Mechanism of Reentry Induction by a 9V Battery in the Rabbit Ventricles

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Abstract

Although application of a 9V battery to the epicardial surface is a simple method of ventricular fibrillation induction, the fundamental mechanisms underlying this process remain unstudied. We used a combined experimental and modelling approach to understand how the interaction of direct current (DC) from a battery may induce reentrant activity within the rabbit ventricles and its dependence upon battery application timing and duration. A rabbit ventricular computational model was used to simulate 9V battery stimulation for different durations at varying onset times during sinus rhythm. Corresponding high-resolution optical mapping measurements were conducted on rabbit hearts with DC stimuli applied via a relay system. DC application to diastolic tissue induced anodal and cathodal make excitations in both simulations and experiments. Subsequently, similar static epicardial virtual electrode patterns were formed which interacted with sinus beats, but did not induce reentry. Upon battery release during diastole, break excitations caused single ectopics, similar to application, before sinus rhythm resumed. Reentry induction was possible for short battery applications when break excitations were slowed and forced to take convoluted pathways upon interaction with refractory tissue from prior make excitations or sinus beats. Short-lived reentrant activity could be induced for battery release shortly-after a sinus beat for longer battery applications. In conclusion, the application of a 9V battery to the epicardial surface induces reentry through a complex interaction of break excitations following battery release with prior induced make excitations or sinus beats.

Key Words

Cardiac modelling; ventricular fibrillation; optical mapping; bidomain; reentry.
1 Introduction

Ventricular fibrillation (VF) remains a significant cause of sudden cardiac death worldwide. Despite decades of research, the processes involved in the initiation of VF from sinus rhythm are still subject to intense investigation. The induction of VF via direct current (DC) stimulation to the heart’s surface with a simple 9V battery is a widely-used method. However, the precise mechanisms of arrhythmogenic interaction of DC from a battery with cardiac tissue, and potential interspecies differences, are currently unknown. Acquiring such mechanistic insight will help optimise VF induction via this method and, importantly, shed light on fundamental arrhythmia induction processes.

The induction of VF by simply touching a 9V battery to the epicardial surface is most widely-used in the experimental laboratory [8-11,15-17,22,24], although it is also often used in a clinical research environment [23,27]. In these cases, the exact location of application, the timing relative to the cardiac cycle and the duration of application are widely varying parameters. Nonetheless, VF appears to be reliably induced. Despite its success, however, there appears to be little, if any, knowledge regarding the mechanisms by which a 9V battery causes the ventricles to fibrillate. Mechanisms which may contribute to VF induction include battery induced make and/or break excitations, virtual electrode polarisation, repetitive focal discharge, conduction block, involvement of Purkinje system (PS), stretch activated channels, and interaction with sinus activation patterns.

All reports in the literature describing successful induction of VF via this method are almost exclusively in porcine [8,9,17,22] and canine [11,24] hearts, with some reports of its use in sheep [15] and guinea pig [10], in addition to ex-vivo human hearts [16]. Intriguingly, though, there appear to be no reports of VF induction with a 9V battery in the rabbit heart, potentially due to the noted difficulty in achieving self-sustained VF in the healthy rabbit ventricles [3,12,14]. It is therefore important to understand the fundamental mechanisms by which a 9V battery may
induce reentrant activity within the ventricles and the potential interspecies differences in the
degeneration of these initial reentrant cycles into fully self-sustained VF.

In this study, using a combined computational modelling and experimental approach, we
investigate the mechanism by which a 9V battery induces reentry in the rabbit ventricles. We
examine how the interaction of the battery electrodes with the tissue during application and
release may induce reentrant activity and the dependence of the witnessed effects on application
timing and stimulus duration, as well as the interaction of these effects with sinus beats. Model
simulations allow a precise dissection of the mechanisms at play, with corresponding optical
mapping experiments being used to validate the findings at each stage.
2 Materials & Methods

2.1 Computational Methods

Electrical activity was simulated by the bidomain equations within a finite element model of the rabbit ventricles [7] using the Cardiac Arrhythmia Research Package (CARP) [26]. The model, shown in Figure 1 (left), included histologically-derived fibre architecture and a representation of the PS [7]. Ionic membrane dynamics within the myocardium were represented by a recent rabbit ventricular cell model [13], augmented to include two additional currents to faithfully simulate the membrane response to strong shocks [1], specifically an electroporation current and a hypothetical potassium current that activates at larger positive polarisations beyond +160mV. Membrane kinetics within the PS were represented by a recent Purkinje specific cell model [2]. The model also included transmural and apicobasal electrophysiological heterogeneity in ionic currents, as described previously [4]. Anisotropic tissue conductivities along the fibre and cross-fibre directions were based on previous experimentally-derived values [6] within the intracellular (0.174 S/m, 0.0193 S/m) and extracellular (0.625 S/m, 0.236 S/m) domains, respectively, adjusted to reduce conduction velocity by 25% in each direction in accordance with previous studies [3,4] and to provide a closer match to that witnessed in experiments.

Battery electrodes were defined by constant voltage (9V anode; 0V cathode) circular regions on the epicardial surface of the left ventricle (LV) free wall with diameters 5mm (anode) and 7mm (cathode), separated by 13mm, corresponding to a standard 9V battery, highlighted in Figure 1 (left).

Sinus activation paced the ventricles via the PS at a fixed cycle length of 350ms throughout the entire simulation. Following a variable time (50–300ms) after the 10th paced beat, the battery electrodes were activated for a variable duration (50–700ms), after which activity was allowed to
evolve and vulnerability for reentry induction assessed. Following cessation of the battery stimulus, electrode regions were treated as normal myocardial tissue. The significant computational burden of a full bidomain run precluded a thorough parameter sweep of all shock application and duration timings. For example, simulation of battery application required a bidomain solve with a very fine time-step (2\textmu s) in order to resolve the rapid changes in potential associated with the strong extracellular field. Simulation of a 700ms stimulus took \~128 CPU-hours (Xeon, 2.53GHz), with further post-shock simulation (500-1000ms) then required to assess reentry induction.

Simulations were performed with CARP on the Oxford Supercomputing Centre clusters. Visualisation of results was performed with the custom written Meshalyzer software.

### 2.2 Experimental Optical Mapping

Optical mapping recordings were obtained from ex-vivo Langenndorff-perfused rabbit hearts following the same overall protocol and setup to the simulations for comparison and validation.

All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the Animals (Scientific Procedures) Act 1986 (UK). Hearts were swiftly isolated from young adult New Zealand White rabbits (approximately 1kg weight) killed by an overdose of anaesthetic (pentobarbitone), and then were placed in ice cold normal Tyrode (in mM: 140 NaCl, 5.4 KCl, 1 MgSO4, 5 HEPES, 11 Glucose, 1.8 CaCl2 pH 7.4) solution prior to mounting. Hearts were mounted to a Langendorff perfusion system, stabilized in a custom built optical mapping chamber and perfused via gravity with warm (35-37 °C) normal Tyrode for 5 minutes to resume normal contractions, and loaded with membrane dye rh237(N-(4-Sulfobutyl)-4-(6-(4-(Dibutylamino) phenyl) hexatrienyl) Pyridinium) as a bolus injection by coronary perfusion (5 \textmu m over 5 minutes).
Blebbistatin was used to immobilize the heart (10 μM, recirculated). The heart was illuminated with two LED light sources (530nm, Cairn Research), passed through a 650nm long pass optical filter (Chroma Technology) attached to a 25mm f0.95 imaging lens (Navitar). Signals were recorded with a Photometrics Evolve 128 EMCCD camera with a 1.5ms frame period (64 x 64 pixels, 2 x 2 pixel binning). A series of 3000 images were captured for each experimental run.

A custom chamber was constructed from plexi-glass with two platinum wire loop electrodes attached to the respective terminals of a 9V Duracell Procell alkaline battery to prevent obstruction of the optical field-of-view by the battery itself. Wire electrode spacing (13mm), diameter (5-7mm) and wire loop thickness (1mm) matched those of 9V battery terminals. The LV was lightly pressed against the electrodes prior to imaging. Current was controlled by a relay (sil05-1a72-71d). A second bipolar stimulator was pressed against the atria. A micro-controller (Arduino Uno, Arnuino.cc) was used to control the camera, stimulator and drive the relay.

The heart was paced at its intrinsic sinus period minus 50ms. For each run, the heart was paced for 40 beats prior to application of the battery. The battery was applied for various time intervals so that the initiation and termination of the battery application fell at different time points, or phase, in the pacing cycle. Pacing protocols where the phase of the stimulus was progressively incremented by 25ms were carried out for all preparations. Data was analysed using a combination of custom written software (available on request) based on ImageJ (http://rsb.info.nih.gov/ij/) and Scipy (www.scipy.org) software packages. Fluorescence vs time traces in all images are generated from unprocessed data. Isochronal activation maps were generated from data that was denoised by performing spatial (r=3 pixels) and temporal (5 frame) averaging. Activation times were found by first performing a running correlation operation on each trace with a 150ms segment centred on an action potential upstroke captured from the
middle of the imaging field, and then finding the local maxima of the correlation operation. Qualitatively similar phenomena were seen in all experiments (n = 6). Results presented are representative examples of the observed phenomena.
3 Results

3.1 Battery Application

3.1.1 Battery Application During Diastole - Make Excitation Production

For battery application some time (>150ms) after the last sinus beat, the ventricular tissue is excitable and both anodal and cathodal make excitations are elicited on the epicardial surface in both simulation and experiment, rapidly spreading across the ventricles, as demonstrated respectively in Figure 2A & 2D. As these make excitations propagate whilst the battery is still being applied, the make excitation wavefronts propagate through (and interact with) the static virtual electrode patterns established in the vicinity of the battery electrodes evident in the simulation images of Figure 2A. A single ectopic beat results, but no reentry as the wavefronts rapidly collide with one another due to their unhindered propagation pathways throughout the rest of the ventricles distal to the electrodes.

The individual respective $V_m$ and fluorescent signal traces in Figure 2B & 2C highlight depolarisation of tissue due to make excitation production in surface regions positively (virtual cathode) and negatively (virtual anode) affected by the shock (red, green traces, respectively). Noticeably, a much stronger depolarisation is witnessed in the experimental preparation in the region negatively affected by the shock (Figure 2C green trace) due to intramural differences in polarisation levels picked-up in the fluorescent optical signal. This effect is highlighted in Figure 3 which demonstrates that although the epicardial surface of the model is strongly hyperpolarised in this region, substantial make excitation wavefronts propagate intramurally through tissue which has been left excitable. Due to the well acknowledged effect of photon scattering in the optical mapping technique, fluorescent signal is collected from a widely-distributed 3D volume beneath the epicardial surface [5]. Consequently, information regarding the propagation of intramural wavefronts, which pass within this scattering volume of tissue, is picked-up in the optical signal,
increasing the strength of the apparent depolarisation recorded from this pixel in the optical trace. The phenomena also explains the appearance of more uniform activation in the experimental maps of Figure 2D.

Approximately 100ms after the battery application, a sinus beat is initiated in both model and experiment which subsequently fails to capture as the ventricles are still refractory at this time. As the tissue recovers, a static virtual electrode pattern is established by the battery in the model (Figure 2A, panel 3750ms). A similar static virtual electrode pattern is also evident in the experiments (Figure 2E). Both patterns resemble the characteristic ‘dog-bone’ distribution of surface polarisation under unipolar stimulation [28,29] with regions of depolarisation and hyperpolarisation in the vicinity of the anode and cathode. However, due to the close proximity of the electrodes, combined with the irregular ventricular geometry, a complex distribution of tissue polarisation levels across the LV results. Furthermore, the well-documented effects of photon scattering [5] are also responsible for the virtual electrode pattern in the vicinity of the battery electrodes being less well defined in the experimental image of Figure 2E than in the $V_m$ simulation images of Figure 2A. As shown previously in Figure 3, although the epicardial surface is strongly polarised by the battery, intramural tissue directly beneath it is of intermediate polarisation. Thus, the 3D spatial averaging effects of the fluorescent photon scattering attenuate the strength of the apparent surface fluorescent signals, as described previously, leading to the virtual electrode pattern being significantly less distinct in the experimental image.

Analysis of simulation data in Figure 4 shows how the progression of make excitations varies following battery application when applied at different time instances (100–300ms) following the previous sinus beat, all showing the tissue state 25ms after battery application. When applied to relatively refractory tissue (100–150ms), no make excitations are elicited. However, for application
during diastole (200–300ms), make excitations are elicited by the battery. The initial propagation speed of these excitations is then seen to depend strongly on the recovered excitability of the surrounding tissue. When fully recovered (300ms), more make excitations are elicited which progress much further around the ventricles during the first 25ms of battery application than when the tissue has only just regained excitability (200ms). For example, when applied 300ms after the sinus beat, the corresponding make excitations have propagated right around the ventricles within the first 25ms and are beginning to depolarise the RV which is not the case when the battery is applied 200ms after the sinus beat in which case the wavefronts have progressed significantly less far.

3.1.2 Battery Application During Systole - De-Excitation

For battery application soon after a sinus beat (<150ms), surrounding refractory tissue prevents make excitation production in model and experiment. However, in both cases, the tissue in the region negatively affected by the battery is rapidly de-excited from the plateau potential, whilst the region positively affected by the battery experiences prolonged depolarisation. Continual application of the battery results in similar static virtual electrode patterns being established on the epicardial surface in tissue proximal to the electrodes. A few hundred milliseconds after battery application, a sinus beat is initiated which is successfully captured due to tissue distall to the electrodes having regained excitability. The resulting wavefronts propagate around, and interact with, the virtual electrode pattern established by the battery, in both cases causing brief depolarisation of tissue close to the electrodes.

3.1.3 Reentry Initiation

Self-sustained reentrant activity could not be induced in the model nor in any of the experimental cases during the process of battery application, neither during systole nor diastole. Brief
interaction of sinus beat wavefronts with the static virtual electrode pattern established by the battery also did not induce reentry. A steady-state thus took hold, periodically disturbed by sinus beats.

3.2 Battery Release

3.2.1 Battery Release During Diastole - Break Excitation Production

For battery release during diastole, tissue distal to the electrodes has fully regained excitability with only tissue in the vicinity of the electrodes strongly polarised due to the action of the battery electrodes prior to removal. Upon cessation of the stimulus, the virtual anodes and virtual cathodes induced by the battery electrodes initiate both anodal and cathodal break excitation wavefronts [29]. Similarly to make excitation production upon application, the break excitations induced upon battery release during diastole result in a single ectopic beat, shown in Figure 5A & 5D in the model and experiment, respectively and in the corresponding individual model $V_m$ and experimental fluorescent signal traces (Figure 5B & 5C) from regions positively/negatively (red/green traces) affected by the battery. In both simulation and experiment, propagation is initially highly non-uniform as the break excitations interact with neighbouring refractory tissue due to the virtual electrode pattern established by the battery. Contrary to the case of make excitation propagation, however, the virtual electrode pattern begins to disappear upon stimulus termination. Upon release, as ventricular tissue distal to the electrodes has fully regained excitability the induced break excitation wavefronts (blue arrows in Figures 5A) rapidly sweep across the rest of the ventricles unhindered, colliding with each other and self-terminating. Activation of the rest of the ventricles by the break excitation wavefronts causes a single ectopic beat, leaving the tissue refractory when the next sinus beat occurs (some 150ms following release), which consequently fails to capture. Once the tissue excited by the break excitation has
recovered, however, the next sinus beat is able to capture as normal, highlighted by the individual traces from both simulation and experiment (Figures 5B & 5C, respectively).

3.2.2 Battery Release During Systole

For longer battery application durations, the sinus beat may capture whilst the battery is still being applied (described in Section 3.1.2). In these circumstances, when the battery is subsequently released soon after such sinus beat capture (approximately 100ms after), much of the tissue away from the electrodes (and intramurally) is refractory. Thus, upon release, break excitations can only propagate into the hyperpolarised tissue immediately in the vicinity of the electrodes, upon which they rapidly interact with unexcitable tissue and terminate.

3.3 Conditions for Reentry Induction During Short Battery Applications

3.3.1 Interaction of Make and Break Excitations

Extensive investigations using the computational model allowed us to uncover the conditions under which reentry was most likely induced within the rabbit ventricles by the battery. Such conditions involved the relatively short (<150ms) battery applications in cases where the battery was initially applied to the tissue during diastole, thus successfully inducing make excitations which propagated across the ventricles. Releasing the battery a relatively short time after make excitation initiation allowed the resulting break excitations to initially propagate, but importantly allowed them to also interact with the previous make excitation wavefronts themselves, in addition to the heterogeneous distribution of refractory tissue which the make excitations leave in their wake. Such interaction results in the slowing of the break excitation wavefronts and non-uniform conduction block as they encounter refractory tissue, restricting their pathways, providing the necessary substrate to encourage reentry. Of the 4 different short battery application durations simulated (50, 75, 100 and 150ms), those applied to diastolic tissue (states 200, 250,
300ms post sinus beat), successfully induced initial reentrant circuits in 9/12 cases (75%), whilst longer application durations failed to induce. In the experiments, 12/16 (75%) of short durations shocks (125ms) applied to diastolic tissue (225-325ms post sinus beat) successfully induced reentry.

An example of reentry induction via a short battery application is shown in Figure 6 for simulation and experiment. Both Figure 6A & 6D show the induction of break excitation wavefronts upon battery release, their subsequent convoluted pathways and the formation of self-sustained reentry. In both cases, the battery was applied during diastole and was applied for a relatively short duration. Therefore, not only were make excitations elicited upon battery application, but the subsequent break excitations elicited upon battery release successfully interacted with the refractory tails of the prior make excitations, causing the break excitations to slow and reenter.

The slowing of initial break excitations and their non-uniform propagation patterns is evident in both Figure 6A (3490-3550ms) and the first experimental activation map of Figure 6D. Most evident in the simulation images is that both cathodal and anodal break excitation wavefronts are initiated that begin by propagating into the virtual anode regions associated with each individual electrode. As the surrounding tissue is still partially refractory, critical points are established as the break excitations begin to propagate. As the tissue regains its excitability, the break excitation wavefronts pivot around these critical points and start to reenter [28]. Furthermore, the individual $V_m$ and fluorescent signal traces shown respectively in Figure 6B & 6C also show blockage of sinus beats in both simulation and experiment, both immediately following battery release (when the initial break excitations are active) as well as later during self-sustained reentry.

The specific duration of battery application (when applied during diastole such that make excitations are successfully induced) is very important as it governs the time between make and
break excitation production. Figure 7 considers this interaction, showing the progressive propagation of make excitation wavefronts during battery application (down first column), prior to its release for different application durations. Shown along each row are the subsequent interaction of the resulting break excitations with these make excitations for different battery release times. The battery was applied at the same time (a point in diastole) in each row. Each row therefore shows the evolution of activity when that battery is applied for different durations. For short battery applications (rows 50 – 150 ms), make excitations (dark blue arrows) are still active within the ventricles when the break excitations begin to propagate (turquoise arrows), slowing their initial propagation and restricting their paths. The different stage of make excitation wavefront progression at the point of battery release in these cases is thus seen to govern the initial propagation speed and pathways taken by the break excitations, evident by comparing the evolution of break excitation wavefronts between the 15 – 50 ms images along each row (for battery application duration: 50, 100, & 150ms) in Figure 7. The specific stage of make excitation wavefront propagation at the time of release therefore dictates the heterogeneous substrate which causes reentry. Again, establishment of critical points can be seen here for all cases as the break excitations begin by propagating into the virtual anodes associated with the electrodes upon cessation of the battery stimulus, which encourages the waves to initially reenter.

3.3.2 Interaction of Break Excitations with Prior Refractory Tissue

For battery application during systole, no make excitations are induced for the break excitations to interact with. However, upon battery release, break excitations may still be elicited, propagating initially into the hyperpolarised regions of tissue proximal to the electrodes de-excited by the application of the battery. An example of such an effect is shown in Figure 8 in both model and experiment. Figure 8A shows that the ventricles are still refractory upon battery application (3250ms) which was applied just 100ms following a previous sinus beat. Upon release (just 50ms
following application, at 3300ms), break excitations (blue arrows) initially progress into the regions
de-excitated and hyperpolarised by the action of battery (virtual anodes). Due to the fact that the
battery was applied during systole, tissue distal from the electrodes, unaffected by the virtual
electrodes established in the vicinity of the battery, remains relatively refractory, thus slowing the
propagation of the break excitations as they attempt to capture the rest of the myocardium
(panels 3335, 3375ms). Such slowing of the progression of break excitation wavefronts provides
sufficient time for both the refractory tissue depolarised by the action of the battery, in addition
to tissue distal to the electrodes activated by the prior sinus beat, to recover, allowing the break
excitation wavefronts to propagate into these regions before reentering. Figure 8B shows the
corresponding individual $V_m$, as before, highlighting the production of break excitations only into
the region negatively affected by the battery (green trace). Such a mechanism of reentry
induction was more likely for short duration shocks applied during systole. For example, of the 4
different short battery application durations simulated (50, 75, 100 and 150ms), those applied to
systolic tissue (states 0, 50, 100ms post sinus beat), successfully induced initial reentrant circuits in
7/12 cases (58%), of which 5 were for the shorter shocks (50, 75ms) and 2 for the relatively longer
shocks (100, 150ms). In this case, longer battery applications interacted with the next sinus
activation, and thus reentry induction was governed by the mechanism discussed in the Section
below. In the experiments, 7/17 (41%) of short duration shocks (125ms) applied to systolic tissue
(<150ms post sinus beat) successfully induced reentry. A similar example of the induction of a
number of reentrant cycles in the experimental model is shown in Figure 8D for battery
application during systole (50ms after sinus beat). Activation maps highlight the initial complex
propagation patterns of break excitations followed by the later establishment of a reentrant circuit.
Similar reentrant behaviour to that witnessed in the model is also evident in the fluorescent signal
trace of Figure 8C which also highlights the production of break excitations only into the virtual
anode region.
3.4 Conditions for Reentry Induction During Longer Battery Application

3.4.1 Anti-Arrhythmogenic Conditions

For battery release a long time (200ms) following application to diastolic tissue, all make excitation wavefronts have died away and the heterogeneous refractory tissue that they established in tissue distal from the ventricles has also recovered (panel 3750ms of Figure 2A). Upon release, the break excitations are then free to sweep around the ventricles in a faster-moving, larger wavefront whose path is not restricted by heterogeneous refractory tissue, rapidly exciting the rest of the ventricles and self-terminating (as shown in Figures 6 & 7).

For longer battery applications applied to systolic tissue, the tissue left refractory by the prior sinus beat regains excitability during the battery application period, leaving the rest of the ventricles relatively homogeneously excitable. In this case, battery release induces break excitations which again simply sweep across the ventricles unrestricted, inducing only a single ectopic, but no reentry.

3.4.2 Interaction of Break Excitations with Sinus Beat

For longer battery application durations, although any make excitations have died away, induction of short-lived cycles of reentry was still possible through the interaction of the break excitations with sinus activations. However, the majority of release timings during sinus rhythm resulted in early termination of reentry.

In such a scenario that a sinus beat captures during battery application, if the battery is then released soon after this sinus activation, the majority of the tissue is still refractory. The break excitations upon battery release then either fail to propagate or rapidly annihilate with the sinus
activation wavefronts. For battery removal just prior to a sinus beat, break excitations rapidly render the majority of the ventricular tissue refractory and, in addition to anterograde propagation up the PS, means the next sinus beat fails to capture (demonstrated in Figures 5B & 5C).

However, if the battery is released a longer time (>150ms) following the previous sinus beat (although not too close to the next sinus beat), much of the ventricular tissue distal to the electrodes has regained excitability, allowing break excitations to propagate more freely. Initial reentrant cycles are established, but they tend to terminate quickly. An example of such a mechanism is shown in Figure 9. This Figure shows different examples of the evolution of break excitations (along the rows) when the battery is released at different times following the previous sinus beat (down the first column). As can be seen, when the battery is released after 50, 100 or 150ms following the previous sinus beat (rows 1-3), although break excitations are successfully initiated (turquoise arrows), their propagation is hindered via interaction with sinus activation wavefronts (blue arrows) and their corresponding refractory tails leading to rapid termination of activity. However, when the battery is released 200 or 250ms following the previous sinus beat, the sinus activations have passed and the tissue has recovered sufficiently to allow the break excitation wavefronts to propagate more readily. Initial cycles of reentry are induced (witnessed in the 75ms columns of rows corresponding to 200 & 250ms battery release following previous sinus beat) as the activations are still slowed somewhat by the tissue recovering from the sinus beat, although sustained reentry is not induced.
4 Discussion

Using advanced computational modelling, combined with high-resolution optical mapping measurements, we have uncovered the hitherto unknown conditions under which DC from a battery interacts with myocardial tissue to induce reentry within the rabbit ventricles. We have further presented our findings regarding the highly complex interaction and dependence of these mechanisms upon battery application timing relative to sinus activation and battery application duration.

4.1 Summary of Arrhythmogenic Mechanisms

In the rabbit, although self-sustained VF was not induced at all in model nor experiment with battery application, relatively sustained cycles of reentry were inducible. In all cases of arrhythmia induction, reentry was initiated due to the interaction of break excitations (produced by both anodal and cathodal electrodes upon battery release) with:

(a) refractory tissue due to prior make excitations (elicited upon battery application), requiring relatively short battery applications applied to diastolic tissue;

(b) refractory tissue due to prior sinus beats occurring either before the battery was applied (requiring relatively short battery applications to systolic tissue) or occurring during the battery application (requiring relatively longer application durations).

Reentry was induced in these circumstances as the break excitation wavefronts were slowed and forced to take convoluted pathways upon interaction with the heterogeneous distributions of refractory tissue. Such refractory tissue was located both proximal to the battery (due to the direct effect of the virtual electrodes induced by the battery itself) and distal to the battery (due to the complex propagation patterns of the prior make excitations and sinus beats). The identification and dissection of these arrhythmogenic mechanisms which are highly specific to the
application of DC via a 9V battery to the ventricles represents a novel and intriguing finding uncovered in this study.

It should also be noted that central to the initiation of reentry in all the cases studied here is the initial establishment of critical points at the border of virtual anode and virtual cathode regions associated with each electrode. At the end of the stimulus, anodal and cathodal break excitation wavefronts initially propagate into the virtual anode regions of tissue forming critical points with the neighbouring refractory tissue which was depolarised by the stimulus [28]. These critical points provide the substrate around which the break excitations begin to reenter. Due to the close proximity of the battery electrodes, combined with the heterogeneous distribution of refractory tissue (from prior make excitations or sinus beats), many of these initial reentrant waves rapidly terminate either through interaction with one another or with inexcitable tissue. Those that survive, however, provide the foundation for sustained reentrant activity.

Other mechanisms do not appear to be necessary for reentry induction. The battery produces a single make excitation (if applied to diastolic tissue) and a single break excitation when released, so repetitive discharges do not occur. The virtual electrode pattern is essentially static, developing over a few milliseconds, and is large enough that it provides an obstacle with which sinus wavefronts may propagate around. Ionic gradients are not necessary as qualitatively no mechanistic differences were seen using an electrophysiologically homogeneous model (data not shown). The PS was required for delivering the sinus pulse which could interact with the battery excitations in some cases to produce reentry, but it was not a special component of the ensuing reentrant circuit. Mechanical aspects were not considered, namely stretch-activated channels. These may be opened near the electrode near the boundary of the tissue persistently depolarized
by the battery. However, due to the static nature of the response and the fact that an action potential is generated regardless, and more importantly, that arrhythmias can reliably be induced in motion arrested hearts with a 9V battery, it would not appear that such mechanical effects would alter the outcomes of this study significantly.

4.1.1 Shorter Battery Applications

The arrhythmogenic and anti-arrhythmogenic mechanisms described in this study following short battery application durations are summarised in Figure 10. This figure shows an intentionally schematic representation of the qualitative results uncovered in this study from both model and experiment demonstrating how vulnerability to reentry induction depends strongly on both the timing of the application relative to the prior sinus beat and the duration of the application itself. Reentry can be induced when the battery is applied during systole in the absence of make excitations (which are not induced upon battery application, as tissue is refractory). Here, relatively longer applications are required when the initial application occurs very soon after the sinus beat, as this allows more time for the refractory tissue resulting from the sinus activations to recover, allowing break excitations to propagate in the rest of the ventricles upon release. However, the application must still be sufficiently short such that the break excitations are slowed by tissue which has not fully regained excitability following the sinus beat.

Reentry is also likely induced due to the interaction between make and break excitations following battery application during diastole. In this case, the break excitation wavefronts interact with the repolarisation tail of the prior wave which has been induced by the make excitation. When applied a longer time after the previous sinus beat, the make excitations have propagated more rapidly through the ventricles as the rest of the tissue has regained more excitability, meaning that
battery application duration needs to be shorter to ensure sufficient interaction between make and break excitations.

4.1.2 Longer Battery Applications

For longer battery application durations, all tissue refractory from the prior sinus beat and that tissue refractory from the potential make excitations (if applied to diastolic tissue) is recovered. Break excitations will thus propagate relatively uniformly and unhindered upon release, again leading to a single ectopic, but no reentry, unless they interact with additional sinus beats occurring during battery application. However, often the break excitations either directly interact with sinus wavefronts (if released just after sinus capture) and mutually annihilate, or else get blocked by relatively homogeneously refractory tissue established by the sinus wavefronts captured during application. Nonetheless, such interactions were frequently noted to be sufficiently complex to result in 1–2 cycles of reentry, although generally not sufficiently complex to induce fully self-sustained reentry here in the rabbit. The interaction of break excitations with prior make excitations is more arrhythmogenic than with prior sinus activation wavefronts due to the non-uniformity of the make excitation propagation pathways, relative to the more spatially uniform nature of sinus activation patterns, even in the presence of the virtual electrode pattern induced by the battery.

4.2 Relation to Other Species

The focus of this study has been the healthy rabbit ventricles, which are widely recognised to be difficult to induce sustained VF in [3,12,14]. The lack of VF induction in our simulations and experiments likely explain the lack of evidence in the literature for the use of a 9V battery for such purposes in the rabbit, as opposed to its widespread use in species such as pig [8,9,17,22] and dog.
which are known to be easier to induce more complex arrhythmias in [25] due to their relatively larger effective electrical size [19]. Therefore, the larger effective size of other species may prevent the rapid self-termination of initial reentrant cycles induced by the battery, which may then likely degenerate into more complex, self-sustained fibrillatory-like activity. We believe that the fundamental interaction of the battery with the myocardial tissue to produce make/break excitations and their subsequent interaction with one-another and with sinus activations highlighted here, will hold and be equally as relevant across different species, as such phenomena are governed primarily by the bidomain nature of cardiac tissue [28,29].

**4.3 Application to Practical Use within the Experimental Lab**

The practical use of a 9V battery for VF induction is often conducted in a less controlled environment than that represented in the simulations and experimental setups presented in this study. Creating a constant, steady contact of the battery electrodes with the epicardial surface is often difficult due to movement of the heart, particularly problematic in a Langendorff setup and in the absence of mechanical uncouplers. In reality, this will result in slight movements of the battery electrodes, periodically losing contact with the surface. Consequently, this will result in the production of additional make/break excitations each time contact is lost and re-established. Despite this significantly more complex, and less predictable, scenario, we believe that the fundamental mechanisms of make/break excitation interaction uncovered in this study will be the driving force leading to reentry initiation.

Furthermore, in the practical use of the battery, it is often necessary to slightly “jiggle” the battery to induce sustained arrhythmia in human Langendorf preparations [personal communication Prof K Nanthakumar]. We have performed test simulations of this action (data not shown) and seen
how such a jiggling of the battery back and forth produces a similar induction of make/break excitations upon each individual movement. Despite the witnessed complex interaction between make and break excitations following each successive movement, the propagation dynamics of the final set of break excitations still depends strongly on the duration of the final application and timing of release. Successive back-and-forth movements may also induce complex activations during the application which in more arrhythmogenic species may indeed be sufficient to increase the likelihood of sustained reentry induction either through make-break excitation interactions, or interactions with sinus activations.

4.4 Study Limitations

Due to restrictions in mesh resolution of our unstructured finite element mesh, we modelled the battery electrodes in the simulations as solid ‘discs’ on the epicardial surface whereas experimentally (and using a real 9V battery) these electrodes are rings of the same diameter, but of ~1mm width. We have performed test simulations using higher resolution slab meshes which have demonstrated the very close similarity in $V_m$ distribution patterns around the electrodes during battery application when represented by either rings or discs. These similarities are due to the fact that the area within the disc is effectively caged, so its activity has little effect, in addition to the established edge effects of disc electrodes [20] from where current mostly flows.

Furthermore, our model does not include a representation of electrode polarisation impedance at the tissue/metal interface [21] which may slightly affect current flow from the electrodes during battery application/ removal. However, as has been shown experimentally [18], the effects of electrode polarisation impedance are most relevant for weaker shocks, below the normal anode make threshold where this effect can then cause artifactual anode break excitations. In our case,
the stimulus strength was always sufficiently strong to cause anode make and break excitations to
diastolic tissue (without including electrode polarisation impedance) and thus this small additional
effect would not be expected to significantly affect our results. This issue is further supported by
the close match between make/break excitation phenomena witnessed here between simulation
and experiment.
5 Conclusions

Through close agreement of model simulations and experimental data, we demonstrate the mechanism of reentry induction by DC application through a 9V battery as involving the interaction of break excitations upon battery release with refractory tissue from prior make excitations elicited upon battery application or prior sinus beats. Reentry induction was most likely during shorter battery applications (<150ms) and interaction with prior make excitations. Although not witnessed here in the healthy rabbit ventricles, degeneration into sustained VF is likely in other more arrhythmogenic species.
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References


Figure Captions

Figure 1: Computational ventricular model showing positioning of battery electrodes (left) and PS network (right). Approximate fibre orientation direction is also shown close to the electrodes.

Figure 2: Battery application during diastole. Panel A shows epicardial distribution of $V_m$ in model at different times following battery application (at 3400ms) with make excitation wavefront propagation indicated by blue arrows. Panel D shows experimental activation map (right) along with electrode locations (left) of excitation patterns following similar battery application episode (at 1680ms). Stimulus durations were both of relatively long duration: 700ms and 500ms in the model and experiment, respectively. Individual temporal model epicardial $V_m$ (B) and fluorescent signal traces (C) from locations shown by coloured stars in panel B (3405ms) and squares in panel D. Make excitation induction is seen immediately following battery application in both model and experiment which subsequently excites the rest of the ventricles, and causes the next sinus beat to be blocked. Following recovery of the tissue from this initiation excitation, a static virtual-electrode pattern is seen around the ventricles in model (panel A, 3750ms) and experiment (panel E). Colour bar scale in panel E is relative to fluorescent intensity of action potential amplitude recorded during sinus pacing. Approximate fibre orientation is shown by purple arrow.

Figure 3: Intramural polarisation levels and wavefront propagation in battery application during diastole (shown in Figure 2). Panels show $V_m$ levels within the 3D model where a clipping plane (highlighted in the figure centre) has been used to expose the intramural tissue state beneath the cathode at different instances in time following battery application (at 3400ms).

Figure 4: Progression of make excitations following battery application in model. Epicardial distributions of $V_m$ at 25ms following battery application, applied at varying instances in time.
(100–300ms) following sinus beat. As battery is applied a longer time following the prior sinus beat (to progressively more recovered tissue), make excitations propagate faster, reaching further around the ventricles after 25 ms.

**Figure 5**: Battery release during diastole. Epicardial distribution of $V_m$ in model (A) at different times following battery release (at 3700ms) with induced break excitation wavefront propagation indicated by blue arrows. Panel D shows experimental activation map (right) along with electrode locations (left) of excitation patterns following similar battery release (at 2600ms). Individual temporal model epicardial $V_m$ (B) and fluorescent signal traces (C) from location shown by coloured stars in panel B (3700ms) and squares in panel D. Battery release induces break excitations which activate the entire ventricles resulting in a single ectopic beat (but no reentry) in both simulation and experiment. Following the ectopic, sinus rhythm returns in both cases.

**Figure 6**: Reentry induction due to interaction of make and break excitations. Epicardial distribution of $V_m$ in model (A) after a relatively short application (duration 75ms), applied during diastole, with induced break excitation wavefront propagation indicated by blue arrows. Panel D shows two experimental activation maps (right), along with electrode locations (left), of excitation patterns following a similar episode of battery release (application duration 146ms). Individual temporal model epicardial $V_m$ (B) and fluorescent signal traces (C) from location shown by coloured stars in panel B (3475ms) and squares in panel D. In both model and experiment, battery is released sufficiently soon after application such that the induced break excitations (upon release) interact with the refractory tails caused by the prior make excitations, facilitating reentry.

**Figure 7**: Interaction of make and break excitations. Epicardial $V_m$ distributions showing the evolution of break excitation wavefronts at different time instances in the first 100ms (columns)
following battery release for different total battery application durations from 50ms to 200ms
(rows) when initially applied 200ms after the previous sinus beat (during diastole). Make
excitations shown by blue arrows, break excitations by turquoise arrows. As the battery is applied
for longer (going down column 1), the make excitations induced upon its application have
advanced further and the ventricles are progressively more recovered when the battery is
released, allowing the subsequent induced break excitations to propagate more freely, thus
encountering less refractory tissue.

Figure 8: Reentry induction following battery application during systole. Epicardial distribution of $V_m$ in model (A) after a relatively short application (duration 50ms), applied during systole, with induced break excitation wavefront propagation indicated by blue arrows. Panel D shows two experimental activation maps (right), along with electrode locations (left), of excitation patterns following a similar episode of battery release (application duration 130ms). Individual temporal model epicardial $V_m$ (B) and fluorescent signal traces (C) from location shown by coloured stars in panel B (3300ms) and squares in panel D. As the battery is applied to refractory tissue, no make excitations are produced. However, both model and experiment show rapid de-excitation of tissue negatively affected by the shock (virtual anode) in addition to depolarisation of tissue positively affected by the shock (virtual cathode). The relatively short battery application time means that break excitations elicited upon battery release interact with tissue still recovering from the prior sinus beat, providing the substrate for reentry.

Figure 9: Interaction of break excitations with sinus activations. Epicardial $V_m$ distributions showing the evolution of break excitation wavefronts at different time instances in the first 150ms (columns) following battery release at different times between 50-250ms following the previous PS sinus beat (having occurred during battery application). Battery initially applied 150ms
following a sinus beat; thus total application durations ranged from 250-450ms. If the battery is
released too soon after the previous sinus beat (<150ms), the induced break excitations have little
excitable tissue to propagate into and thus rapidly terminate. If released later following the
previous sinus beat (>150ms), distal tissue has recovered sufficiently to allow initial break
excitation propagation which is slowed slightly, facilitating reentry.

*Figure 10*: Schematic summary of vulnerability to reentry induction following short battery
application durations for simulations. Dependence of different arrhythmogenic mechanisms to
battery application timing following previous sinus beat and for different application durations is
highlighted. Solid pink circles demonstrate successful induction in simulations, with open circles
representing failed reentry attempts.
make excitations

hyper-polarised tissue

intramural propagation

3402ms 3405ms 3410ms
During systole and during diastole, make excitations.

Battery application time post prior sinus beat:
- 100ms
- 150ms
- 200ms
- 250ms
- 300ms

-150mV to 100mV
time of battery release after previous sinus beat

battery release

0ms 15ms 30ms 50ms 75ms 150ms

0ms 15ms 30ms 50ms 75ms 150ms

0ms 15ms 30ms 50ms 75ms 150ms

0ms 15ms 30ms 50ms 75ms 150ms

0ms 15ms 30ms 50ms 75ms 150ms

0ms 15ms 30ms 50ms 75ms 150ms
battery application time post sinus beat

- systolic tissue
- diastolic tissue

battery application duration

- 0ms
- 50ms
- 150ms
- 300ms

systolic tissue

- arrhythmogenic
- break-prior refractory tissue interaction

diastolic tissue

- arrhythmogenic
- make-break excitation interaction

break-prior refractory tissue interaction