Detubulation of rat ventricular myocytes

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Formamide-induced detubulation of ventricular myocytes isolated from the hearts of rats, which were humanely killed using a Schedule 1 procedure, will be demonstrated. This technique was described in the talk 'Subcellular heterogeneity in ventricular myocytes' presented in the symposium 'Cardiac Electrophysiology: Heterogeneity in the Heart' at this meeting (Orchard *et al.* 2002) and used in the study described by Brette *et al.* (2002).

Brette, F. *et al.* (2002). *J. Physiol.* **544.P**, 46*P*.

Orchard, C.H. *et al.* (2002). *J. Physiol.* **544.P**, 46*P*.

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All procedures accord with current UK legislation.

A simple device for alternating the direction of electrical stimulation of muscle preparations

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When delivering electrical stimuli to muscle preparations, one electrode normally acts as the anode, one as the cathode. During chronic stimulation it may be advantageous to reverse the cathode and anode on alternate stimuli, to minimise the build-up of electrolysis products, and avoid electrode damage. Most commercially available stimulators do not have the facility to do this. We have, therefore, developed a simple circuit to reverse the polarity of the stimulating electrodes on alternate pulses.

Figure 1 shows the circuit diagram. The output of the stimulator is passed via a double pole change-over reed switch, to the bath electrodes. The position of the switch is controlled by a programmable microcontroller (PIC16F877, Microchip, USA). This device is programmed to respond to a pre-pulse from the stimulator by flipping the state of a digital output. Thus before each stimulus the pre-pulse from the stimulator triggers the microcontroller, which operates the reed switch, reversing the polarity of the electrodes.

For stimulators lacking a pre-pulse, it would be possible to trigger the microcontroller from the stimulus itself, programming it to delay the operation of the reed switch until a fixed period (e.g. 100 ms) after the stimulus.

This circuit provides a simple and cheap (< £50 components) method of providing alternating stimuli from commonly available and economical commercial stimulators. The versatility and ease of programming of the PIC16F877 microcontroller enable it to be used for a wide range of applications. This will be demonstrated using a multi-purpose circuit board (EasyPic), which was developed in-house.

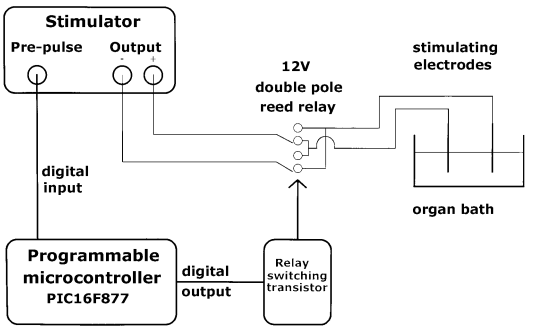

Figure 1. Diagram of the circuit used to produce stimuli that alternate in direction.

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Cellular open resource (COR): a new environment for cellular and multicellular modelling of cardiac electrophysiology

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Computer models have become an invaluable tool for both experimental and theoretical research into cardiac electrophysiology. These models have, over the years, matured from simple representations of action potential shapes to mechanistic models with developing predictive power. Theoretical work on the sodium–calcium exchanger has, for instance, predicted its stoichiometry some five years before its experimental confirmation (for details, see Noble, 1995). The iterative interaction between simulation and experimental work is a vital part of model development (Noble & Rudy, 2001). OxSoft Heart (Noble *et al.* 1998) offers an environment for such an iterative process and has for more than 15 years been widely used as a research tool, as well as a teaching tool. It has, however, been recently discontinued, due mainly to technical issues that prevent DOS-based programs written in Borland Pascal from working on the latest generation of processors. COR (Cellular Open Resource) aims at pursuing the approach taken by programs like OxSoft Heart, while providing an up-to-date environment for cellular and multicellular modelling of cardiac electrophysiology.

Currently, a number of academic groups offer interfaces for cardiac modelling (both at the cellular and multicellular levels). LabHEART (Puglisi & Bers, 2001) and iCell (Demir, ssd1.bme.memphis.edu/icell) provide advanced ionic cell modelling, but they are restricted to a limited number of single-cell models. Both have static interfaces, which make for useful teaching tools (specific interfaces help the setting of tasks), but do not offer enough flexibility for research. Software packages like Cell Editor™ (Physiome Sciences, Inc., www.physiome.com) are more appropriate for the latter, since they allow for the refinement and development of new single-cell models. Cell Editor™, however, does not currently support multicellular modelling. Also – it is proprietary software. Cellular and multicellular models can very appropriately be implemented using CMISS (Hunter, www.cmiss.org). However, CMISS is a package for the advanced user, requires significant training, and is best applied to complex problems involving structural electromechanics models.

Based on the above, COR has set out to provide a package that is simple to use, both as a research and teaching tool. It aims at offering the same level of flexibility as programs like OxSoft Heart (voltage and current clamps, *I–V* curve, ability to pause and resume simulations for interactive parameter changes, etc.). Unlike most of the current interfaces, models are not hard-coded, but stored in files using the CellML™ format (Hedley *et al.* 2001). This provides COR with an 'out of the box' access to a large database of cardiac single-cell models, such as sino-atrial node pacemaker cells (Zhang *et al.* 2002), atrial myocytes (Nygren, 1998), Purkinje fibres (Varghese & Winslow, 1994), and ventricular myocytes (Luo & Rudy, 1994; Noble *et al.* 1998), as well as the ability to share new models with other CellML™-compliant programs like Cell Editor™ and CMISS. Models are parsed for correctness before being converted into machine code for the Intel x86 family of processors. The computation of the model is therefore optimised, which is essential considering that COR supports the simulation of multicellular problems (which are intrinsically computationally demanding). Unlike CMISS, COR uses a finite difference technique, instead of a finite/boundary element method, which demands less user effort in setting up the problem. 1D and 2D problems correspond to a strand and a tissue section of single cells, respectively. 2D tissue architecture can be freely defined or inherited from histological samples using bitmaps to define a particular mesh. Individual cells properties and intercellular coupling can be assigned to pre-defines models or values, and they can be interactively modified. This provides an easy to use, yet powerful, environment for cellular and multicellular simulations.

Last but not least – COR is freely available (source code included) from cor.physiol.ox.ac.uk

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A simple method for the assessment of vascular reactivity in humans to changing blood levels of carbon dioxide

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Hyperventilation, which commonly occurs during orthostatic stress both in patients with tendencies to syncope and in normal volunteers, leads to cerebral vasoconstriction and systemic vasodilatation. In the accompanying communication (Norcliffe *et al.* 2002) we present evidence that in individuals with a high sensitivity of vascular resistance to changes in CO_2 there is a high incidence of syncope during orthostatic stress. This demonstration shows the method used for assessing CO_2 reactivity in both the cerebral circulation and in a forearm.

Blood flow in the cerebral circulation is assessed in the middle cerebral artery, determined using the technique of transcranial ultrasonography (Multi-Dop® X4, TCD-8.01, DWL Elektronische System GmbH, Sipplingen, Germany) whereby a pulsed ultrasound beam insonates the middle cerebral artery through a thinning of the temporal bone, the transcranial window. Forearm blood velocity is assessed by a second ultrasound beam along the direction of the brachial artery. Blood pressure is determined using a Finapres photo-plethysmograph (Finapres, Ohmeda, Winconsin, USA), calibrated frequently using an automated sphygmomanometer (Hewlett Packard 78352C, Boebringen, Germany). Cerebral perfusion pressure is estimated from brachial pressure, corrected if necessary for height differences. Vascular resistance in the two beds is calculated as mean arterial pressure divided by mean blood velocity. As the Doppler device does not give absolute values of blood flow, vascular resistance can only be assessed in relative terms.

Arterial blood P_{CO_2} is assumed to be equal to the end-tidal value and this is determined using an infrared analyser (model Binos 1, Leybold-Haraeus Limited, Koln, Germany). The levels of CO_2 are increased by hypoventilation, aided by the use of increased dead space, and decreased by hyperventilation. CO_2 sensitivity in the two vascular beds is expressed as the percentage change in vascular resistance divided by the change in end-tidal CO_2 concentration.

This technique is used to assess the reactivity in the two vascular beds to CO_2 and results of research using this method are given as a communication (Norcliffe *et al.* 2002).

Norcliffe, L.J. *et al.* (2002). *J. Physiol.* **544.P**, 84*P*.

All procedures accord with current local guidelines.