Neural Activity in the Parietal Eye of a Lizard

Abstract. Electrical signs of activity in response to illumination of the parietal eye of the American chameleon, *Anolis carolinensis*, have been investigated. The responses were of two types. Under conditions of direct-coupled amplification, with glass pipette electrodes recording extracellularly from the retinal surface, the response consisted of an increase in negativity maintained throughout prolonged illumination. With capacitance-coupled amplification and metal electrodes, brisk mass discharges of nerve impulses were detected at the onset and cessation of illumination. During illumination a less vigorous maintained discharge was observed.

The parietal organ is an eyelike structure located in an orifice between the parietal bones in the skulls of some lower vertebrates. The organ is highly developed in the cyclostomes, but it is also present in some reptiles and amphibians. In certain lizards the organ has a translucent cornea, a lens, and a retina containing cells that resemble the photoreceptors and ganglion cells of vertebrate lateral eyes (*1*). This retina is unlike that of the lateral eyes in that it is not inverted; that is, the incoming light falls on the photoreceptor-like cells without first passing through neural elements. The cells resembling photoreceptors have recently been studied by means of the electron microscope (*2, 3*). Like the rods and cones of vertebrate lateral eyes they have lamellated outer segments.

In spite of the anatomical similarity of the parietal organ to lateral eyes, their function has been a matter for conjecture. In 1890 Ritter (*4*) concluded that the parietal eyes of several native American lizards are degenerate and have no function. Dendy (*5*) described the more prominent parietal eye of *Sphenodon* but was unable to demonstrate that it was functional. Recent results of obscuration and extirpation experiments in other lizards are equivocal. Evidence (*6*) does indicate, though, that the photophobic activity of the animal may be decreased if the eye is rendered inactive by any means [the crayfish is affected in much the same way after removal of its abdominal ganglion photoreceptor structures (*7*)]. Among the functions that have been suggested for the parietal organ are secretion of hormones, manufacture of vitamin D, and thermoreception (see *3, 6*, and *8* for bibliography). It has also been suggested that, since the organ resembles an eye in structure and has a nerve leading into the brain, there should be nervous activity associated with illumination (*3*). Our work supports the latter hypothesis.

The investigation was carried out mainly on the adult American chameleon, *Anolis carolinensis*, but a few experiments were performed on other types of lizards (*Lacerta muralis, Lacerta viridis*, and an unidentified species of iguana) with results essentially the same as those described below. The animal was immobilized on a splint with Tackiwax (Central Scientific Co.). A cotton wick soaked with frog Ringer's solution was inserted into the mouth. This wick was in contact with a silver chloride-coated silver tube and served as the indifferent electrode. The cornea and lens were dissected from the eye and the recording electrodes were placed on the exposed retina by means of a micromanipulator. Direct-coupled (d-c) recordings were obtained by using glass pipettes with a tip diameter of about 2 μ which were filled with frog Ringer's solution. Glass-insulated platinum-iridium microelectrodes (*9*) were used with capacitance-coupled (a-c) amplification.

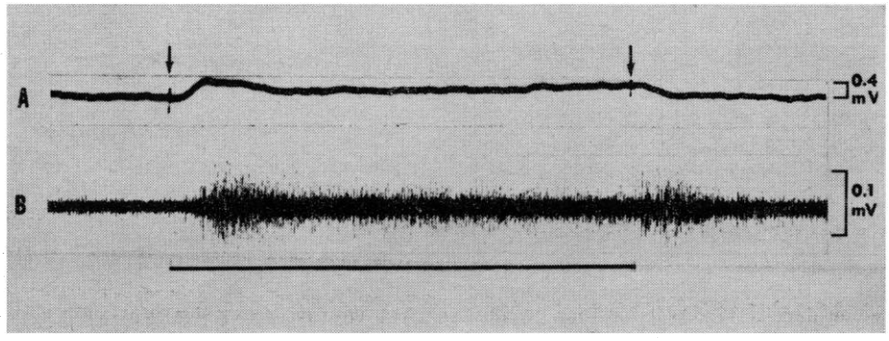

Fig. 1. Responses to illumination from the parietal eye of *Anolis*. *A*, d-c recording. Stimulus duration 3.5 seconds. Arrows indicate artifacts resulting from shutter operation. *B*, a-c recording. Stimulus duration 4.0 seconds. For both records: heavy line under *B* indicates presence of stimulus. Positive potential at the recording electrodes is down.

Two types of responses to illumination were recorded from the parietal eye: slow potentials (electroretinograms) and mass discharges of nerve impulses. The electroretinogram (ERG) was obtained in almost every preparation, and in addition was recorded from several eyes which had been excised from the lizards' skulls. The mass discharges of nerve impulses were difficult to obtain, being recorded in six cases, about 5 percent of the total number investigated. Nerve impulse activity was recorded only when the microelectrode appeared to be near the surface of the retina, never when it was deeper, and these responses were not obtained when the stimulating light was shifted from the parietal eye to other parts of the head (including the lateral eyes). It has not been possible to isolate the activity of single neurons.

The ERG is illustrated in Fig. 1*A*. About 0.1 second after the onset of illumination the electrode on the retina becomes negative with respect to the indifferent electrode. It then becomes somewhat less negative, and this potential is sustained for the duration of the illumination, following which it returns to the resting level. Figure 1*B* depicts the discharge of nerve impulses in response to illumination. About 0.1 second after the onset of illumination a vigorous discharge is observed ("on" response). This discharge subsides somewhat, but is maintained during the illumination. The cessation of illumination is followed by another burst of activity ("off" response) that can be inhibited by reillumination (not depicted). The amount of nervous activity increases with light intensity. Occasionally the "on" response has been observed to be more vigorous than the "off"; however, no cases were seen in which the "off" response was totally absent. Light and dark adaptation affected both the ERG and the impulse discharges qualitatively in the same way they affect lateral eyes. Reduction of the red and infrared end of the spectrum with broad band filters did not reduce the response to light, so it seems likely that the eye is not primarily an infrared or thermal receptor.

We would like to stress three of our findings: (i) The parietal eye responds with nervous activity to stimulation by light. (ii) As in the nerve response of the more complex lateral eyes of vertebrates, there is an "on" response which is maintained during illumination and an "off" response (*10*). (iii) No "off"

component was observed in the ERG. In this respect the parietal eye resembles the eye of the scallop, *Pecten irradians,* which has a vigorous "off" response but lacks an "off" component in the ERG (*11*).

Further investigation of the parietal eye, which has only two neural cell types (photoreceptors and ganglion cells), may contribute to a clearer understanding of retinal processes. Techniques are now being developed to study the action spectra of the eyes and to isolate the responses of single units (*12*).

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Thermodilution Method for Measuring Cardiac Output of Rats by Using a Transistor Bridge

Abstract. Featuring an interlocking bridge amplifier, our new method measures cardiac output in animals from man to the rat, and one can perform many multiple assays with safety in the same animal with accuracy and reliability as reported, using room-temperature saline, whether the animal is in the normal or hyperthermic condition.

Numerous workers (*1, 2*) have established the thermodilution method to be sufficiently accurate and reliable for measuring the cardiac output of dogs, it being comparable to the Fick and dye-dilutions methods. However, the limited sensitivity of these methods has required a relatively large quantity of very cold saline for injection, which limits their use to larger animals for study. Our method features significantly greater sensitivity, requires only 0.05 ml of room-temperature (about 25°C) saline for injection, and can be used with animals as small as a rat or even a mouse or hamster. Although the principle has been utilized for recording dilution curves in dogs and human beings (*3*), this report describes the instrumentation and more exacting procedure required for the study of the albino rat.

The circuit employed is a transistor-amplified dual interlocking bridge as shown in Fig. 1, with an amplification of 20 to 90, depending on the transistor selected. Both interlocking bridges are balanced simultaneously by the 1000-ohm, ten-turn potentiometer. The output connects to a medium- or high-gain commercial d-c amplifier and recorder of almost any type.

The switch S-1 can be turned to either the calibrating or recording position, so that by placing the thermistor in a solution of measured temperature one can adjust the 5000-ohm potentiometer to give an identical pen position on the recorder for that selected temperature, such as 37.4°C, for example. The switch S-4 momentarily shorts the output to null to verify accurate bridge balance, and the other switches, S-2 and S-3, are the contacts of a multiple switch to simultaneously connect the power supplies.

Any of several transistors can be used for Q-1 if they are PNP types of relatively high gain. For example, CK722, CK721, 2N107, 2N190, 2N1265, and 2N109 have been found satisfactory by test, especially the 2N109. Exhaustive experimentation has shown the circuit to be remarkably stable and linear if a reasonable precaution is used to select a transistor with low noise and a low open-circuit current. Transistor testing devices are useful, but trial tests in the circuit are more practical and pertinent. It should be especially observed that the transistor input connection is not, and should not be, grounded. There should be a single ground connection at the output as indicated. It may be desirable to adhere the case of the transistor to a metal plate, or embed it in plastic within a small metal receptacle attached to a metal plate or the chassis. Though not necessary for ordinary environmental fluctuations, this added heat sink

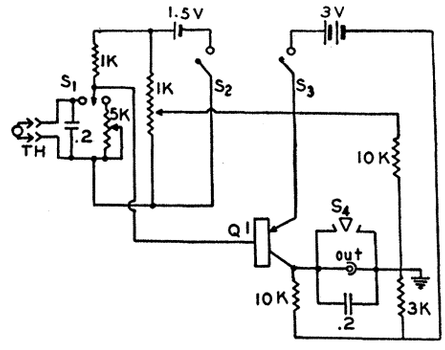

Fig. 1. Diagram of the preamplifier and interlocking bridge used with the measurement of cardiac output by thermodilution.

tends to damp and integrate any temperature fluctuations of the transistor over long measurement periods.

When a Sanborn Twin-viso d-c amplifier and recorder was used, at full gain setting (X1), a temperature change of 0.05°C gave a 40-mm deflection (80 percent of full scale). Other ranges can be used for calibration or measurements; a 1.0°C temperature change gave a 40-mm deflection with a gain attenuation of X20. Our temperatures were calibrated with a Bureau of Standards thermometer. With assays of different thermistors, we found that thermistors rated at about 2000 ohms (500 to 5000) at 25°C were most effective and efficient in this particular circuit, when used in the tip of a PE-10 polyethylene plastic tubing. The time constant of a well-constructed 2000-ohm thermistor was 0.4 sec, well beyond the required response speed for cardiac output measurements.

Fifty albino rats, weighing 350 to 550 g and anesthetized with pentobarbital sodium (24 mg/kg) injected intraperitoneally, were used in the animal studies. With each animal a 6-cm-long PE-50 catheter was inserted in the jugular vein to the right atrium, and the PE-10 thermistor-tipped tubing was passed down the right carotid artery to the aorta. For each assay, 0.05 ml of normal saline at room temperature was injected into the right atrium. This venous catheter was allowed to fill with blood before each injection, and a volume correction was made for this dead space. Between injections, the circulating blood was allowed to equilibrate thermally. Calculations of cardiac output were made by using the modified and corrected formula of Fegler (*1*).

The cardiac outputs of the total group of animals ranged from 196 to 299 ml/min per kilogram, and averaged