potential is transformed into the digital form of the action potential. The positive receptor potential appears to be necessary for the occurrence of an action potential. The hy-

pothesis that the positive receptor potential is the generator of the action potential is consistent with these data.

In *Dionaea* a single receptor potential was never observed to elicit more than one action potential. This property is not characteristic of most other receptors which have been studied. A single action potential is observed in response to stimulation in the Pacinian corpuscle (see Gray, 1959). In this case, a receptor potential has been measured and is thought to be the generator of the action potentials occurring in the corpuscle nerve. The duration of the corpuscle receptor potential is very short and this is thought to account for the singular action potential response.

The function of the negative receptor potential in the preying system is not clear to me. The marked difference between the waveforms of the negative and positive receptor potentials plus the fact that they can be elicited either separately or simultaneously suggests that they may originate from independent sources. This is not meant to imply, however, that they are or are not caused by the same types of mechanism(s) ionic or otherwise. The negative receptor potentials are strikingly effective in preventing certain fast-rise large amplitude stimuli from eliciting action potentials. If action potentials are required for closure, prey which deliver *very fast-rise* stimuli to a trap *in situ* would not contribute a stimulus toward the number, n, which are required for closure.

Two characteristics of the *Dionea* stimulus-response relation recall the stimulus strength–duration curve of nerve axon (see Katz, 1939). In *Dionaea*, shorter duration positive receptor potentials are elicited by faster-rise stimuli for which the action potential threshold increases. Hence, action potential threshold increases as the duration of the positive phase of the receptor potential decreases. Similarly, in nerve the threshold for electrical stimulation increases as stimulus duration decreases. Furthermore, in *Dionaea*, action potentials are not elicited for the very short duration positive transient of the negative receptor potential. Similarly, in nerve, action potentials are not elicited by electrical stimuli of very short duration regardless of stimulus amplitude.

The increase of action potential threshold with faster-rise stimuli does not appear to render the trap less sensitive to stimulation by moderately fast-rise stimuli. On the basis of information from the contour plots, the sensitivity of the preying system would be expected to be essentially independent of stimulus rise time over a rise time range of about two log units. Over this range, "receptor sensitivity" increases for faster-rise stimuli. The increase in sensitivity is apparent from the fact that a constant stimulus amplitude line on the contour plot intersects isoresponse lines of increasing value over a rise time range of 10^4 to about 10^2 msec. This increase of receptor sensitivity should tend to compensate for the increase in threshold of the action potential.

There are certain stimuli for which receptor sensitivity is a maximum. These stimuli elicit receptor responses of maximum amplitude for minimum stimulus amplitude. The loci of these stimuli lie on a line through the minima of the isoresponse lines. For a given amplitude, stimuli whose loci deviate to a greater extent to the left or right of this line elicit receptor potentials of smaller positive amplitude. To the left of the line as the amplitude of the responses decreases, the waveforms of the responses gradually change and come to resemble negative receptor potentials. This gradual transition suggests that the observed waveforms may be the sum of a positive response and a negative response component. In the case of very fast-rise stimuli, the magnitude of the negative component would be relatively large and would tend to dominate the net response.

Slow-rise stimuli of reduced amplitude fail to elicit a receptor response. An explanation for this phenomenon is suggested by some of the properties of the sensory system illustrated by records B and C of Fig. 4. The receptor system adapts for stimuli of maintained constant amplitude. Furthermore, the rate of onset of a receptor potential is directly related to the rate of onset (rise time) of a stimulus. Presumably a system with these properties would give no response to stimulation when the rate of onset of a response was equal to or exceeded by the rate of adaptive decay.

The data allow little more than speculation about the stimulus memory in the preying process. There is no indication that the memory is closely associated with the non-propagated receptor response. It is apparent that not all stimuli elicit action potentials and that a critical number of action potentials are required for closure of the trap. From these observations it appears more likely that the memory stores information on the number of action potentials elicited rather than on the number of stimuli received. This suggests that the memory may be remote from the sensory hairs and that the action potentials "carry" sensory information to the memory site. The nature and location of the memory remain as one of the more challenging questions posed by Venus's fly-trap.

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