Comp. Biochem. Physiol. Vol. 96A, No. 4, pp. 473–477, 1990
Printed in Great Britain

0300-9629/90 $3.00 + 0.00

A COMPUTER-AIDED PHYSIOLOGICAL MONITORING SYSTEM FOR CONTINUOUS, LONG-TERM RECORDING OF CARDIAC ACTIVITY IN SELECTED INVERTEBRATES

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(*Received 22 January* 1990)

Abstract—1. A computer-aided physiological monitoring system (CAPMON) is described which permits long-term, continuous recording of cardiac activity in selected crustaceans and molluscs.

2. Infra-red phototransducers used with the system are simple to attach and allow non-invasive measurement of physiological function, thereby minimising disturbance to the test organism.

3. Applications of the CAPMON system in pollution studies and in clinical settings with Man, are discussed.

INTRODUCTION

Since the mid-1970s there has been a growing awareness of the value of physiological monitoring in the assessment of pollutant impacts (Vernberg *et al.*, 1977; Widdows *et al.*, 1980). The rational basis for such monitoring has recently been discussed (Depledge, 1989). A prerequisite for identifying abnormal responses associated with pollutant exposure is a detailed knowledge of the normal repertoire of physiological responses of the animal under investigation. However, most attempts to gain such information have been severely hampered by the lack of suitable transducers capable of acquiring data over long periods (weeks or months), without harming the test organisms. This perhaps explains why the vast majority of techniques reported in the literature are seldom taken up and used in subsequent studies. Other problems are that some methods require the development of considerable technical skills to operate expensive, sophisticated equipment. Furthermore, with the majority of techniques available, it is impractical to monitor the physiology of more than 2 or 3 animals at a time.

The method described here permits cardiac activity of 8 animals to be monitored non-invasively, continuously for long periods using an automatic, computer-controlled system. The apparatus was developed for use with reptant decapods, but has also been used successfully with thin-shelled bivalves and polychaete worms. The versatility of the system permits a great variety of applications, including use in pollution studies and, with Man in clinical settings.

MATERIALS AND METHODS

Overview of the system

The system comprises a set of 8 transducers for the measurement of rate functions (heart rate, scaphognathite rate, ventilation rate, etc.), a signal-conditioning interface and a standard, IBM compatible computer (PC). Rate functions per minute as recorded simultaneously from the 8 transducers and displayed on the monitor screen in graph form. The data acquired is also stored on disc for analysis later. A schematic representation of the system is shown in Fig. 1.

The transducers

Depledge (1984a) developed a non-invasive transducer for measuring cardiac and ventilatory activity in decapods which operated by emitting low intensity, visible light into the test animal and detecting variations in reflected light intensity associated with heart beat or scaphognathite activity. The same principle has been employed here, but has been refined by using a smaller, lighter transducer operating in the near i.r. range (Fig. 2).

Principle of operation. The reflective optocoupler consists of an i.r. light emitting diode (LED) and a phototransistor detector. Both elements are mounted parallel to each other, facing in the same direction (Fig. 2a). The phototransistor detects variations in i.r. light intensity and generates a light-dependent current. This current, which is a function of the reflected light, is then amplified and filtered prior to input to the transducer/computer interface.

Attachment of the transducers. To record cardiac activity of a crab the transducer is affixed over the cardiac region of the carapace (Fig. 2b). In various trials, it was found that a convenient way to achieve this is by using a rapid-setting, non-toxic adhesive (e.g. a "superglue" such as Histoacryl Blau, Braun Melsungen AG., West Germany) to fix a 1.5 cm diameter plastic ring to the animal. The ring itself is fitted with a screw allowing the transducer to be secured in position (Fig. 2b). This procedure involves contact with the animal for a matter of seconds and allows connection and disconnection the transducer at will, so that animals can be held for several months (or even years), in various conditions with recordings being made periodically. The transducer is connected to the computer interface by fine, flexible wire so that the animal is virtually unrestrained within its aquarium. The transducer package is water-tight and permits continuous recording both in air and water. It weighs approx. 600 mg.

The transducer/computer interface

The circuitry incorporated in the interface is shown in Fig. 3 and is described below.

An operational amplifier U1A (part of TL 084) is coupled as a standard, non-inverting amplifier with adjustable gain (P1) from 260 to 2600 times. U1B is coupled as a 2 pole filter with F 3 dB at 10 Hz. C4 and R9 act as a high pass filter with F 3 db at 10 Hz. U1C is coupled as an inverting

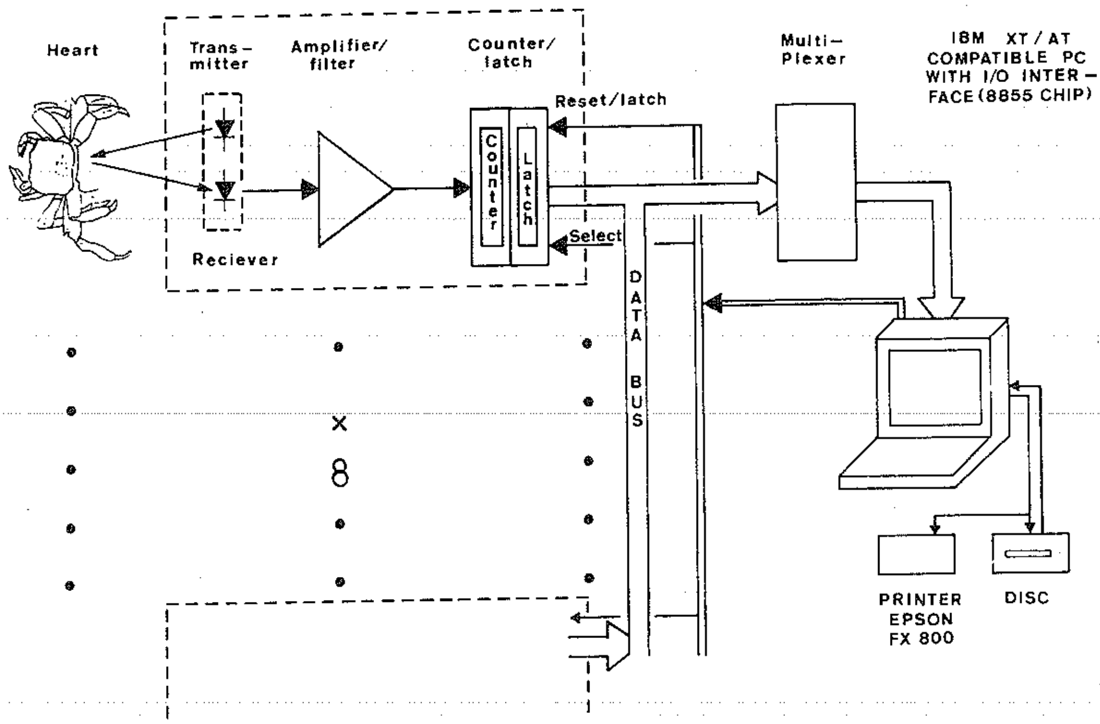


Fig. 1. Overview of the computer aided physiological monitoring system.

amplifier with a gain of 10 times. A "noise" limiting system (D1 and D2) reduces "noise" on the baseline. U1D is coupled as either an inverting or non-inverting buffer so that the best signal to noise ratio can be selected.

R15-D3-D4 is a protection circuit for U2A (a Schmidt trigger 4093). U2A converts the signal to a pure square wave. U3A (a retriggerable monostable, multivibrator 4098) prevents the passage of pulses with a higher repetition rate than 40 Hz. This prevents multiple triggering by "noisy" heart beats.

U4 (a 12 bit binary counter 4040) counts pulses from U3A. Initially, the counter is reset by the computer (PCO) and rate counting begins. At the end of each counting period, counts are fed to U5 (a three state latch 4034). U4 is reset and initiates the next count. The output from U5 is transferred to Port A on the PCI0 interface board (located in the computer), so that the software programme can read rate values.

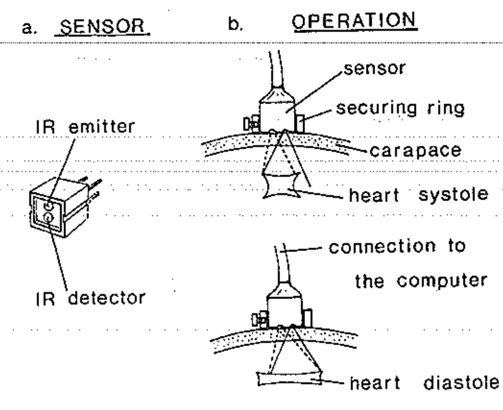


Fig. 2. Infrared phototransducer. (a) The sensor. (b) Sensor attachment.

Data acquisition and storage

A "Setup" programme allows variations in reflected i.r. light intensity (associated with heart beats, etc.) to be viewed as if input into an oscilloscope. The display comprises the signal from the transducer amplifier and the output from the trigger circuit (Fig. 4). It is then possible to adjust the amplifier gain so that triggering coincides with each heart beat. Typical heart beat patterns which appear on the computer monitor screen are shown in Fig. 4(a–c). In some cases a double upward peak is seen (Fig. 4b). Since the counter mechanism determines rate by measuring time between peaks, such double peaks might result in counting errors. This problem has been overcome by the incorporation of an inverting circuit in the transducer interface. U1D (Fig. 3) acts as a differential amplifier and the switches are coupled so that the signal can be inverted (Fig. 4c). This enables the single negative peak to be counted instead, thereby yielding the correct rate.

When the system is operating, data is acquired and stored with the aid of two software programmes written in Turbo Pascal (4.0) and using the graphics toolbox.

A second programme (designated CAPMON—computer-aided physiological monitoring) displays the heart rate per minute (or some other rate function) of up to 8 animals simultaneously in 8 different "windows" (Fig. 5). There is also the option of selecting any of the windows for enlargement (Fig. 5). All the data are saved on floppy disks and can be viewed again using a display programme (CAPDIS). Also, the raw data can be exported to Lotus 123 for manipulation and statistical analysis.

EVALUATION OF THE METHOD

Direct observation of heart beat via a small hole drilled in the carapace confirmed that beating coincided with the peaks displayed on the monitor. Also, the rate of beating was the same as counted by the computer system. Problems have seldom been encountered, but the operator should be aware that if transducers are incorrectly positioned or become loose during operation, spurious counts may arise. To avoid this, it is worthwhile periodically calling the "Setup" programme to check the heart beat pattern and to ensure that each beat is triggering the counter.

The system has now been operated in excess of 1000 hr in standard laboratory conditions without difficulty. It has also been used in a very basic field laboratory in sub-tropical Hong Kong with equal ease. The system has been adapted by research students to investigate cardiac activity rhythms in the freshwater bivalve, *Anodonta*.

DISCUSSION

The computer-aided physiological monitoring system described here is relatively simple to construct, is easy to use and has been found to be extremely reliable. The transducers are non-invasive, leaving the test organism completely intact. Transducer attachment can be completed within a few seconds, thus minimising disturbance to the animal. Recordings can be obtained with equal ease in air or water. The fact that 8 animals can be monitored simultaneously is a great advantage over earlier systems (Depledge, 1978). Monitoring can be conducted 24 hr/day for indefinite periods. Thus, the system lends itself to chronic studies of pollutant toxicity or investigations of tidal and diurnal rhythms. As one diskette is filled with data, so it can be replaced with another. The advent of small, self-contained battery-operated, portable computers now enables field use of the system.

Important aspects of the system are its versatility and adaptability. For example, a variety of transducers measuring rate functions in different ways (E.C.G., impedance techniques, pressure transducers, etc.) are readily interfaced with the computer and data handled using the soft-ware programmes mentioned above. Applications in diverse areas of biology are feasible, notably in pharmacology and chemical toxicity testing. Also, the potential for using the system in a clinical setting to monitor cardiac activity (via finger pulse plethysmography) is clearly enormous. The opportunity to monitor 8 patients simultaneously for a relatively low cost, without risk of infection must surely be attractive, particularly to those working in less well-equipped hospitals in remote localities where sophisticated monitoring systems are not currently available, but would be of value.

Major questions which will determine whether or not the equipment will be widely used are:

(1) Can rate functions (such as heart rate, ventilation rate, etc.) be used to provide useful and meaningful indicators of physiological state?

(2) Can the system be reproduced easily for a reasonable cost?

With regard to the first point, numerous authors have found heart rate to be a useful indicator of changes in physiological state in crustaceans, molluscs and fish (Coleman, 1974; Lunn *et al.*, 1976; Depledge, 1984b, 1985; Taylor, 1988). The potential

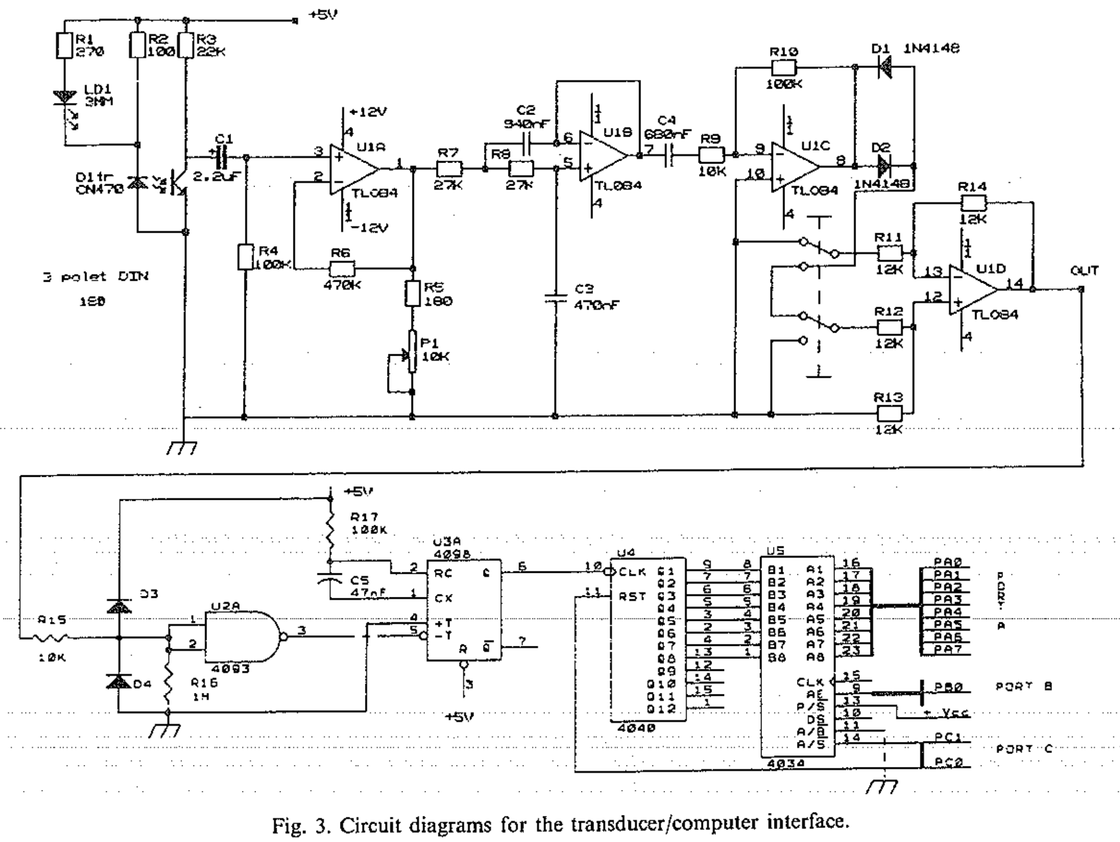


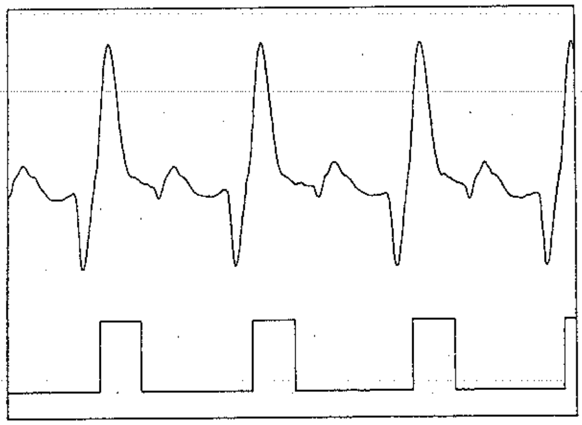
Fig. 3. Circuit diagrams for the transducer/computer interface.

of such monitoring is now being recognised by both industry and environmental protection agencies as a means of assessing the biological effects of pollutants. For example, in Finland, the Helsinki City Water and Wastewater Authority have been monitoring heart rate in salmonid fishes to detect changes in the quality of surface raw water (Aalto and Smeds, 1985). Incorporation of the system described here into such a monitoring programme would remove the necessity for electrode implantation, as is currently undertaken, and allow 8 organisms to be monitored simultaneously.

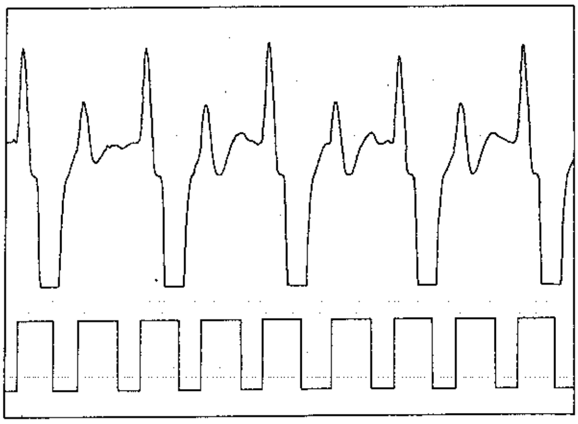
In medicine, heart rate and cardiac activity patterns are key indicators of the condition of the patient and are included in all general clinical assessments (Swash and Mason, 1984).

Cost has been borne in mind during the development of the CAPMON system. Most laboratories now have access to an IBM compatable computer. Thus, the remaining costs centre around the

(a)



(b)



(c)

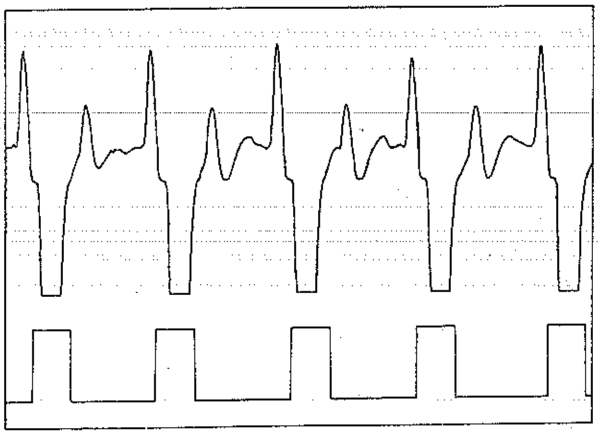


Fig. 4. Heart beat patterns and counter triggering. (a) Typical heart beat pattern (upper trace) and associated counter triggering (lower trace). (b) A typical heart beat pattern and associated "double" triggering giving rise to erroneous counts. (c) Heart beat pattern inversion to avoid "double" triggering. The signal from (b) has been electronically inverted to ensure that each heart beat is counted once only.

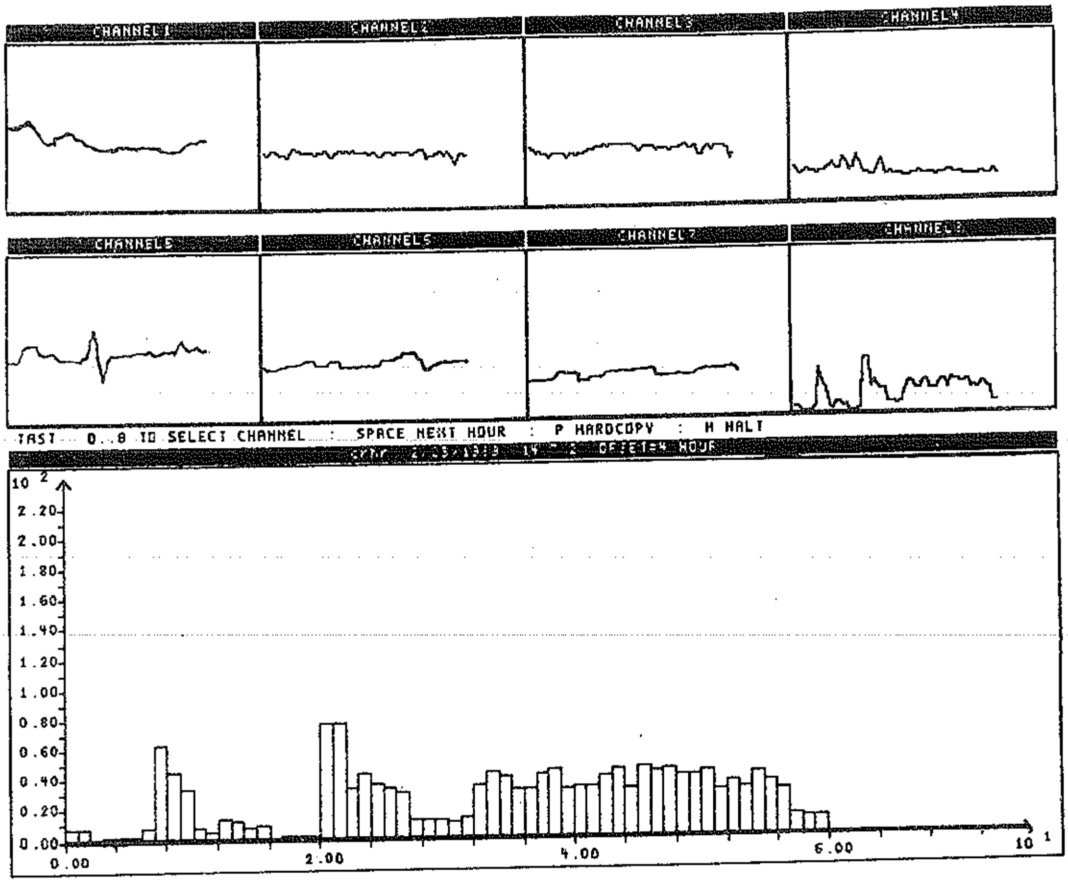


Fig. 5. Display of the heart rates of 8 animals recorded simultaneously. Each of the 8 windows displays heart rate (vertical axis) over one hour (horizontal axis). The display restarts at the beginning of each new hour. All the data are stored on floppy diskettes. Any window can be selected and enlarged in the lower part of the display.

transducers, interface and computer software. At current prices, such components may be purchased for approx. US$500.

It is hoped that the system (or a modified version of it) will be widely adopted to further the use of physiological monitoring as a means of assessing the well-being of animals in Nature.

Acknowledgements—We are grateful to Lene Hau, Torben Andreasen, Alf Aagaard, Lilian Hansen and Lars Bjørn for assistance with the development and testing of the CAPMON system. We are indebted to the Carlsberg Foundation and the Shell Oil Company for their financial support.

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