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APPARATUS FOR MEASUREMENTS OF PHYSIOLOGICAL PARAMETERS IN FISH

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Key words: fish physiology, in vivo experiments, apparatus, ECG, water flow pressure, gill movement

Abstract: A special apparatus for simultaneous recording of a fish gill movement, ECG and water flow through a fish mouth was designed, developed and experimentally tested. A pair of single channel force transducers with linear dependence of the output voltage upon the load 0.5 mV/mN over the nominal range of 0-70 mN at a bridge excitation of 5V was used for recording the curves of gill movements. Wires fixed with the fishing hooks into the fish body and on the other side connected to the amplifier were used for ECG recordings. A pressure transducer recorded a water flow through a fish mouth. We recommend our set up as a simple and useful tool for recording the described physiological parameters in experiments with fish.

Naprava za merjenje fizioloških parametrov pri ribah

Kjučne besede: ribja fiziologija, poskusi v živem, naprava, ECG, tlak vodnega pretoka, pomik škrg

Izvleček: Razvili, izdelali ter testirali smo posebno napravo za merjenje gibanja ribjih škrg, EKG, ter pretoka vode skozi ribja usta. Gibanje ribjih škrg smo spremljali s parom mehansko-električnih pretvornikov, ki linearno merita mehanske obremenitve v razponu 0-70 mN. EKG smo snemali prek izoliranih žic, ki so bile s trnki na eni strani pritrjeni v ribjo kožo na drugi strani pa v ojačevalnik. S posebnim merilcem pritiska smo spremljali pretok vode skozi usta testirane živali. Izdelana aparatura je preprosta, praktična in poceni pripomoček za spremljanje določenih fizioloških parametrov pri ribah.

1. Introduction

In vivo experiments on fish represent a problem when vital parameters have to be recorded in a simulated "natural" environment. There is not much commercially available recording equipment for simultaneous measurements of respiratory activity and cardiac function. However, various measuring equipment has been developed of different authors for the certain experimental purposes /1-3/. We designed, developed and experimentally tested a special setup for simultaneous recording of fish gill movements, ECG and water pressure change in the mouth of fish. The pressure change produces the necessary water flow through the gills by coordinated mouth and gill movements.

2. Material and Methods

The frame of the apparatus was made of a plastic block fixed on a plastic plate. Into a plastic block a cone was drilled with a small hole in the front side of the cone. The broad part of the cone was opened to the back. On both sides of the back half of the cone wall a narrow, and horizontally oriented fissure was drilled. Plastic holders for the fish body were placed a few centimetres from the back opening of the cone.

The force transducer was made up of full Wheatstone bridge composed of four semiconductor strain gages (resistance in ohms: 500.0±0.3%), bonded on a specially designed cantilever. A full Wheatstone bridge equipped with terminals for connection of wires leading to the connector /4-6/ was mounted in a tool made of Plexy Glass. The tool was fixed into the narrow horizontally orientated fissure of the apparatus framework cone. Horizontal orientation of the cantilever was changed by the arm of the tool and adapted to a fish gill. Horizontal force induced by a fish gill caused an elastic deformation of the cantilever and thus resulted in a change of the output voltage. The output voltage directly represents the information about a mechanical load applied on the cantilever /7-9/. Tinny wires fixed with the fishing hooks into a fish body insulated on one side and on the other side connected to the amplifier were used for ECG recordings.

The water flow through the fish mouth was recorded by using a commercially available semiconductor Wheatstone bridge pressure transducer (Statham, USA). The transducer was pushed into a fish mouth through the hole at the front part of the cone. Signals from the transducers were amplified (D. Peterec, SLO) and connected to the A/D converter (Axon instruments, USA) and an IBM compatible PC. The data were processed with the software *Axoscope* 3.1 freely available on the Internet. The experiments on fish were provided in fresh oxygenated water cooled to 15°C.

3. Results and Discussion

In our experiment we had no trouble in positioning of a fish into the cone of our apparatus as fish simply swam into the cone so that fixation was unnecessary (Fig.1).

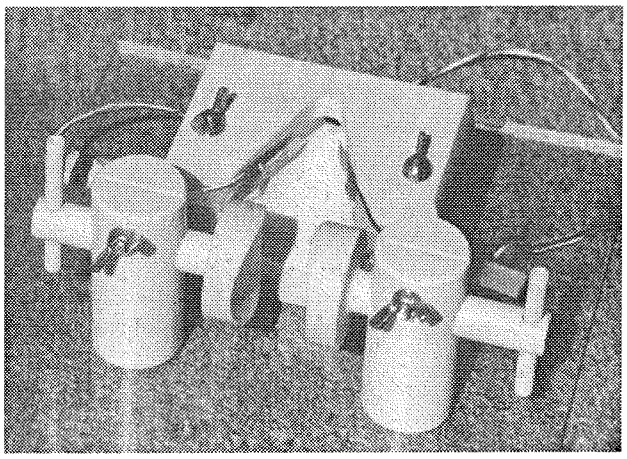

Fig.1. Experimental apparatus. The fish is placed into the equipment so that its movements are restricted, but not completely prevented. On the front side a flow transducer is placed into the fish mouth. One mechano-electrical transducer is placed on each side of the apparatus in order to measure the gill movements.

Nevertheless, we restricted the fish movements gently by the adaptable holders attached on each side. Introduction of the pressure transducer into a fish mouth and the fixation of the ECG wires were effortless.

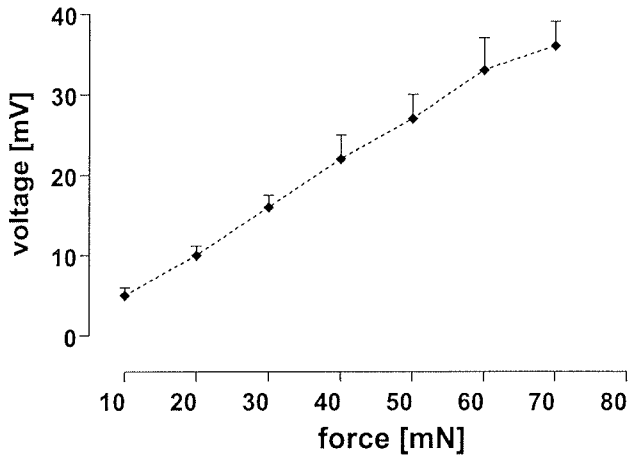

Fig.2. The force transducer has linear voltage response in a range of forces from 0-70 mN

The pair of force transducers has linear dependence of the output voltage upon the load of a gill movements 0.5 mV/mN over the nominal range of 0-70 mN at a bridge excitation of 5V (Fig.2) The linear response of the force transducer could give us the data about the real forces of the gill movement in case the force is calibrated beforehand. A similar transducer was successfully used also for other applications where the contractions of an isolated muscle, rings of pig coronary artery, movement of an animal leg were measured /4-9/.

We obtained reproducible ECG recordings of fish heart beating (Fig. 3). The recordings of the gill movements and those of the water flow pressure through the fish mouth were also reproducible, and enabled the study of correlation of water pressure changes and gill movements.

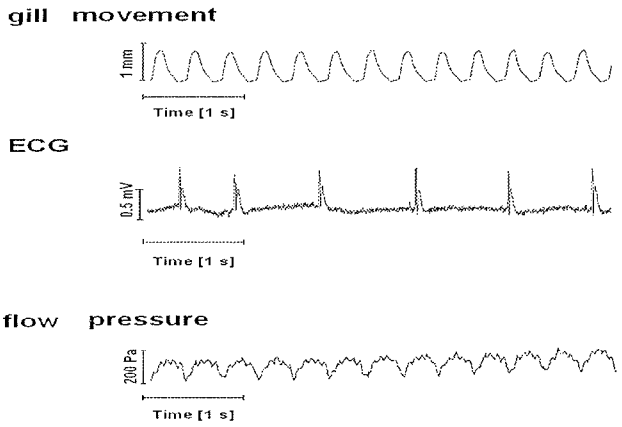

Fig. 3. Experimental data from fish in control conditions. Besides the amplitude of gill movements, the ECG and water flow pressure through the fish mouth can be measured reliably.

The results presented in Fig. 3 suggest that the fish in our experiment was under substantial stress due to its prior exposure to equinatoxin II, a haemolytic toxin that exposed lethal effects in vivo /10-12/. The speed of gill movements was over 100 per minute.

In our experiments we just wanted to demonstrate the measurement characteristics of our equipment. Of course during the experiments with the fish we should take care about lowering the discomfort and stress to a fish that could influence the obtained measurements. The advantage of our equipment is open construction, so different kinds of manipulations with the fish are possible. The fish is quite free and the experimental equipment gives it a more natural environment than some other setups. In the latter cases the treated fish were usually closed in a glass tube of the experimental equipment /1-3/ and far more stress was put on them. Therefore it is difficult to distinguish between the effects of the stress due to the experimental equipment and the effects of the tested substances, in the case of toxicological experiments on fish.

4. Conclusion

According to the presented results we recommend our setup as a simple, inexpensive and suitable tool for *in vivo* experiments on fish even in their natural environment.

5. References

/1/ J. Caprio, Olfaction and taste in the channel catfish: an electrophysiological study of responses to amino acids and derivates, J. Comp. Physiol., 123, 1978, 357-71.

/2/ K. H. Holland, J. H. Teeter, Behavioral and cardiac reflex assays of the chemosensory activity of channel catfish to amino acids, Physiol. Behav., 27, 1981, 699-707.

/3/ E. E. Little, Conditioned cardiac response to the olfactory stimuli of amino acids in the channel catfish, Ictalarus punctatus. Physiol. Behav., 27,1981, 691-697.

/4/ M. Bunc, D. Suput, J. Rozman, Force transducer for measurement of muscle contraction, J. Med. Eng. Technol., 23, 1999, 222-224.

/5/ J. Rozman, M. Bunc, Sensor of forces in a small volume contracting tissues, Inf. MIDEM, 2003, in press.

/6/ M. Bunc, J. Rozman, Electronic brace for the measurements and eliciting of muscle contractions in a dog's ankle, Inf. MIDEM, 31, 2001, 106-109.

/7/ J. Rozman, B. Zorko, M. Bunc, Recording of electroneurograms from the nerves innervating the pancreas of a dog, J. Neurosci. Methods, 112, 2001, 155-162.

/8/ M. Bunc, R. Frangez, J. Rozman, D. Suput, The effects of equinatoxin II on respiration–possible mechanism of the toxin lethality, Pflugers Arch., 439, 2000, 129-130.

/9/ J. Rozman, M. Bunc, A. Zupan, Evaluation of the strength of elbow flexors in patients with neuromuscular diseases, J. Med. Eng. Technol., 2001, 25, 235-239.

/10/ P. Maček, D. Lebez, Isolation and characterization of three lethal and hemolytic toxins from the sea anemone Actinia equina, Toxicon, 27, 1988, 441-451.

/11/ M. Bunc, G. Drevenšek, M. V. Budihna, D. Šuput, Effects of equinatoxin II from Actinia equina (L.) on isolated rat hearts: the role of direct cardiotoxic effects in equinatoxin II lethality, Toxicon, 37, 1999, 109-123.

/12/ M. Bunc, R. Bregar, D. Šuput, The importance of hemolysis in the lethal effects of equinatoxin II, a protein from the sea anemone Actinia equina (L.), Pflügers Arch., 440, 2000, 151-152.

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