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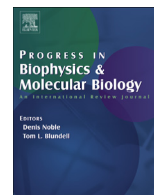

Original research

An automated system using spatial oversampling for optical mapping in murine atria. Development and validation with monophasic and transmembrane action potentials

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ABSTRACT

We developed and validated a new optical mapping system for quantification of electrical activation and repolarisation in murine atria. The system makes use of a novel 2nd generation complementary metal-oxide-semiconductor (CMOS) camera with deliberate oversampling to allow both assessment of electrical activation with high spatial and temporal resolution (128×2048 pixels) and reliable assessment of atrial murine repolarisation using post-processing of signals. Optical recordings were taken from isolated, superfused and electrically stimulated murine left atria. The system reliably describes activation sequences, identifies areas of functional block, and allows quantification of conduction velocities and vectors. Furthermore, the system records murine atrial action potentials with comparable duration to both monophasic and transmembrane action potentials in murine atria.

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1. Introduction

Atrial fibrillation is the most common sustained arrhythmia in man and a common cause of stroke and cardiac deaths. Although good progress has been made in the characterisation of factors that cause AF (Camm et al., 2012a,b; Schotten et al., 2011; Wakili et al., 2011), there is still an unmet need for better therapies to prevent incident and recurrent AF (Camm et al., 2012a,b; Kirchhof et al., 2013). Unravelling the mechanisms conveying the genetic basis of atrial fibrillation (Benjamin et al., 2009; Ellinor et al., 2012; Gudbjartsson et al., 2007) is a promising and relatively new avenue to novel preventive and therapeutic targets. Genetically altered murine models are popular tools for the study of molecular disease mechanisms, including of atrial fibrillation. Such models allow the characterisation of the functional and molecular consequences of defined genetic alterations, thereby allowing us to identify arrhythmia mechanisms attributable to such modifications. This type of research extends the association studies in patients and populations which are limited by comorbidities, ethical restraints and low sample availability (Riley et al., 2012). The small size of murine hearts, particularly the atria, poses a challenge for detailed electrophysiological assessment, and especially for high density mapping of electrical activation and repolarisation. Evolving knowledge of existence of regional heterogeneities calls for the development of a technique with high spatial resolution, so that the extent of heterogeneity can be identified (Di Diego et al., 2013; Waldeyer et al., 2009). Additionally, the high frequency and fast repolarisation properties of the murine atrium necessitate high temporal resolution.

Optical mapping is an established technique for high spatial and temporal resolution non-contact investigation of electrical excitability of cardiac cells, tissues and whole organs (Efimov et al., 1994; Lee et al., 2011; Rohr and Salzberg, 1994; Salama et al., 1987; Wu et al., 2001). Optical mapping of cardiac tissue uses voltage-sensitive dyes to visualise action potentials as changes in

Abbreviations: ADC, Analogue to digital converter; AF, Atrial fibrillation; APD, Action potential duration; CL, Cycle length; CMOS, Complementary metal-oxide-semiconductor; CV, Conduction velocity; LA, Left atrium; LED, Light emitting diode; MAP, Monophasic action potential; OAP, Optical action potential; SEM, Standard error of the mean; TAP, Transmembrane action potential; TIFF, Tagged image file format.

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fluorescence, as an index for changes in transmembrane potentials (Salzberg et al., 1973). Numerous improvements have been made to overcome technical difficulties associated with optical mapping of cardiac tissue including removal of contraction artefacts in large and small vertebrates by using uncouplers (Dou et al., 2007; Fedorov et al., 2007; Jou et al., 2010; Wu et al., 1998) and sensitive optical dyes (Efimov and Fahy, 1997; Ehrenberg et al., 1987; Rohr and Salzberg, 1994). As such, this technique has provided important insights into the mechanisms of atrial arrhythmias in murine models (Arora et al., 2003; Blana et al., 2010; Kirchhof et al., 2011a,b). The complex electrophysiology of atrial fibrillation (Eckstein et al., 2013; Kirchhof et al., 2011a,b; Verheule and Tuyls, 2013), renders a higher spatial resolution of murine atrial activation and repolarisation mapping technology desirable. This allows us to pinpoint further, the regional origins and patterns of AF activation (Mandapati et al., 2000). Several foci of AF activation have already been identified in genetically altered mouse models (Benes et al., 2014; Faggioni et al., 2014). However, even with the best available systems, the need for higher spatial and temporal resolution combined with the small signal amplitude generated in the thin atrial murine tissue still poses technical challenges.

To further improve temporal and spatial resolution in cardiac optical imaging of murine atrial tissue, a second-generation complementary metal-oxide-semiconductor (CMOS) camera is utilised and novel algorithms developed to record and analyse atrial activation and repolarisation in the isolated, perfused murine atrium at high spatial resolution. A spatial oversampling technique is implemented to generate high-resolution activation maps at the same time as high quality repolarisation information. The algorithms used to generate isochronal maps and to perform APD measurements are largely automated to limit user bias and to facilitate high throughput analysis. Atrial optical action potentials are compared with epicardial atrial monophasic action potentials and atrial microelectrode transmembrane action potentials from isolated hearts to validate the findings.

2. Methods

2.1. Heart isolation

All surgical procedures were performed according to the Animals (Scientific Procedures) Act, 1986 and were approved by the Home Office and the local authorities. Hearts were rapidly excised by thoracotomy from WT adult mice (3–7 months old), on a 129/Sv or MF1 background, under terminal anaesthesia (200 mg/kg pentobarbital sodium) administered by intraperitoneal injection.

2.2. High resolution mapping using novel methods of automated data processing

Following isolation, hearts were mounted on a vertical Langendorff apparatus (Hugo Sachs, Germany) and the aorta retrogradely perfused with a standard bicarbonate buffered Krebs–Henseleit solution (see below), containing the voltage sensitive dye Di-4-Anepps (50 μM; Biotium, California, USA) and the excitation-contraction uncoupler blebbistatin (5 μM; Cayman Chemical, Michigan, USA), a well-characterised substance used for optical mapping (Fedorov et al., 2007; Kirchhof et al., 2011a,b). The dye was stored in 25 μl aliquots of 5 mg/ml. For each experiment an aliquot was mixed with 1 ml of Krebs solution and injected through a bolus port in the Langendorff system over a period of 5 min. The flow rate of the Krebs solution was kept at 4 ml/min and at 36 °C. After 5 min of infusion, the atria were dissected and placed in a superfusion chamber for image acquisition. A summary of the system set-up used for image acquisition is shown in Fig. 1. Preparations were continuously superfused with Krebs and blebbistatin to reduce contraction and paced incrementally (300 ms–80 ms paced cycle length) via platinum electrodes placed in the tissue bath at twice the diastolic voltage threshold at 2 ms pulse width. Stimuli were generated using an isolated constant voltage stimulator (Digitimer, UK) driven by an analogue to digital converter with spike2 software (Cambridge Electronic Design, UK). The atria were field illuminated by two twin LEDs at 530 nm (Cairn Research, UK). These were required to provide sufficient field illumination from all angles. A lens with a high numerical aperture (Schneider Kreuznach Xenon 0.95/25, NA = 0.52) was used to collect as many photons as possible. Wide field macroscopic images of emitted fluorescence (630 nm) were captured at a sampling frequency of 1 or 2 kHz using a novel, high speed, high resolution camera (128 × 2048 pixels, single pixel area: 6.5 μm by 6.5 μm, ORCA flash 4.0; Hamamatsu, Japan, Figs. 1 and 2). The camera reads the data from the centre of the sensor. Temporal resolution can be increased with reduced spatial resolution only in the vertical direction, as a trade-off. This allows for the full window of the sensor to be used in the horizontal direction. To reduce the read noise of the camera, the 4 × 4 binning option was used (Fig. 2B and C).

Images were extracted and collated using WinFluor V3.4.9 (Dr John Dempster, University of Strathclyde, UK) so that the fluorescence intensity from a specific region of interest (4 × 4 pixels) could be viewed as a single continuous waveform. This permitted the identification and monitoring of a greater number of optical action potentials (OAPs) across the entire surface of the mouse atrium at high spatial resolution. In addition, selected images could be exported into uncompressed Tagged image file format (TIFF) for the generation of whole atrial isochronal activation maps.

For the analysis of OAPs and generation of isochronal activation maps, data were automatically processed using custom made algorithms produced in MATLAB. For measurements of action potential duration (APD), an initial sign change was necessary as the emitted fluorescence intensity is inversely proportional to transmembrane voltage (Loew, 1996). Baseline subtraction was achieved by applying a 'linear top hat filter' to remove any fluorescence drift. At each cycle length, a maximum of 25 action potentials were averaged to calculate the mean action potential waveform for a selected region of interest (Fig. 1B). Previously we found that 25AP signals were sufficient to measure APD reliably. Fewer than 25 APs could be used, but this was dependent on the signal quality from each experiment. For example, a very good signal would require less averaging, but to keep the algorithm consistent 25 signals were routinely used. The baseline (100% repolarisation) was determined as the mean fluorescence signal recorded over 10 ms prior to depolarisation. The point of activation was defined as the peak rate of change (dF/dtmax) and APD30, 50 and 70, were evaluated by measuring the time from dF/dtmax to 30%, 50% and 70% repolarisation respectively (Fig. 1B). For each APD, the value usually falls between sampling time points, 1 ms apart for 1000 fps, 0.5 ms for a 2000 fps. The repolarisation time was estimated by connecting these two closest sampling time points above and below with straight lines. A simple linear equation can be used to determine the time for each APD.

Isochronal activation maps were produced after initially cropping the exported image stack to isolate the region of interest; this was the only step that required user input. Fluorescence intensity from every pixel was automatically analysed in the *z* (time) axis. The background was removed and signals were smoothed and then filtered using a Savitzky–Golay filter, a moving average filter which preserves signal morphology (Bachtel et al., 2011; Savitzky and Golay, 1964). Signals were differentiated to detect the time of activation, which was repeated until a 2D array (image) was compiled with each element (pixel) corresponding to a specific

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