Long-Term Activity Recording in Small Aquatic Animals

Abstract. *We have built an activity-recording device based on the principle of detecting slight changes in the heat conductance of an aquatic environment by means of a thermistor. A major advantage of the device is that it can be used with a simple event recorder, thus combining high sensitivity with low cost. The equipment has been tested with automatic mechanical stimulation, and has been used for long-term recording of the swimming of small marine crustaceans. Problems of stability and reliability have been solved, and potential applications of the device seem widespread.*

The recording of locomotor activity in small aquatic animals presents difficulties because the water movements produced are too weak to be detected by devices previously described (*1*). One solution to this problem has been the use of optical devices (*2*), but this procedure interferes with the light conditions during the experiment. The activity sensor we have designed eliminates this difficulty by detecting variations in heat conductance of an aquatic environment associated with slight water movements. In principle, the device is remotely related to an activity sensor designed by F. E. J. Fry (*3*) for use with fish.

The thermistor (*T1*) serves as a heat source (Fig. 1). The heat produced by the Joule effect is lost at a constant rate if the water temperature is constant and the water is motionless; at the same time, the temperature of the thermistor reaches a steady state. The slightest disturbance of the water increases the heat loss; the temperature of the thermistor drops, and its electrical resistance thereby increases. The thermistor *T1* is incorporated into a Wheatstone bridge which includes two variable resistances (*R1* and *R2*) and, in addition, a second thermistor (*T2*) identical to the first. This second thermistor is shielded from water movements by a constricted plastic cylinder (*SH*) provided with a slit (*SL*) to avoid accumulation of air bubbles. Both thermistors are subject to the same long-term temperature variations, thereby eliminating, within limits, the artifacts which might be produced by temperature variations.

The voltage variations across the Wheatstone bridge are related to the movements of water in which the thermistors are immersed. To record these voltage variations directly might seem to be the simplest way to register activity. In long-term experiments, however, it would require a very expensive potentiometer to eliminate the problems of baseline drift. The circuitry (Fig. 1) permits adaptation of the Wheatstone bridge for use with an event recorder which is standard equipment in studies of activity rhythms. This procedure also eliminates the need for further data reduction.

The transducer is based on the following principle. The capacitor (*C*) is charged through the transistor 2N396A. While the water is motionless, the bridge is adjusted so that the voltage applied to the base of transistor 2N396A is slightly positive relative to the emitter; the voltage across the capacitor (*C*) is then low. If the animal moves, the base of transistor 2N396A becomes negative relative to the emitter, thus increasing the voltage across the capacitor. When the voltage across the capacitor reaches a constant threshold value, the unijunction transistor 2N1671B discharges the capacitor through the relay (*RE*). Thus

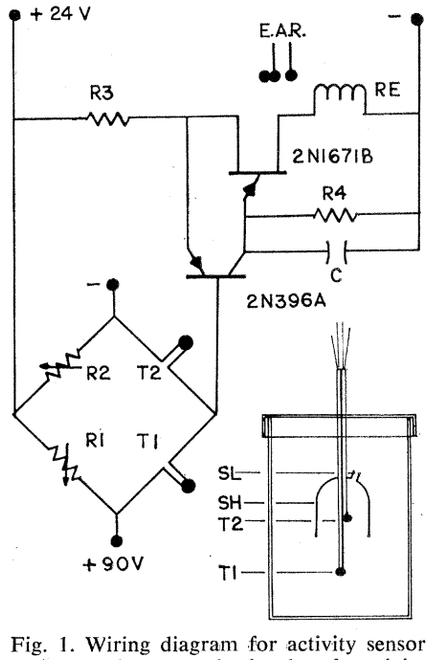

Fig. 1. Wiring diagram for activity sensor and transducer, and sketch of activity chamber. Following are details of the electronic components used: *T1* = *T2* = 100 kΩ at 25°C; *R1* = *R2* = 10 kΩ; *R3* = 350Ω; *R4* = 1.5 MΩ; *C* = 20 μfarad; *RE* is a Potter and Brumfield RS–5D relay (single-pole, double throw, 2-amp, 6-volt, d-c).

an electrical pulse is sent to the event recorder (Esterline Angus Model AW operation recorder). As long as the base of the transistor 2N396A remains negative, the capacitor charges and discharges continuously, the frequency being related to the activity of the animal.

In order to keep the temperature of the thermistor only slightly above water temperature, we use high-resistance thermistors (100,000-ohm at 25°C). In our experimental conditions, the core of the thermistor was about 3°C above water temperature. The specific configuration of the Wheatstone bridge (Fig. 1) allows adaptation of the high-resistance thermistors to the relatively low input resistance of the transistor 2N396A.

The activity sensor responds to any temperature gradient between the two thermistors. To avoid temperature gradients it is essential to maintain the activity chamber at a relatively constant temperature, and desirable to keep the surrounding air saturated with water vapor to limit evaporation from the free water surface. The two thermistors are rigidly connected, thereby making the sensor unresponsive to moderate vibration of the unit. To assure stability of the system, the voltage supplied to both the bridge and the transducer should be stabilized to about ±1 percent.

The equipment was tested for reliability and stability over a 4-week period. During that time, intermittent water movements were automatically produced at regular intervals. The recording was of uniform intensity throughout the test. The equipment was also tested for spontaneous firing due to voltage drift; none was observed with the present circuitry.

An extended activity record (Fig. 2) was obtained with the device shown in Fig. 1. The isopod was collected intertidally on 17 March 1966, and kept in constant conditions until the recording was begun on 25 March. The experiment was terminated on 9 June 1966, at which time the animal was alive but behaviorally abnormal (general lethargy, coupled with failure to bury in sand when stimulated). The terminal damp weight of the animal was 28 mg; the length was 7 mm. During recording, the animal was in a glass chamber (5 cm in diameter, 8 cm high) filled with seawater and a 5-mm layer of sand, in continuous dim light (about 0.1 lux) at a tem-

perature of 20° C. No food was provided.

Previous studies of populations of this species indicate that freshly collected animals show an activity rhythm characterized by two (usually unequal) bursts of activity per day, synchronized with the tides on the shore at the point of collection. This rhythm can be entrained in the laboratory by mechanical stimuli (*4*). Several features of the record in Fig. 2 are of biological interest: (i) a tendency for more intense activity to recur at intervals of about 14 to 16 days; (ii) a free-running period of the rhythm which shortened during the recording; and (iii) an apparent drifting together of two components of the rhythm about midway through the record. A simultaneous recording from another, adjacent animal which survived about 60 days indicates that neither the days nor the times of most intense activity coincided for the two animals (most intense activity for the other animal: 10 to 12 p.m., 4 April to 9 April; and midnight to 4 a.m., 8 and 9 May). Such results demonstrate that these features of the recordings are not due to influences of the environment on either the animals or the activity sensors.

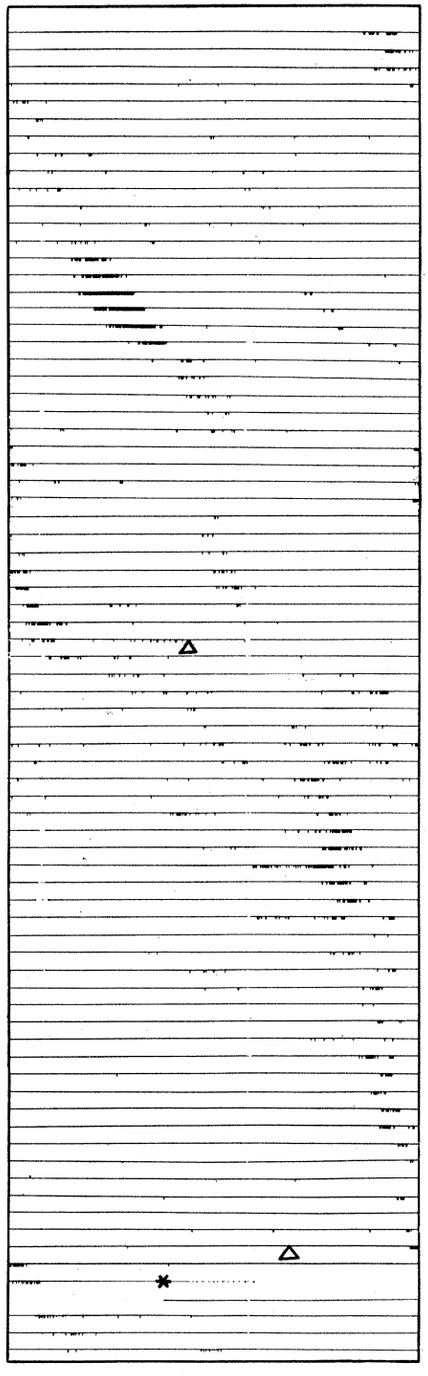

Fig. 2. Record (77 days) of the swimming activity of an isolated adult isopod, *Excirolana chiltoni,* under constant conditions. Successive 24-hour strips of record (midnight to midnight) mounted beneath each other. Triangles indicate times at which distilled water (about 30 ml) was added to replace evaporation; asterisk indicates pen failure, corrected about 24 hours later.

A subsequent recording from another isopod indicates that repetition of sessions of intense activity at intervals of about 2 weeks is not a unique feature of the behavior of the animal whose activity is shown in Fig. 2. During a recording from 9 June to 5 July 1966, this third animal showed very intense activity between 4 and 8 a.m. 11 to 13 June, and between 5 and 8 a.m. 25 to 28 June.

The device described here could obviously be used in its present form for long-term, inexpensive monitoring of the activity of larger aquatic organisms than are considered here (for example, fish of any size in a larger aquarium). Modification of the apparatus for use with much smaller aquatic animals also appears to be possible by using thermistors of much smaller thermal inertia, or by inserting an additional transistor in the transducer circuitry. Such modification has not yet, however, been attempted; environmental factors, including temperature, vibration and evaporation, as well as instrumental noise and drift will ultimately place a lower size limit on the organisms for which the sensing principle is useful (*5*).

A. A. HEUSNER*
Center for Health Sciences, University of California, Los Angeles
J. T. ENRIGHT†
Department of Zoology, University of California, Los Angeles

References and Notes

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* Present address: Institut de Physiologie, Faculté de Médecine, Strasbourg, France.
† Present address: Department of Oceanography, Scripps Institution of Oceanography, University of California, La Jolla.

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Electrophoretic Heterogeneity of Mammalian Galactose Dehydrogenase

Abstract. *Electrophoretically distinct forms of galactose dehydrogenase were demonstrated in various tissues of the rat. Phylogenetic comparisons revealed considerable variation with species in mammalian liver zymograms; virtually no activity was demonstrable in fish, pigeon, and frog liver. Ontogenetic studies of the rat revealed sequential appearance of liver isoenzymes.*

D-Galactose can be converted to D-xylulose by rat liver enzymes (*1*); the first enzyme involved, galactose dehydrogenase, has been purified 100-fold and its properties have been studied (*2*); it is a nicotinamide adenine dinucleotide (NAD)–requiring enzyme found in the soluble cellular fraction of the liver of several mammalian species. We now describe electrophoretically distinct forms (isoenzymes) of galactose dehydrogenase in rat tissues, compare the electrophoretic patterns of liver extracts from different species, and describe changes that occur in the developing rat liver.

Vertical starch-gel electrophoresis was performed with 0.0053*M* phosphate buffer, *p*H 6.7, at 5 volt/cm for about 15 hours at 4°C. A thin layer of gel was then sliced and incubated at room temperature for about 45 minutes in a mixture, modified from that used for staining lactic dehydrogenase (LDH) isoenzymes (*3*), that contained phenazine methosulfate (0.02 mg/ml), NAD (1 m*M*), nitroblue tetrazolium (0.5 mg/ml), sodium cyanide (1 m*M*), tris buffer, *p*H 8.4 (50 m*M*), and galactose (50 m*M*).

Tissues of Sprague-Dawley rats were homogenized in 0.01*M* phosphate buffer, *p*H 7.0, and centrifuged at 32,000*g* for 90 minutes. The dialysed supernatant was separated by starch-gel electrophoresis. Liver microsomal prep-