laceae, *Betula*; Caprifoliaceae, *Lonicera*; Caryophyllaceae, *Dianthus*; Compositae, *Blennosperma, Cichorium, Stephanomeria, Taraxacum, Tragopogon*, and *Wyethia*; Convolulaceae, *Calystegia*; Crassulaceae, *Sedum*; Cruciferae, *Brassica, Diplotaxis*, and *Nasturtium*; Cucurbitaceae, *Cucurbita*; Ericaceae, *Arctostaphylos* and *Rhododendron*; Euphorbiaceae, *Euphorbia* and *Pedilanthus*; Hippocastanaceae, *Aesculus*; Leguminosae, *Cercis, Medicago, Melilotus, Phaseolus*, and *Trifolium* (3); Liliaceae, *Allium, Aloe, Brodiaea* (2), and *Zygadenus*; Limnanthaceae, *Limnanthes*; Malvaceae, *Gossypium*; Myrtaceae, *Callistemon* and *Eucalyptus* (2); Oleaceae, *Forsythia, Ligustrum*, and *Syringa*; Onagraceae, *Clarkia* and *Fuchsia*; Passifloraceae, *Passiflora*; Polemoniaceae, *Navarretia* and *Phlox* (2); Ranunculaceae, *Aquilegia* and *Delphinium*; Rosaceae, *Amelanchier, Prunus* (4), *Pyracantha, Pyrus, Rubus*, and *Spiraea*; Rutaceae, *Citrus* and *Xanthoxylum*; Salicaceae, *Populus* and *Salix*; Saxifragaceae, *Escallonia* and *Ribes*; Scrophulariaceae, *Anthirrhinum* and *Collinsia*; Solanaceae, *Capsicum, Nicotiana*, and *Petunia*; Tiliaceae, *Tilia* (2); Ulmaceae, *Ulmus*; Violaceae, *Viola*.

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21. Sponsored in part by a grant-in-aid from Homer Park, Park Apiaries, Palo Cedro, California, and a faculty research grant to R.W.T. We thank G. Webster, H. G. Baker, and B. Heinrich for useful comments on the manuscript.

19 March 1975

Plant "Primary Perception": Electrophysiological Unresponsiveness to Brine Shrimp Killing

Abstract. *A test of the "primary perception" hypothesis proposed by Backster in 1968 was made by recording electrical activity from the leaves of* Philodendra scandentia *while randomly ejecting the contents of micropipettes filled with brine shrimp or distilled water into boiling water. Test conditions conformed to those published by Backster or communicated in personal exchanges. Data were analysed from five experiments, in each of which recordings were made from four plants in the presence of three brine shrimp killings and two control water ejections. Inspection of the data and analysis by two statistical methods revealed no relationship between brine shrimp killing and electrical "responsiveness" of philodendron.*

With publication of Backster's report (*1*) on a perception capability of plant leaves observed when brine shrimp (*Artemia salina*) were killed, the possibility that communication by unknown physical or chemical means might exist between plant and animal forms was reactivated. Backster's observation has been received enthusiastically by the public but has been disregarded until recently (*2*) by the scientific community. Since neither response is appropriate to an experimentally supported contention, we repeated Backster's basic experiment and designed an additional test (*3*) based on popular accounts of Backster's unpublished work (*4*). Care was taken to duplicate Backster's conditions and in some instances to use more stringent controls. No support for Backster's "primary perception" hypothesis was obtained in either of our experiments.

Philodendron scandens oxycardium were initially grown by the Kenneth Post Laboratories of the Cornell Department of Floriculture and Ornamental Horticulture (*5*). Seven days before use they were transferred to a holding room with a westerly exposure near the experimental laboratory. On the day of the experiments the plants were moved to two light-tight rooms in the laboratory, each of which housed a Faraday cage to electrically shield the plants plus a three-channel polygraph (Grass model 7) for recording simultaneously from two plants and from a 110,000-ohm fixed resistance to simulate an unchanging plant.

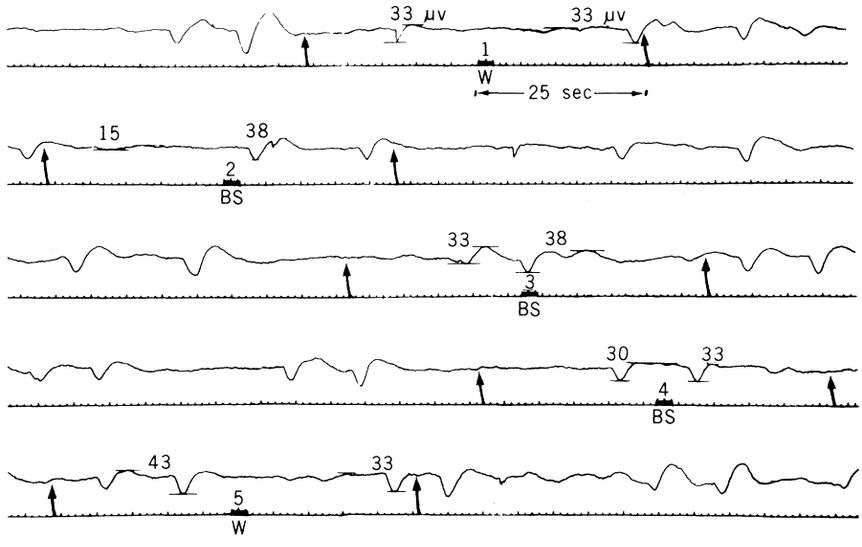

Fig. 1. Representative leaf recordings obtained on curvilinear paper during five ejections (1 through 5) indicated by the signal marker deflection just below the numerals. Two ejections were water (*W*) and three were brine shrimp (*BS*). Vertical arrows mark the beginning of 25-second control periods and the end of 25-second ejection periods. Analysis was made by comparing maximum peak-to-peak voltage deflections regardless of polarity (marked by horizontal lines and recorded in microvolts) for control and ejection periods.

Artemia (*6*) were incubated in a distant room; on the experimental day they were observed microscopically for vigor and then transferred to a laboratory room located between the two recording rooms and containing the shrimp-killing apparatus. These three rooms opened into a monitoring area in which a video tape system recorded the operation of the shrimp-killing apparatus.

The apparatus consisted of five disposable pipettes which were mounted vertically on a modified kymograph drum. An electrical impulse, initiated by a programmable tape reader, activated a solenoid which squeezed a pipette bulb and ejected the contents of the pipette into a beaker of boiling water. Simultaneously, the tape reader marked the recording charts by activating the polygraph signal markers. The solenoid reset and the drum automatically rotated until the next pipette was in position for the next activation. Failure of the ejection apparatus could be detected through the video system or by discoloration of calcium anhydride indicator (Drierite) spread beneath the rotating drum to detect pipette leakage. The tape reader was programmed by means of 16-mm film in which five holes were punched at intervals determined from a random table of digits. When read by the tape reader the film provided five activations of the termination system in 15 minutes. Five different films were made; one was selected by chance before each run.

One-half hour before each recording session four plants were gently washed with distilled water to remove any dust, and the potting soil was saturated with water. Polygraph amplifiers were warmed up one-half hour and then calibrated. The polygraphs operated from a-c mains equipped with radio-frequency filters. Tests were conducted to ensure correct operation of the total system.

Two plants were placed within each Faraday cage, and a pair of gauze-coated electrodes (*7*) for each plant was arranged to contact gently both sides of a leaf having surface area greater than 1.5 times the electrode area. The electrodes were held in place by two arms of a burette clamp pre-

Table 1. Assembled data scored as +, −, or 0, depending on whether the voltage deflection following ejection was larger, smaller, or unchanged with respect to the control. Only differences greater than 10 μv (magnitude of slow drift of the system) were used. Contents of the pipettes are noted as BS (brine shrimp) and W (water). The symbol (+) indicates a coincidence of change in potential and killing of brine shrimp (a "hit" according to Backster).

Plant	Pipette number				
	1	2	3	4	5
			Experiment 6		
	BS	W	BS	W	BS
18	0	−	−	+	0
19	−	0	0	−	(+)
20	−	−	0	0	0
21	−	+	(+)	+	(+)
			Experiment 7		
	BS	BS	BS	W	W
22	−	0	0	0	0
23	0	−	−	0	0
24	0	0	−	−	0
25	0	0	0	+	−
			Experiment 8		
	W	BS	BS	BS	W
26	0	(+)	(+)	0	0
27	−	0	−	(+)	0
28	0	(+)	0	(+)	+
29	+	0	0	0	0
			Experiment 10		
	W	BS	BS	BS	W
34	0	(+)	0	0	0
35	−	0	−	0	0
36	0	0	0	0	0
37	0	0	(+)	0	0
			Experiment 12		
	BS	BS	BS	W	W
42	0	−	−	0	0
43	0	0	0	0	0
44	−	−	0	0	0
45	0	0	0	0	−

viously insulated with a vinyl covering. Special care was taken to ensure that there was no physical damage to the leaf. After experimentation, the leaf was checked for injury, and the plants were then maintained for 14 days so that latent injury arising from handling or electrical currents could be assessed; none was seen.

To reduce electrical interference and to effect differential recording, the plants were grounded by inserting bare stainless steel electrodes into the potting soil, care being taken to prevent contact between the soldered junctions and salt or soil. The three leads to a given plant were twisted together to further minimize pickup of electrical interference. Shielded cables connected the polygraph a-c preamplifiers to the plants and to the fixed resistance within the cages (*8*). Each fixed resistance was connected from one side of its input channel to ground. This control channel thus operated in a nondifferential mode, making it more sensitive to electrical interference. The fixed resistance with its associated recording channel served as a local control against artifacts caused by fluctuation in line voltage or by radiated electrical interference. To determine the constancy of the electrode-leaf preparation, resistance was measured by means of a Simpson test set before and after the recording session.

Polygraphs were placed in the "use" mode, the doors to each of the recording rooms were closed, and 10-minute base-line recordings of all channels were made. During this period, the programming film was mounted in the tape reader. Ten to 20 active *Artemia* were drawn into three of the disposable pipettes. The remaining pipettes were filled with distilled water and all five pipettes were placed on the kymograph drum in an order drawn from a random numbers table ensuring the chance injection of *Artemia* or distilled water. This sequence was recorded but was not referred to until the polygraph records were scored.

At the end of the 10-minute base-line period, polygraph records were checked for sensitivity, and the end of the control period was marked. Both recording room doors were closed and all other systems were activated. The door to the room containing the shrimp-killing apparatus was then closed, and we departed from the laboratory suite for approximately 18 minutes. During this period, we engaged in activities other than those pertaining to the experiment until an alarm watch signaled the end of the experimental period.

Upon returning to the experimental area, we immediately placed the polygraph amplifiers in the calibrate mode and turned off the shrimp-killing apparatus. The pipettes were checked for content, the calcium anhydride observed for color change, and the video tape reviewed.

Due to recording failures, three of our eight sessions were disqualified before analysis of the records. Our method of analysis and recordings from one plant are illustrated in Fig. 1. Results are tabulated in Table 1, where the data can be examined for coincidence of brine shrimp ejections and positive scores ("hits"). A summary appears in Table 2.

Data for both the brine shrimp and water ejections were scored as +, −, or 0, depending on whether the voltage deflection after ejection was larger, smaller, or unchanged with respect to the control. Statistical analysis of the scored data was carried out by a normal approximation of the binomial model and the null hypothesis of $P = .5$. No significant deviation was found either with the 0's excluded ($P_{BS} = .27$ and $P_W = .39$) or with the 0's divided equally between the +'s and −'s ($P_{BS} = .35$ and $P_W = .44$).

Comparison of the unscored amplitude data for brine shrimp and water ejections by use of the Mann-Whitney nonparametric test for unpaired observations revealed no interdependence ($P = .19$). Similar comparisons were made of the amplitude data of the brine shrimp ejections with their control periods and the amplitude data of the water ejections with their control periods ($P = .12$ and $P = .50$, respectively). No significant deviation from the null hypothesis that brine shrimp and water ejections produce no effect was found.

Table 2. Summary of +, −, and 0 scores used in binomial analysis. Data were pooled on the basis of no interaction between simultaneous recordings within groups of four plants.

Ejection	Number of scores		
	+	−	0
Brine shrimp ($N = 60$)	10	14	36
Water ($N = 40$)	6	8	26

Considerable effort was made to conduct these experiments in accordance with the published account and directly communicated suggestions of Backster. One variation from his published method was our use of high-gain a-c coupled amplifiers instead of psychogalvanic response (PGR) d-c coupled amplifiers, a change which Backster viewed as acceptable before our study. The a-c amplifiers have the advantages of stability and supression of in-phase interfering signals, and they pass essentially no current through the preparation. At the gains we used (5 μv/mm) the system was capable of recording the smallest potentials so far reported for plants. Indeed, the regular waveforms seen in Fig. 1 appeared in more than 30 percent of our recordings and are analogous to those reported by Pickard (*9*). It is conceivable that if the effects Backster recorded were due to a pure series resistance change in the leaves and this resistance was the only resistance in the leaves, the a-c amplifier would not have detected the change. Such a model would be at variance with electrophysiological views that leaves are better represented as a combination of series and parallel resistances.

In contrast to Backster's method, our controls had the advantage of being obtained from the experimental leaf-electrode combination within seconds of the experimental period rather than from completely different preparations studied on different days. We obtained no evidence of similarity of "responses" among plants constituting a particular group of four. Thus, each plant was treated as an independent preparation, giving a total of 60 brine shrimp ejection periods compared to Backster's 13. Rigorous methods of statis-

tical analysis were used. When the binomial test was applied to Backster's data, they were found to be statistically significant by our criteria.

We believe that we matched, and in several instances improved on, Backster's experimental techniques, such as controls, shielding, number of observations, methods of analysis, and number of shrimp killed per injection. We obtained no evidence of primary perception in plants. While the hypothesis will remain as an intriguing speculation one should note that only the limited published data of Backster support it.

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References and Notes

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4. J. Collier, *Reader's Digest* **102**, 151 (February 1973); R. Martin, *The Wall Street Journal* **179**, 1 (2 February 1972); J. Robbins and C. Robbins, *Nat. Wildl.* **9**, 21 (November 1971).
5. The growing medium consisted of equal parts of sterile soil, peat, and pearlite; 3 ounces of 20 percent superphosphate fertilizer was added per bushel. Illuminance was natural daylight. The maintenance program consisted of Peters 20 : 20 : 20 water-soluble fertilizer alternated weekly with KNO_3 at 1.0 pound per 100 gallons of water, Malathion sprayed weekly, and plants watered daily. The temperature was 70°F during the day, and 60°F at night.
6. *Artemia salina* were obtained in dehydrated egg form (Longlife Hatch Pack, Longlife Aquarium Products, Harrison, N.J.) and were grown to maturity in a Longlife brine shrimp hatchery according to the directions included with the unit.
7. The electrodes were constructed of polished 0.027-inch stainless steel, shaped to dimensions of 1.0 by 1.4 inches, with a soldering tab extending beyond the end at a small angle to the plane of the electrode. Leads consisted of 4 feet of No. 26 PVC type B wire and were attached to the tab with silver-based solder. The entire finished electrode was polished to eliminate sharp areas and its contact surface was covered with 12-ply gauze which had earlier been impregnated with a hot solution of 0.25*M* NaCl in 1.25 percent Difco Bacto agar and then trimmed to meet the electrode border.
8. The recording electrodes were connected to Grass model 7 polygraphs equipped with 7P5A wideband electroencephalogram preamplifiers. Routinely the system sensitivity was maintained at 5 μv/mm with bandpass filters set at 0.15 to 15.0 hertz (half-amplitude frequencies with slopes of 12 db/octave). The manufacturer's input impedance rating under push-pull operation was greater than 3.0 megohms and the common mode rejection ratio was 1 : 1600.
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10. We are greatly indebted to E. Daniels for technical assistance and illustrations. We thank B. Schilling, A. Morrison, R. Wallace, and R. Marshall for technical assistance, capital equipment, photography, and constructive criticisms, respectively. Supported by a grant from the Mary Reynolds Babcock Foundation, Inc. We are indebted to C. Backster for his encouragement and technical advice.

25 February 1975

Central Projection of Optic Tract from Translocated Eyes in the Leopard Frog (*Rana pipiens*)

Abstract. *In* Rana pipiens *embryos, eye anlagen were moved to the evacuated ear position, where they continued to differentiate and sent their optic nerve fibers into the hindbrain. Upon entering the medulla, the optic fibers turned caudally, penetrated the spinal cord, and traversed the dorsolateral white matter to the caudal end. We found this pattern of growth in every animal; the optic fibers did not enter the tecta. These results suggest the existence within the neural tube of a three-dimensional gradient system to which embryonic optic fibers are responsive and which may guide the normal development of the visual pathway.*

During development of the vertebrate visual system, nerve fibers from the ganglion cells in the retina achieve an orderly projection onto specific populations of cells in the central nervous system. The pathway that the fibers take in reaching the central nuclei is identical in different individuals within a given species; this suggests that the fibers are guided systematically in their growth through the central nervous system. Most previous investigators of axonal guidance in the visual system have utilized the regenerative capabilities of the optic tract in adult anurans and teleost fishes (*1*). These studies have revealed that severed or slightly deflected optic nerve fibers can often reestablish their central connections. But little is known about the mechanisms in embryonic development that are responsible for directing the initial growth of axons from the retinal ganglion cells within the eyecup to their final destination in the central nervous system. Is this initial projection an intrinsic property of the optic nerve fibers themselves or, instead, does it depend on directional guidance from the embryonic brain during its early development?

We investigated this question of axonal guidance during embryonic development by drastically altering the pathway between the retina and the cells within the central nervous system upon which the optic nerve fibers normally terminate. We present here the results of our experiments in which eye primordia of leopard frog (*Rana pipiens*) embryos were relocated in the position normally occupied by the ear anlage. In this way we have been able to cause the optic nerve from the transplanted eye to enter the hindbrain, thus completely altering the normal diencephalic route from the retina to the optic tectum in the midbrain (*2*).

The transplantation operations were performed on Shumway stage 16 to 18 *R. pipiens* embryos (*3*). The left ear capsule was removed and either the left (type 1) or the right (type 2) primordial eye was inserted in its place. A small part of the forebrain tissue adjacent to the optic stalk was routinely taken in the removal of the eye primordia. This forebrain tissue fused with the hindbrain, thus forming a tissue bridge between the medulla and the eyecup which facilitated penetration of the optic nerve from the transplanted eye into the central nervous system. The experimental animals were raised individually until late tadpole or postmetamorphic stage. A modified Holmes silver stain was used to examine the morphology of the transplanted retina and optic nerve. The optic tract from the transplanted eye was traced within the central nervous system by intraocular injection of 1 or 2 μl of tritiated L-proline [1.3 μc/ μl in H_2O; New England Nuclear (6.8 c/m*M*) or Schwarz/Mann (3 c/m*M*)] 24 hours prior to killing. These specimens were then fixed in Carnoy's fluid, serially sectioned at 10-μm thickness, and examined by light microscope autoradiography.

The translocated primordial eye develops into an externally normal eye (Fig. 1A). Light microscope examination of the retinas in these transplanted eyes revealed cellular layering and cell densities that appear similar to those in the retinas of the normal (nontransplanted) eyes. Thus, translocation of the eye to a different region on the animal's head does not qualitatively alter the normal development of the organ. Furthermore, the retinas of these translocated eyes are capable of visual function. In two preparations, we have inserted gold- and platinum-coated indium micropipettes (tip diameter $\simeq$ 6 μm) into the transplanted optic nerve as it enters the cranial cavity. We recorded action potential activity in these nerves in response to small spots of light in the visual field of the transplanted eye.

Figure 1B demonstrates the altered geometry of the brain of a typical postmetamorphic animal that had a type 2 transplant operation. The optic nerve from the normal (left) eye approaches the brain ventrally. Most of the fibers in the normal tract cross the midline in the anterior region of the diencephalon and then proceed in a dorsocaudal direction along its lateral margin to enter the right optic tectum (*4*). The optic nerve from the translocated eye, however, does not enter the diencephalon; instead, it penetrates the medulla at approximately the level normally occupied by the eighth cranial nerve. The left tec-