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Doctor of Philosophy Dissertation

Mathematical Modelling of
Gastric Electrophysiology

Peng Du

Supervisors
Professor Andrew J Pullan
and
Dr Leo K Cheng

A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

Auckland Bioengineering Institute
The University of Auckland
Auckland, New Zealand
2011
Abstract

This thesis investigates the electrophysiology of the stomach, using a joint experimental and mathematical modelling approach.

Normal gastrointestinal (GI) motility is coordinated by multiple cooperating mechanisms, both intrinsic and extrinsic to the GI tract. A fundamental component of the GI motility is an omnipresent electrical activity termed slow waves, which are initiated and propagated by the interstitial cells of Cajal (ICCs) and smooth muscle cells (SMCs). The role of ICC and/or SMC pathophysiology in GI motility disorders is an area of ongoing research.

This thesis begins with an overview of the functions of the GI tract and slow wave electrophysiology. High-resolution electrode arrays were designed and manufactured using the printed-circuit-board (PCB) technology. The performance of the PCB electrodes were validated against the performance of epoxy-embedded electrodes in porcine subjects, in terms of amplitudes (0.17 vs 0.52 mV), velocity (15.9 vs 13.8 mm s\(^{-1}\)), and signal-to-noise ratio (9.7 vs 18.7 dB). The PCB electrodes were then used to record gastric slow waves from a number of human subjects. Automatic slow wave activation times identification and velocity calculation techniques were applied to analyse the recorded slow wave data. Analysis of the human data revealed that the gastric slow wave activity originates from a pacemaker region (average amplitude: 0.57 mV; average velocity: 8.0 mm s\(^{-1}\)) in the stomach, and continues into the corpus (average amplitude: 0.25 mV; average velocity: 3.0 mm s\(^{-1}\)), and then the antrum (average amplitude: 0.52 mV; average velocity: 5.7 mm s\(^{-1}\)).

The focus of this thesis then shifts to mathematical models of slow wave activity. An existing SMC model was adapted to investigate the effects of gastric electrical stimulation (GES) protocols, in conjunction with experimental recordings in rat
antral SMCs. The simulations using the adapted SMC model showed that effective GES protocols could be adapted to include frequency-trains (40 Hz) of short pulse-width (3-6 ms); In a separate study, an existing ICC model was adapted to include a voltage-sensitive inositol 1,4,5-trisphosphate receptor model, which modelled entrainment of slow waves in a network of ICCs; Two coupling mechanisms were also proposed to link the slow waves in the ICC and SMC models.

A continuum approach was used to model slow waves in tissue and whole-organ models. The monodomain equation was used to simulate slow wave propagation in a grid of SMCs coupled to a cell automata model, which was used to quantify the entrainment of normal slow wave activity and entrainment of slow waves by a 3.5 cpm GES protocol. The simulation results demonstrated the highest ‘zone of entrainment’ that could be achieved by the GES protocol was 78% of the modelled tissue area; Next, the bidomain equations were applied to simulate entrainment of slow waves in a wild-type (normal) and a degraded (serotonin receptor knockout) ICC networks obtained from mouse tissue. The ICC network models demonstrated that slow wave propagation was influenced by ICC loss. In addition, compared to the degraded ICC network, the normal ICC network model demonstrated a higher peak current density (1.94 vs 1.45 μA mm$^{-2}$) as well as $[Ca^{2+}]_{i}$ density (0.67 vs 0.41 mM mm$^{-2}$), which could help to explain functional impairments that arise when ICC populations are depleted; The human recordings were used to create slow wave activation in a whole-organ stomach model. The whole-organ model was used as a platform to simulate gastric slow wave propagation, as well as to incorporate physiological characteristics that could not directly measured using the HR technique, such as the variation in the resting membrane potentials of gastric tissues.

The final set of modelling studies employed the forward modelling technique to simulate the resultant body surface potential, i.e., electrogastrogram (EGG) of gastric slow waves. A virtual EGG analysis showed that the frequency of EGG matched the underlying slow waves (3 cpm) and the peak potential (-0.63 mV) in the EGG signal could be correlated to the timing of the full antral activation. This thesis concludes with a discussion on the results and potential future research directions in this field.
Acknowledgements

I am grateful to my supervisors, Professor Andrew Pullan and Dr Leo Cheng, for their guidance, encouragement, and challenge. Andrew, the inertia of your support was more than sufficient to jolt me over the finish line, and now it is our turn to support you in your battle with ‘the beast’.

Special thanks to my colleagues, Dr Greg O’Grady and Dr John Egbuji.

I am thankful for the support provided by the following people. To Professor Wim Lammers for guidance during the ‘dark age’ of our recording work and continued support thereafter; to Mrs Linley Nisbet for technical assistance; to Professor John Windsor for the opportunity to conduct research at Auckland City Hospital; to Professor Jiande Chen for the many innovative ideas; to Dr Tom Abell and Dr Chris Lahr for the opportunity to conduct research at The University of Mississippi Medical Center; to Professor Gianrico Farrugia and Dr Simon Gibbons for the many images of ICC networks and insights into cell physiology. Finally, I would also like to thank the members of my advisory committee for your support, especially to Dr Mark Trew for proofreading;

I would like to express my deep gratitude to my family and friends for their support, which constantly reminded me of the high aspiration of the pursuit of knowledge. Dear parents, congratulations on eradicating the last Bachelor’s only degree holder in the family. Karmun, I consider finishing my degree before you - for once finally, as an accomplishment.

Funding from The University of Auckland is greatly appreciated.
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<td>cpm</td>
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<td>CICR</td>
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<td>CM</td>
<td>Circumferential muscle (layer)</td>
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<td>CMISS</td>
<td>Continuum Mechanics, Image analysis, Signal processing and System Identification</td>
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<td>Common mode sense</td>
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<td>DRL</td>
<td>Driven right leg</td>
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<td>FP</td>
<td>False positive</td>
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<tr>
<td>GEMS</td>
<td>Gastric Electrical Mapping Suite</td>
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<td>Gastrointestinal (tract)</td>
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<td>HR</td>
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<td>IP$_3$</td>
<td>Inositol 1,4,5-trisphosphate</td>
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<td>LM</td>
<td>Longitudinal muscle (layer)</td>
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<td>MMC</td>
<td>Migrating motor complex</td>
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<td>ODE</td>
<td>Ordinary differential equation</td>
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<td>PCB</td>
<td>Printed circuit board</td>
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<td>PPV</td>
<td>Positive prediction value</td>
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Chapter 1

The Digestive System

On a grilling summer’s day in a quiet Mississippi water-side restaurant called the Cock of the Walk, after realising the fatal mistake of not recognising that entrée really means the main course in the United States, I braved myself for the entrée (Fig 1.1(a)) - two arduous hours later, not much had changed (Fig 1.1(b)), except by now I was feeling very bloated and a little sick.

Figure 1.1: The (in)famous fried catfish and chips, pickles, and onion rings; delicious in taste but lethal in dosage.

Still feeling hungry?

Even though I stuffed myself silly that day, I suspect my experience was far from unique. Amazingly, there was still room for a light dessert half an hour later. Of course, we vary in our meal intakes daily, eating too much we accumulate the excess nutrients as the dreaded body fat; eating too little we could seriously impair
1. The Digestive System

our normal bodily functions. This eternal seesaw of the desire to eat and the guilt of gluttony underlies one of the most primal urges of the human body and dictates many aspects of our everyday lives: from McDonald’s to the Michelin stars; from Jenny Craig to the unrelenting ‘how to lose 10 kgs in 2 weeks’ email spams - particularly right after the holiday periods. A search for the key word ‘how to lose weight’ on Google yielded 188 million hits, whereas a search for the key phrase ‘recession’ yielded a comparatively few 33 million hits. In fact, during the height of the latest bout of recession, KFC New Zealand, a fast food outlet chain, experienced a close to 10% quarterly growth-rate in the year 2010 [131]. The moral of the story? We are a lot more concerned with our stomachs than we care about the economy.

Yet, have you ever wondered how your stomach actually works? For the past three years, I have sought to address one aspect of this question by recording the bioelectrical activity in the stomach, and employing mathematical models to study the stomach. Many discoveries were made, some confirming what was already known, while others uncovered fascinating findings for the first time. This thesis is a compilation of the developments and applications of the techniques that I have employed to understand the electrophysiology of the stomach in an integrated manner.

The organ system responsible for digestion of food is called the digestive system, which plays a critical role in the uptake of energy, nutrients, and water. We eat on average three meals and drink eight cups of water daily, yet the stomach continuously makes room for more food and water intake, we feel hungry, and then we want to eat more. After having a meal, a healthy human stomach is capable of digesting the meal and emptying its contents by more than 30% after one hour, 75% after two hours, and 99% after four hours of ingestion. People with stomach emptying rates consistently and significantly less than those values are actually classified as having a medically significant delayed stomach emptying symptom [171].

The digestive system is only one of the organ systems in the human body, which comprises billions of cells with highly specialised functions and round-the-clock maintenance of bodily functions such as growth, repair, and reproduction. The other major organ systems include the following systems: circulatory, cardiovascular, lymphatic, endocrine, integumentary, muscular, nervous, reproductive, respiratory, skeletal, and urinary systems. This categorical approach serves a refined scope in which
researchers could focus their study on a particular physiological system. However, it is important to realise that in reality, the functions of the organ systems are highly integrated. For instance, the endocrine systems also in many ways influence the functions of the digestive systems via hormones. Therefore, it is imperative that when studying the digestive system, the cross-linking functions between the physiological systems are also considered.

1.1 The Gastrointestinal Tract

The gastrointestinal (GI) tract is a continuous tube comprising several distinct organs that run over several metres in length ($\sim5\ m$) from the mouth to the anus (Fig 1.2). There is still a surprising amount we do not yet know about the physiology of the GI tract, despite its role of supplying nutrients and water to sustain all of our normal bodily functions. While the organs along the GI tract, e.g., the stomach and intestines, are functionally discrete, their behaviours are highly coordinated, affording a sophisticated overall level of integrated function, which is tightly regulated by many facets of the body systems, such as the endocrine, nervous, and neuromuscular activities.

The integrated activity of the GI organs provides many essential bodily functions: storage, digestion, absorption, excretion, and protection. By definition, digestion is the process by which ingested food is broken down into basic nutrients and water, ready for absorption and subsequent use in the functions, repair, and growth of bodily tissues [44]. Digestion begins in the mouth, where food is first masticated, i.e., chewed, and mixed with saliva. The resulting food bolus is swallowed, through the oesophagus, and then reaches the stomach. The anatomy of the stomach is further examined here, since it is the principal organ of interest of this thesis. The stomach appears as an expandable muscular cavity with a curved shape connecting the oesophagus to the small intestine, sitting just beneath the diaphragm in the human body (Fig 1.2). The longer side of the stomach is termed the greater curvature, and the shorter side of the stomach is termed the lesser curvature. The stomach is anatomically divided into the fundus, corpus, antrum, and pylorus. Each anatomical zone of the stomach is understood to have specific functions and movements to store
or to digest the gastric contents. In this way, the stomach acts as an intermediary between the behavioural acts of eating and the physiological acts of digestion [7]. The fundus acts as a storage space of food, but it is usually filled with air. Digestion of ingested food takes place in the corpus and antrum. The pylorus acts as an outlet sphincter of the contents in the stomach. The stomach uses sphincter muscles to actively control the flow of its contents into the small intestine, and under normal circumstances, prevents its contents from regurgitating up the oesophagus. In fact, the etymology of the word ‘stoma’ was defined as ‘orifice, small opening in an animal body’, which was later specified to refer to the stomach in the modern sense sometime in the 14th century [70].

The process of digestion involves both mechanical movements, such as the mixing of food particles, and chemical reaction, such as pH regulated enzymic reactions. The key result of these mechanisms is to break down food into a slurry of fine particles termed chyme, which is released from the stomach into the small intestine at a controlled rate through the pylorus [7]. Chyme is continuously propelled along the intestines while absorption of nutrients and water takes place mainly via a densely folded (to increase surface area) small intestinal wall. The large intestine, i.e., colon, is primarily concerned with desiccation and compaction of the waste products from the digested food in the GI tract, with storage in the sigmoid colon and rectum prior to excretion through the anus.
Figure 1.2: The major organs along the gastrointestinal (GI) tract. The stomach is situated between the oesophagus and the small intestine. The top of the stomach is classified as the fundus. The midsection body of the stomach is termed the corpus, and the remaining of the stomach between the corpus and the pylorus (outlet) is termed antrum. Food is stored and mixed with gastric secretions in the stomach, and then emptied into the small intestine and colon for further digestion and absorption of nutrients. Source image obtained from [151].
1.2 Transport of Food

A key function of the GI tract is its ability to transport food bolus/chyme through the body. The movements by which the GI tract moves its contents is termed GI motility, which exhibits many highly complex modes and patterns. For example, the sequential contraction and relaxation of the GI tract is called peristalsis, which propagates a mechanical wave and propels food in the antegrade direction towards the end of the GI tract. The magnitude of peristaltic contractions can be vigorous, particularly in the gastric antrum, as demonstrated in Fig 1.3; here, the peristalsis of the stomach manifested as a partial occlusion due to the organised muscular contractions in the gastric antrum. The peristalsis travelled in the antegrade direction, towards the pylorus, where the occlusion was up to ~90% compared to the diameter of the antrum in the first frame (Fig 1.3). The vigorous peristaltic contractions of the gastric antrum serve to break down food, and to mix the gastric contents with gastric secretions such as the stomach acid.

Another important contractile activity in the stomach is the slow volume-reducing contractions of the gastric fundus [142]. The fundus stores food and maintains a gastro-duodenal pressure gradient that propels the gastric contents into the small intestine [80, 142]. The stomach empties its content by antral peristaltic waves and at a rate controlled by pyloric sphincter opening [18]. The two modes of contractions ensure that food is well digested by the gastric acid before it is emptied from the stomach at a slow but controlled rate for absorption in the intestines.
Figure 1.3: Gastric motility captured via an endoscopic video camera. (a) Frames from an endoscopic video of the contraction in the gastric antrum. The contractions can be seen as an almost complete occlusion of the gastric lumen, propagating in the antegrade direction. If the stomach was full, food particles would be moved with this peristalsis activity. If the terminal end of the stomach (the pylorus) stays open then the food particles are propelled into the intestine, whereas if the pylorus is closed then the food particles exhibit complex retrograde turning motions that serve to further break down food particles and mix them with gastric acids. (b) The contraction of the antrum was also quantified by measuring the degree of contraction by the ratio of the contracted diameter to the diameter at 0 s from the images. The diameter of gastric pylorus contracted up to 90% over a period of 15 s. Courtesy of Dr Shou Tang, the University Mississippi Medical Center (UMMC), Jackson, MS, USA.

Although normal gastric motility is the end-result of several cooperating mechanisms, which include myenteric, neural, hormonal, and paracrine factors, the principal focus of this thesis is the underlying electrophysiological mechanisms that initiate and coordinate motility. A key motivation for studying the electrophysiology of the stomach is the hope to emulate the success of cardiac electrophysiology in the GI field. Like the stomach, the heart is also a muscular organ and it too contains an underlying electrical event that governs the contractions of the heart. Cardiac electrophysiology has evolved into a highly sophisticated body of research, deriving a multitude
of clinically significant findings relating to the patterns of the electrical activation in
the heart, from which both diagnostic procedures such as electrocardiograms (ECG),
and treatment procedures such as radio-frequency ablation, have been designed and
applied as routine treatment procedures. A similar body of research techniques can
be adapted in gastric electrophysiology, and to help facilitate the understanding and
possibly treatment of a multitude of GI diseases.
Chapter 2

Gastrointestinal Electrophysiology

Many GI functions are dependent on the structure and physiology of the underlying tissues in the GI wall. For example, peristalsis of the stomach is mediated by the layers of muscular tissues that are part of the gastric wall, and secretion of gastric acid is mediated via specialised oxyntic cells that are embedded in the lining of the stomach [7]. Therefore, an accurate understanding of GI electrophysiology, in particular gastric electrophysiology, can only be gained by studying the integrated activities of the multitude of physiological processes and structural information of cells, networks, and tissues in the stomach. This chapter presents a detailed introduction of the structures and functions of the GI tract, with an emphasis on the functions of the electrically active cells in the GI tract (Section 2.2 and 2.3). The clinical relevance of GI electrophysiology is discussed in Section 2.4. The aims of this thesis are presented in Section 2.6.

2.1 The Gastrointestinal Wall

The GI wall consists of a number of distinct layers of tissues [155]. Not all parts of the GI tract have the same composition, however, the general structure is illustrated in Fig 2.1. The mucosa is the innermost layer of the GI tract, and it consists of epithelial lining the lumen of the GI tract, underpinned by a thin layer of smooth muscle known as the muscularis mucosa. The next layer is the sub-mucosa, which consists largely of loose connective tissue, nerves, and blood vessels that support and supply the
mucosa. Beyond the sub-mucosa lies the muscularis externa - a thick muscular coat that is responsible for affecting the mixing and movement of digestive contents. In the stomach, the muscularis external consists of smooth muscles arranged in three layers, the outer longitudinal muscle (LM), the middle circular muscle (CM), and the inner oblique muscle (only in some part of the stomach). The CM layer contains smooth muscle fibres arranged in rings around the GI tract, while the LM layer contains smooth muscle fibres aligned in the direction along the GI tract. A rich plexus of nerves, the myenteric plexus, termed Auerbach’s plexus, lies between these major muscle layers [155]. The small intestine contains only LM and CM layers, and in the human colon the LM layer is mostly coalesced into three bands termed the taenia coli [7]. The outermost layer of the GI tract is the serosa, which mainly consists of connective tissue, and serves as a structural outer coat.
Figure 2.1: Gastric wall anatomy. (a) An illustrated stomach and the major anatomical landmarks. (b) An illustrated cross section of the gastric wall. (c) An electron microscopic image (16,000× magnification) of gastric circular smooth muscle cells and an interstitial cell of Cajal. The smooth muscle cell is innervated by the interstitial cell of Cajal via a gap junction formed between the two types of cells. Both (a) and (b) were obtained and reproduced from [38, 35, 37], and (c) was reproduced from [98].
2.2 Gastric Smooth Muscles

Gastric motility is mediated by the gastric smooth muscle cells (SMCs), which are elongated with an expanded central region and tapering ends in shape. The individual SMC form fibre that is usually 2 to 5 \( \mu m \) in diameter and 20 to 500 \( \mu m \) in length [7]. Like all cells, SMCs are enveloped by a bilipid and semi-permeable membrane which acts as a selective barrier that divides the intracellular space (also known as the cytoplasm) from the extracellular space, \( i.e. \), tissue matrix. The constituents of the cytoplasm include many and cellular organelles and chemicals, of which the most important to motility are charged particles known as ions, notably calcium (\( Ca^{2+} \)), potassium (\( K^+ \)), sodium (\( Na^+ \)).

There are many types of ion channels/conductances embedded in the cell membrane of a SMC, which act as selective passageways for the ions. As the concentrations of these ions differ between the cytoplasm and the extracellular space, an electrochemical gradient, known as the (trans)membrane potential (\( V_m \)), develops across the cell membrane. For a single ion species, there is an electrical potential at which the net flux due to the electrochemical gradient becomes zero. The \( V_m \) at this point is known as the Nernst potential of this ion species, and is usually denoted as \( E_x \), where \( x \) denotes the symbol of the ion species. In a SMC at rest, \( i.e. \), no contraction, the resting membrane potential is quantified under the assumption of electroneutrality, \( i.e. \), there is a balance of charges across the cell membrane [7]. The SMCs at the resting membrane potential, which is around -68 \( mV \) in antral SMCs [78], can maintain the basic cellular functions without producing any active contractions.

Preceding a contraction, the configurations the protein sub-units in the SMC ion channels change to allow a flux of ions across the cell membrane. This initial flux is induced by a change in the \( V_m \), caused by the cell-to-cell spread of depolarising electrical events known as slow waves (Fig 2.2 SMC trace). The resultant fluxes of \( Ca^{2+} \), \( K^+ \), and \( Na^+ \) rapidly depolarise the \( V_m \) towards -30 \( mV \), and subsequently \( Na^+ \)-type channels inactivate and \( Ca^{2+} \)-type channels remain open, maintaining \( V_m \) at a plateau phase [155]. In some regions in the GI tract, contraction of SMCs is initiated by slow waves, and it has been postulated that the force of contraction may
be dependent on the amplitude of slow waves [7]. Neural, hormonal, and paracrine influences also have a major bearing on the contractile response, and without these additional modulating influences, the amplitude of the slow wave does not typically exceed a certain voltage threshold to enable vigorous contractions to occur [141]. For example, activation of the L-type $Ca^{2+}$ conductance during the plateau phase of slow waves leads to continuous influx and significant rise in intracellular calcium concentration ($[Ca^{2+}]_i$), which triggers the contractile elements in the GI SMCs [141]. The subsequent inactivation of $Ca^{2+}$-type channels and prolonged activation of $K^+$-type channels repolarise the $V_m$ back to the resting membrane potential.

![Figure 2.2: Superimposed experimental traces of slow wave activity in an interstitial cell of Cajal (ICC) and a nearby smooth muscle cell (SMC) in guinea-pig stomach. Slow waves generated within both types of cells occurred synchronously at a rate of 3 cycles-per-minute. Data traces modified from [78].](image)

In general, slow waves modulate mainly the phasic contractions in the GI tract. The other important type of contraction in the GI tract is the tonic contractions, especially the gastric fundus, where the SMCs are understood to be depolarised and influenced greatly by neural factors [154]. Furthermore, it can be said that modes of phasic contractions depend a lot on the state of the underlying tone in the GI tract [154]. In large animals, a separate mode of contractions called migrating motor complex (MMC) [116, 167] is MMCs are categorised into three phases. Phase I involves relative absence of contractions; Phase II is a period of irregular contractions; and phase III involves a period of uninterrupted intense contractions which occur more frequently during period of fasting [194]. It has been demonstrated that two hormones - motilin and ghrelin, are both correlated with MMC, which suggests a strong role of hormones in the onset of MMC [194].

Furthermore, additional voltage-independent pathways have also been found to
influence SMC motility. For example, the Rho/Rho-kinase signalling pathway has been found to alter SMC $[Ca^{2+}]_i$ sensitivity [154, 16]; this pathway offers an added layer of complexity to our current understanding of SMC motility. Specifically, the Rho/Rho-kinase pathway may also modulate neurotransmission between ICC-MY and SMC in the gastric fundus [16]. A concurrent MRI and high-resolution manometry also demonstrated that the MMC helps to setup a pressure pump mechanism controlled by pyloric opening and period of relative quiescence in antral contractile activity [91].

Slow waves in SMCs occur at \sim 3 cycles-per-minute (cpm) in the human stomach, 10-12 cpm in the duodenum, and 8-9 cpm in the terminal ileum [155]. The propagation patterns of slow waves in the large intestine are less well defined [63, 85, 153]. The systematic propagation of slow waves in some parts of the GI tract at these frequencies confer a critical coordinating effect on GI motility patterns, which follow the slow wave pattern in much of the GI tract. For over half a century, slow waves generated by the SMCs in the GI tract were believed to be the key underlying electrophysiological basis of GI motility; however, an hypothesis made by a Nobel prize laureate, with the help of a special mutant mice almost century later, would forever change our understanding of GI slow wave activity and herald this field into a new age.

## 2.3 The Interstitial Cells of Cajal

‘Nerve-like cells at ends of motor neurons in organs innervated by peripheral nerves.’

Santiago Ramón y Cajal, 1911

It was previously assumed that slow waves were autonomously generated and propagated within the GI SMCs, either through nerve activation or some intrinsic mechanisms unique to the SMCs [168]. In the recent two decades, this view has been overturned by the elucidation of the functions of the interstitial cells of Cajal (ICCs), which reside within and between the smooth muscle layers. The ICCs were first described in 1911, as ‘nerve-like cells at ends of motor neurons in organs innervated by
The Interstitial Cells of Cajal

peripheral nerves’ by the Spanish Nobel prize laureate Santiago Ramón y Cajal [17]. The close association between ICCs and nerve terminals throughout the musculature prompted Cajal’s original hypothesis that ICCs were involved in the neuromodulation of GI motility.

The breakthrough in understanding that ICCs are essential for normal GI motility followed the serendipitous discovery that mice injected with a neutralising antibody to c-Kit (a type of protein receptor in ICC) developed lethal hypomotility, accompanied by intestinal dilation [118]. Subsequently, it was documented that $W/W^v$ mutant mice, which had spontaneous c-Kit mutations, lacked ICC-MY as well as intestinal slow waves [85]. Since these seminal studies, numerous investigations have confirmed that ICCs are critical for the generation and propagation of slow waves in the normal gut [155].

The term ‘slow wave’ is one of the many terms that have been used to describe the electrical activity of the GI tract. Historically, the term slow wave was first introduced to describe the electrical activity in SMCs [168]. Later, with the identification of the role of ICCs in GI electrophysiology, slow wave was used as a general term to describe the electrical activity in both ICCs and SMCs. Over the years, different terminologies have been introduced to describe the same electrical activity in ICC and/or SMC. Previous studies have also attempted to associate different terminologies of slow waves to the anatomical locations in which the slow waves are generated [49, 94]. In some instances, the slow waves generated by the ICCs have been referred to as pacemaker potentials. To avoid possible confusion when referring to the electrical activity, a list of the major terms that have been used to describe the electrical activity in the ICCs and SMCs is given in Table 2.1.

One naming convention has been to term the electrical activity in the SMCs as the response to the electrical activity in the ICCs, e.g., electrical response activity (ERA) is the response to electrical control activity (ECA). In this thesis, we have adopted the modern terminology of using slow wave to describe the electrical activity of both ICCs and SMCs [155]. The reason is that SMCs do not independently produce slow waves without the ICCs, and therefore the response electrical activity of the SMCs can be seen as an extension of the slow wave activity that originate in the ICCs (Fig 2.2). Where appropriate, we will specify whether the slow wave activity refers
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<table>
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<tr>
<th>ICC electrical activity</th>
<th>SMC electrical activity</th>
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<td>Driving potentials</td>
<td>Regenerative potential</td>
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<td>Electrical control activity (ECA)</td>
<td>Electrical response activity (ERA)</td>
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<td>Pacemaker potential</td>
<td>Follower potential</td>
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<td>Slow wave</td>
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Table 2.1: Terminologies that have been used to describe the electrical activity in ICC and SMC. Where appropriate, the terms were paired by the pacemaker activity of the Interstitial Cell of Cajal (ICC) and the subsequent response activity of the smooth muscle (SMC). The table was adapted from [155]. Slow wave can be used to refer to the electrical activity in both SMC and ICC.

In a recent review, an author has raised a number of issues which could disprove the functions of ICCs [157]: (i) ICCs do not directly drive SMCs, but instead stabilise slow wave propagation by setting the resting membrane potential gradient in the smooth muscle layers; (ii) the ENS directly innervates SMCs; (iii) ICCs are not mechano-sensitive; and (iv) ICCs impairment in motility dysfunctions is not established. These points were well addressed in an editorial in the same issue [138]. The main points of the editorial were: (i) simultaneous recordings of ICC and SMC clearly demonstrated that slow waves originate from the ICCs [49], as well as setting the resting membrane potential of the SMCs [65]; (ii) the ENS influences both ICCs and SMCs; (ii) a recent study has demonstrated a mechano-sensitive sodium channels expressed in both ICCs and SMCs [99]; (iv) given the three aforementioned points, as well clinical evidence, ICC impairment has been clearly linked to motility dysfunctions (see Section 2.4).

Several different classes of ICCs have been described based on their anatomical locations in the GI wall [155]. The myenteric ICCs (ICC-MY) surround the myenteric plexus and are the primary pacemakers of the gut. The intramuscular ICCs (ICC-IM) lie intermixed in the smooth muscle layers of the stomach and colon, and primarily serve in neuromodulation, although they also have the capacity to act as secondary pacemakers under certain circumstances such as with potent neural drive [76]. Other populations of ICCs lie near the deep muscular plexus at the sub-mucosal surface of
the small intestinal circular muscle coat (ICC-DMP). Another class of ICCs is located in the septa between bundles of SMCs, termed ICC-SEP.

In combination, these ICC populations are now understood to play a number of essential roles in GI motility. Many studies have now confirmed that ICCs are responsible for generation of slow wave activity, through a coordinated system of intracellular events [155]. First, slow waves conduct passively from ICCs to SMCs via gap junctions and they cannot actively propagate in smooth muscle layers [34]. However, it is important to note that even though the conduction of ICC to SMC is passive, the SMC contains voltage-dependent ion channels which respond to the passive depolarisation by ICC, and may regenerate slow wave in the circumferential muscle layer [78, 34]. Slow waves can also be regenerated by other populations of ICCs, e.g., ICC-IM. Second, studies have also since confirmed a major role for ICCs in neuromodulation, and in mediating cholinergic and nitrenergic neurotransmission in particular [180]. Third, ICCs also function as mechanoreceptors, as evidenced by their direct responses to stretch via mechanosensitive ion channels, and ultrastructural evidence showing their tight association with vagal afferent in the oesophagus and gastric fundus [99, 146]. Finally, ICCs also set the resting membrane potential of SMCs via gas-mediated signalling, particularly via the influence of carbon monoxide [65].

2.4 The Clinical Relevance of Slow Waves

The disorders of ICCs and SMCs play a major role in functional GI diseases. In particular, ICC loss and network degradation have now been documented in association with motility disorders in some segments of the GI tract [59, 87]. Likewise, therapeutic interventions seeking to prevent ICC loss, or replenish their numbers, and restore their functions have become an important research focus in recent years [87, 153]. In addition, efforts to restore normal GI motility through electrical stimulation therapy are also ongoing; these efforts are discussed briefly as an example of a research field that is beginning to benefit from a conjoint experimental and mathematical modelling approach.
2. Gastrointestinal Electrophysiology

2.4.1 Role of ICCs in Motility Disorders

The association between ICC loss and dysmotility has been documented in the conditions of gastroparesis and slow transit constipation. Gastroparesis is a condition in which the stomach fails to empty normally in the absence of an anatomical obstruction, leading to symptoms that include nausea, vomiting, bloating, and malnutrition in severe cases. The rate of gastric emptying in gastroparetic patients is usually significantly affected, sometimes with less than 23% emptying after two hours, whereas normal emptying rate should be approximately 75% [120, 171]. Diabetes is a contributor of gastroparesis, with between 11-18% long-term diabetics reporting symptoms consistent with gastroparesis [178, 145]. As the prevalence of diabetes increases, so does the prevalence of gastroparesis, with hospital admissions in the United States having risen by >150% in the recent decade, partly due to the present epidemic of type II diabetes [178]. ICC loss is now a recognised hallmark of diabetic gastroparesis, and is understood to result from disease influences that both promote ICC death, such as the inhibition of the protective enzyme heme oxygenase-1, and that reduce ICC survival or regeneration, such as inhibition of the ICC-promoting hormones insulin and insulin-like growth factor [139]. However, the mechanisms by which ICC loss impairs GI motility remain under investigation.

It is also understood that the loss of ICC in humans is almost always associated with a loss of enteric neurons [59]. This further complicates the effects of ICC loss on the GI motility because the enteric nervous system also exerts a significant influence on the functions of the GI tract. There is also an issue with whether the symptoms of gastroparesis are a direct result of ICC loss in the underlying tissue or as a result of the insult caused by prolonged obstruction of the lumen and/or retention of food [59]. Experimental studies using mice have suggested that inflammation of the GI tissue could lead to a decrease in ICC count and the ICC could be restored after the insult [40, 20]. However, the obstruction of the GI tract is unlikely to be the only factor for a reduction in ICC, particularly in some human diseases associated with an abnormal count. For example, in intestinal pseudo-obstruction there is often a dilation of the GI tract, and only subclasses of ICC have been shown to be selectively affected, but not uniformly reduced in the obstructed tissue [59]. Nevertheless, despite
The Clinical Relevance of Slow Waves

The need to further clarify the details of the mechanisms of ICC loss, the functional outcome of ICC loss is clear - it leads to diminished GI motility.

Another common disease associated with ICC loss is slow transit constipation, which exhibits a pathologically increased transit time of colonic contents. It is a difficult disease to manage clinically, as many patients are refractory to standard medical therapies such as increasing dietary fibre intake and laxative use. A pan-colonic decrease in ICC count occurs in some patients with severe constipation [117]. Interestingly, recent studies on the ongoing loss of ICCs has revealed a constant regeneration of ICCs from progenitor cells in the normal human colon, and a gradual decline that can take place over a number of years due to an increased rate of loss and/or a reduced rate of regeneration [59]. Again, the exact pathophysiological relationship between ICC loss and increased colonic transit, and the means by which symptoms might result, remains uncertain.

2.4.2 Electrogastrography

The ability to non-invasively record the underlying electrical event in the human body for medical diagnosis is one of the key applications of electrophysiology. For example, since the advent of the ECG, the speed and the accuracy by which the clinicians can diagnose a patient with cardiovascular distress have improved significantly [9, 147]. In a similar application, the electrogastrography (EGG) also attempts to record the resultant slow wave activity of the stomach and/or intestines by placing electrodes on the body surface [25]. However, EGG has several technical difficulties to overcome before it can be adopted as a routinely used diagnostic tool [25]. This following section briefly outlines the two main challenges currently facing EGG.

The first challenge facing EGG is the relative weak signal-to-noise ratio (SNR) of the GI slow waves. Whereas the amplitude of the extracellular activity of cardiac electrical activity is around 4 mV [172], the amplitude of GI slow waves is generally much lower [104, 132]. One of the consequences of a lower SNR is the lower amplitude of the resultant EGG signals, which makes the recordings difficult to interpret in the presence of movement artifacts and environmental noises. The second challenge with EGG is the difficulty in trying to relate EGG with the activation state of the underlying tissue. An EGG analysis has traditionally been limited to frequency
dynamics, aiming to identify periods of slow wave activity in which the frequency falls outside the normal range (\(\sim 3\) cpm in the human stomach and \(\sim 15\) cpm in the intestine) [95]. The frequency components in an EGG signal can be identified using signal processing techniques such as the fast Fourier transform [176]. The frequency-based approach has been attempted by researchers for many years, however it is rarely used clinically because of the lack of consensus regarding its application, its poor specificity, and its poor correlation with other functional and symptom disease markers [1, 176]. Another reason EGG is difficult to interpret is because EGG is an integrated representation of the underlying slow wave activity [26]. More studies are required to relate the activations of slow waves in the stomach to the resultant EGG. It remains to be seen whether EGG can be adopted as a standard clinical practice. One potential avenue of progress is to establish the characteristics in the EGG signal in relation to the underlying slow wave activation in the stomach. As will be elucidated in this thesis, improved electrode technology, such as multi-electrode body surface mapping approaches used in cardiology, signal-processing advances, and mathematical modelling could prove valuable in seeking to categorically define the clinical potential of the EGG [44].

2.4.3 Gastric Electrical Stimulation

The success of implantable cardiac stimulator has provided a positive impetus for attempting GI electrical stimulation therapy, as a treatment option to restore normal motility and transit, and thereby in an attempt to improve symptoms such as nausea and bloating. This form of intervention has been shown to improve symptoms in many patients with gastroparesis, but its mechanism of action is an area of on-going research, and a controlled data to support its use is currently lacking [164, 135, 134]. There are two main types of GI electrical stimulation therapies. The first type is gastric electrical stimulation (GES). A standard GES protocol delivers a high frequency (\(\sim 14\) Hz), low energy stimuli to the stomach. The second experimental GI electrical stimulation therapy that has received considerable attention is ‘gastric pacing’, in which low-frequency (\(\sim 0.05\) Hz), and high-energy stimuli are used to entrain the propagation of slow waves. In another application of GI electrical therapy, GES and/or gastric pacing have also been employed with the aim of artificially disrupt-
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Inducing normal slow wave activity in obese patients, to induce delayed emptying and therefore reduce food intake. However, there is currently weak clinical evidence on the efficacy of GES and/or gastric pacing in obesity control, and further research is required [186].

Existing implantable GES devices (including experimental prototypes) typically deliver GES protocols, as they have been adapted from existing cardiac pacemakers or neural simulators, which are only capable of delivering stimuli of less than 500 μs pulse width. For example, the Enterra GES device (Medtronic Inc, Minneapolis, MN) (Fig 2.3), has been granted Humanitarian Exemption by the United States Federal Drug Administration Agency for use in the treatment of gastroparesis. Other experimental implantable simulators have also employed GES protocols to manage morbid obesity symptoms [135, 189]. While the typical GES protocols used in these existing devices can successfully modulate cardiac myocyte or neural functions, different protocols may be required to effectively modulate gastric SMC functions [189].

Figure 2.3: (a) Medtronic Enterra gastric electrical stimulator device. (b) Implantation procedure of the Enterra stimulator at the University of Mississippi Medical Center (UMMC), Jackson, MS, USA. Two silver coloured leads are sutured into the serosal of the stomach in the antrum region, and the stimulator is usually placed in the abdominal cavity in a sub-cutaneous pocket (Courtesy of Dr Thomas Abell).

One idea to improve the existing GES protocols is to deliver gastric pacing protocols with long pulses of electrical energy. Gastric pacing requires greater energy,
and a central research challenge in developing new GES devices lies in achieving desired clinical outcomes, such as improved motility, at a suitably low level of energy consumption [113, 186, 189]. Although one study has previously employed a long-pulse stimulation protocol in gastroparetic patients, the device in this study required external battery-powered simulators too large to be implanted [120]. Identifying new protocols that achieve desired clinical outcomes at low energy consumption has proved to be a tedious and demanding task, as trial protocols must be selected from a vast parameter space involving many variables [113, 189]. To date, trial-and-error has been the dominant research strategy to evaluate potential protocols, requiring laborious animal model testing. Defining promising protocols is further complicated by the recent advent of multi-channel gastric pacing, which may be an important strategy to improve the energy efficiency of GES devices [22]. In multi-channel stimulation, the electrical pulses are delivered to multiple localised regions of smooth muscle, and the channel doses must be individualised yet coordinated, with optimal positioning of the pacing electrodes. There is still uncertainty in the approach to optimise the timings of stimulus delivery in multi-channel stimulation, and it is one area that advances in experimental protocols and mathematical modelling could help answer.

2.5 GI Mathematical Models

Mathematical modelling of GI slow wave activity has been gaining recognition as a significant research strategy in both basic science and clinical research [25, 44]. A validated mathematical model offers a virtual medium in which hypotheses regarding normal and abnormal physiology can be exhaustively investigated, and the effects for treatment strategies predicted, without sole reliance on animal and human experimental models.

Early GI mathematical cell models generally had a fewer number of parameters and with no or little biophysical basis, than models that are used today with descriptions of individual ion conductance, e.g., the Noble 1962 model, Beeler-Reuter model, etc [147]. These cell models are known as phenomenological models [127, 158, 2]. A chain of coupled relaxation-oscillators is one of the earliest mathematical models of
slow wave activity [127]. During the early 1970s a network of bi-directionally coupled relaxation-oscillators was used to simulate GI slow waves [158, 159]. These earlier relaxation-oscillator models simulated coupled slow wave propagation, which was referred to as ‘frequency pulling’ in the original literature [158]. Further research and development of the mathematical models of GI slow waves have been steadily gaining complexity as more experimental evidence regarding the electrophysiological roles of the ICCs and SMCs.

Nevertheless, theses phenomenological models were used to simulate the basic characteristics of slow wave propagation, such as the frequency and direction of propagation [25, 44]. Of the existing phenomenological models, the Aliev model was one of the most prominent cell models that has been used to simulate both gastric and intestinal slow waves [2, 25]. The Aliev model was also the first model to distinguish the functional difference between ICC and SMC [2]. The amplitude of the simulated slow waves using the Aliev model needed to be scaled to match the experimental data [13]. However, the major drawback of phenomenological models was the lack of a strong cellular biophysical basis, i.e., the parameters could not be directly measured from experiments; thus, it was difficult to simulate or provide predictions without first gaining an understanding of the physiological components that contribute to the activity itself [25].

Recent understanding of the different types of ion conductances have motivated the creation of a new generation of mathematical cell models that captured the intracellular processes in biophysical detail. In particular, the Hodgkin and Huxley (H&H)-based approach to modelling individual ion conductances has now been adopted to simulate the improved understanding of ICC and SMC ion conductances. Recently, several new biophysical GI cell models have been introduced, which quantitatively model the slow wave activity by incorporating detailed mathematical descriptions which include experimentally defined ion conductances [32, 33, 61, 60]. Compared to other GI cell models in literature, these models have been chosen for the basis of their representation of the state-of-the-art intracellular processes with biophysical basis, which would provide a more accurate representation of gastric slow waves in accordance with experimental data [32, 33]. In addition, intracellular processes such as $Ca^{2+}$ dynamics are also described in these new cell models. Unlike
phenomenological models, these biophysical cell models allow physiological meaningful quantities such as ion concentrations to be evaluated in physically realistic quantities.

A major impediment to gastric electrophysiology is a lack of understanding of some of the basic intracellular mechanisms that contribute to slow wave activity. One of the key questions of GI modelling is entrainment. In isolated cell cultures of the ICCs, slow waves are generated at different intrinsic frequencies, but in an intact network, frequencies of the slow waves synchronise to the single fastest frequency in the syncytium, in a process known as entrainment of slow wave activities [155, 96, 86]. Entrainment occurs actively, in a manner such that the signal strength of slow waves does not dissipate as they propagate for distances of up to many centimetres in the stomach as well as in the intestines [96]. Entrainment achieves two important phenomena in the stomach: first, the slow waves in the stomach occur at a single frequency (≈3 cpm); second, a constant velocity is achieved over successive cycles of slow wave activity [132], and the intestinal slow wave activity is understood to be entrained in a piece-wise manner into ‘frequency plateaus’ of decreasing entrainment frequency in the aboral direction [155, 102]. Without normal coherent entrainment, the slow wave activities of both the ICC networks and smooth muscle layers can become disorganised, and the resulting dysrhythmia is considered to be a contributing pathophysiological factor in many hypomotility disorders such as gastroparesis [114, 117, 59].

The mechanism that leads to entrainment is still an area of ongoing research. The current consensus is that $Ca^{2+}$-related ion conductances, e.g., the dihydropyridine-insensitive $Ca^{2+}$ conductance and the non-selective cation conductance in ICCs play a central role in initiating slow waves [96]. The current focus is on how $Ca^{2+}$ is released from the intracellular stores of $Ca^{2+}$, namely the mitochondria and the ER, in response to an extracellular voltage source. There are currently two main competing but not mutually exclusive views regarding $Ca^{2+}$ activation. In the first view, the synthesis of the inositol 1,4,5-trisphosphate (IP$_3$) is $V_m$-dependent [129, 174]. Therefore, when the $V_m$ of an ICC is depolarised by an electrically-coupled neighboring ICC, elevation of IP$_3$ results in increased release of $Ca^{2+}$ from the ER, leading to slow wave generation [96]. The second view, known as the unitary potential the-
ory, holds that entrainment of slow waves is dependent on a small and randomly occurring depolarisation activity, termed unitary potentials, which are generated in clusters of pacemaker units, and manifest as small amplitude (∼3 mV) fluctuations of ICC $V_m$ [93]. In this scenario, the depolarisation of an ICC by an electrically-coupled neighbouring ICC would increase the chance of unitary potentials occurring, which in turn results in an increased $Ca^{2+}$ release from the ER via IP$_3$-receptors. The mitochondria then responds to the elevated $[Ca^{2+}]_i$ by rapidly uptaking the $Ca^{2+}$ released by the ER, inducing a deficit of $Ca^{2+}$, particularly from the intracellular spaces physically close to the $Ca^{2+}$ conductances. The response of the $Ca^{2+}$ conductances to a decrease in $[Ca^{2+}]_i$ in the nearby intracellular space is an increased opening probability of their channel state, thereby inducing an entrained slow wave activity [61, 60, 96]. The merits and drawbacks of each view in a modelling environment are discussed in Sections 6.2.1 and 6.3.

As research in GI electrophysiology is being improved through relating slow waves across multiple biological scales, there is a need to gain an integrated understanding of how the physiological processes at these scales interact in a biological system. Mathematical modelling and computational techniques have been proposed as a way to provide a virtual medium in which biophysically-based components of GI slow waves can be investigated across multiple biophysical scales, under a general framework termed the multiscale modelling approach. Recent decades of research in cardiovascular electrophysiology have resulted in sophisticated multiscale mathematical models, which have been applied to successfully address complex clinical problems such as the mechanisms underlying atrial fibrillation [191] and ventricular fibrillation [183]. By comparison, the mathematical modelling of GI slow waves is still in its infancy, partly due to the historically limited understanding of the roles of ICCs and intracellular components which give rise to GI slow waves. With the ongoing development of biophysically-based cell models, significant breakthroughs are also poised to occur in the area of tissue modelling. An attractive proposition is to relate the slow wave propagation and realistic tissue structures within a multiscale framework, as has been performed in cardiac modelling research to define the normal and abnormal propagation of electrical events through complex tissue architectures [191, 172]. The concepts of mathematical modelling are discussed in more details in Chapters 7 and
2. Gastrointestinal Electrophysiology

8.

2.6 Motivation and Aims

There are a number of issues facing the current research in GI experimental and modelling studies [25, 44]. The first issue is the lack of an up-to-date description of gastric slow wave activity at the organ level. Most experimental slow waves studies are conducted in excised (then treated by chemical solutions) or cultured GI tissues, and the slow wave behaviours may not the same as those in the intact tissues. The second issue is the lack of a description of the relationship between the electrophysiological events across multiple biological scales, i.e., how cellular activities result in propagation of slow waves in the tissue and the whole organ. The third issue is there are a vast range of stimulation protocol combinations available to investigators, in terms of stimulus locations, amplitude, pulse-width and stimulation frequency, which made the investigation of the efficacy of stimulation protocols a potential expensive and tedious affair. A more efficient tool to characterise the responses of slow waves to those protocols in an efficient manner is therefore desired.

Advancements in mathematical modelling and computational techniques have presented the possibility of a virtual medium in which traditional physical experiments can be conducted in silico, with minimum material cost and high efficiency. Furthermore, the combination of mathematical modelling and experimental approaches can provide an alternative tool to help facilitate this area of research. The following aims were proposed to provide some solutions the current issues in this field:

1. Devise and validate recording platforms to record GI slow waves in both animal and human subjects.

2. Identify appropriate biophysical cell models that could be used to simulate slow waves in both gastric SMCs and ICCs.

3. Apply the cell models to characterise the response of slow wave activity to GES.

4. Make appropriate modifications to the cell models and integrate them into a multiscale framework to simulation propagation of slow waves in tissue blocks and organs.
5. Incorporate the experimental recordings to simulate slow wave activity in a whole-organ model.

6. Quantify the relationship between EGG and gastric slow waves.
Chapter 3

Recording Gastric Slow Waves

The first recording of human gastric slow wave activity was made by Walter C Alvarez in 1922, and he recounted - ‘I had not been working long with the galvanometers before began experimenting to see what could be done on man’ [3]. Strictly speaking, it was actually a woman whose abdominal wall was so thin that Alvarez could see the peristalsis of her GI tract, and he implanted a small calomel electrode (4 mm in diameter and 20 mm in length) to record the first-ever human gastric slow waves [3]. The recording made by Alvarez demonstrated a frequency of approximately 3 cpm. Alvarez also manually marked on the graph the times when a peristalsis movement (or as Alvarez simply called it ‘the gastric waves’) had passed by the electrode, from which he was able to correlate the deflections in the slow wave recording to the mechanical movements of the stomach in that patient (Fig 3.1) [3].

With the advent of the intracellular recording technique in the 1940’s, the first cellular recording of gastric slow wave activity from SMC was made in 1954 [15, 168]. Recordings of cellular slow wave activity have generally been performed on cells in GI tissues excised from the body or cultured in vitro [155]. With the application of more high precision science instruments and the paradigm shift of biological sciences to investigate physiological processes at ever finer scales, researchers in the GI field also turned their attention almost exclusively to the cellular and molecular basis of gastric slow wave activity. On the other hand, the technique of recording gastric slow waves in situ from the GI organs has largely remained stagnant, mostly restrained to a few sparsely distributed electrodes, typically less than eight, sutured into the
3. RECORDING GASTRIC SLOW WAVES

serosa of the GI tract [22].

Figure 3.1: The first-ever human gastric slow wave recording made by Alvarez in 1922. The slow wave activity was measured from the antrum. The visible peristalsis was manually marked by the dots. The figure was obtained from [3].

As more details of the physiology of gastric SMCs and ICCs are becoming more clear, some researchers once again turned their attention to recording gastric slow waves in the in vivo environment, and at larger biophysical scales (0.01 mm at the cellular scale versus 100 mm at the organ scale), partly to investigate the interactions of large networks of ICCs and SMCs in the intact GI tract. This time however, the requirement was for a recording technique that is able to capture the slow wave activity in a large part of the GI organs at high spatiotemporal resolutions. So the next advance in gastric slow wave recording came from the use of multi-electrode arrays [161, 25]. The technique involves placing a spatially-dense array of electrodes over an electrically-active tissue surface, and simultaneously recording the slow waves in the underlying tissue with up to hundreds of electrodes, from which accurate activation pattern could be ascertained. This recording technique is also known as high-resolution (HR) mapping, which has been widely employed in cardiac electrophysiology, providing information that has been critical to an improved understanding of the initiation and maintenance of both normal and dysrhythmic cardiac electrical behaviours [147, 79, 126]. A number of basic dysrhythmic mechanisms have been defined using cardiac HR mapping, including re-entrant spiral waves, rotors, and
multiple wavelet propagation [126].

As discovered by Alvarez, like the heart, the resultant potential of slow waves generated within the GI tract can be measured from the serosal surface of the stomach and small intestines, though at a lower signal amplitude than cardiac electrical events [3]. The lower amplitude of GI slow waves partly contributed to the relatively late emergence of slow wave HR mapping platforms. Pioneering work in slow wave HR mapping was conducted by Lammers et al., who developed the first HR electrode platforms to describe the precise origin and propagation of slow waves in the small intestine [103]. Recently, HR mapping has also been employed to examine the events underlying gastric slow wave dysrhythmias, revealing complex focal activities and waveform re-entry patterns not apparent in earlier studies employing fewer electrodes [105, 106]. Detailed characterisation of slow wave dysrhythmias is becoming a research priority, as they are widely thought to underlie common clinical conditions such as gastroparesis, functional dyspepsia, and post-operative ileus [113, 112, 81].

This chapter begins with an overview of some existing techniques for recording GI slow waves at different biophysical scales, followed by a brief review of the manually assembled epoxy-embedded HR electrode platform, and then details of the design and validation of a new generation HR recording platform using printed-circuit-board (PCB) technology are described. In total, the PCB platform underwent two iterations of designs and validations. Both generations of PCB electrodes were applied in animal and human experiments. The experimental protocols of animal and human recordings are also described in this chapter.

### 3.1 Existing Recording Techniques

The experimental techniques for recording GI slow waves is a rapidly evolving area of research, with significant progress made in recent years. HR mapping is only one of the many measurement techniques used to record GI slow waves. This section briefly outlines the other major recording techniques and their applications, with a particular emphasis on the challenges that each technique resolves and/or still has yet to overcome.
3. Recording Gastric Slow Waves

3.1.1 Intracellular Recordings

Intracellular recording is a standard technique of recording slow wave activity in ICC and SMC. A typical recording session begins with dissecting the tissue from the organ, isolating the muscularis layers and perfusing the musculature with physiological solutions. The slow wave activity of a single cell is recorded by inserting a finely drawn glass-pipette electrode into the cytoplasm, thus forming an electrode connection with a reference electrode outside of the cell, and the $V_m$ can be measured [41].

Intracellular recordings are generally used to investigate the intracellular mechanisms that underpin slow wave activity, by chemically subjecting the cell to different types of ion channel blockers. The precise intracellular mechanisms underlying slow wave activity are still debated, and a clear understanding of the ion conductances and intracellular messengers that contribute to the whole-cell slow wave event is yet to be consolidated.

There remain two significant experimental challenges in GI slow wave intracellular recording. The first challenge is the inter-species variability of slow waves, which is not unique to intracellular recording, but it is particularly important for intracellular recordings because cells convey the fundamental physiological information of slow waves. An example of a significant experimental variation is the inter-species difference is in the expressions of the mechanosensitive SCN5A-encoded Nav1.5 channel, which was found to be consistently expressed in human and dog, variably expressed in mouse, but not expressed at all in pig and guinea pig [166]. Such species differences need to be identified and accounted for when applying modelling studies to experimental contexts, and in future years, species-specific cell models will need be developed.

The second challenge is to develop robust means of recording slow waves that represent the in vivo and in situ state of ICCs and/or SMCs at the cellular level. The critical role of the experimental preparation in determining experimental outcomes is highlighted by recent work investigating the $Ca^{2+}$-activated $Cl^-$ conductance in ICCs [193]. In the past, intracellular ICC electrophysiology studies have often been performed in cell culture preparations. However, the combination of the cell culturing and cell dissociation processes may have dramatic effects on the proteins and signal
transduction pathways that are the focus of the experiments, including key features of the ICC phenotype such as Kit expression [193]. For example, the $Cl^-$ channel, which is expressed in in situ preparations, but is more difficult to detect in in vitro preparations [177]. Modelling studies need to consider the physiological processes that are readily available in situ but may be compromised in in vitro preparations. This avenue of experimental work provides fertile ground for complimentary multiscale modelling studies that seek to explore the integrated functional consequences of ion channelopathies, and that could potentially assist in the development of therapeutic targets in future.

3.1.2 Endoscopic Recordings

A gastric endoscope is a tube-device that can be inserted into the stomach via the oesophagus. A video camera is generally delivered down the oesophagus to guide the equipment placement during endoscopy. Studies have used endoscopic devices as means to deliver electrodes into the stomach and measure slow waves from the mucosa of the stomach [30, 5, 152]. Attempts have been made to clip, suture, or use suction to physically attach electrodes in the mucosa of the stomach [30]. Endoscopic delivery method has also been used as means to deliver temporary GES to the stomach [5]. A cardiac pacing lead was introduced into the stomach via an endoscope, and the tip of the pacing lead was embedded into the antral wall with a clockwise corkscrew motion. Clips were introduced to secure the pacing lead in place on the gastric muscular wall [5]. GES protocols were then delivered to the stomach via the pacing lead and the symptom scores of the patient following the procedure were noted. The idea behind the temporary GES protocol is that it can be used to rapidly assess the response of the patient prior to receiving a permanent GES stimulator, which requires invasive surgery (Fig 2.3(b)). The endoscopic method can also be deployed routinely to measure gastric slow waves. However, the limited space of the endoscope, high impedance of the mucosa, and reliability of the attachment method have been the significant barriers to the development of more accurate HR endoscopic approaches.
3. Recording Gastric Slow Waves

3.1.3 Laparoscopic Recordings

The invasive surgical procedure required to gain access to the serosal surface of the GI tract is a major clinical limitation to the application of HR mapping. The highest quality slow wave recordings have generally been taken from the serosal surface of the target organ, and ongoing experimental efforts have sought to achieve these recordings via less invasive strategies [103].

Upper GI surgical procedures are increasingly being conducted via laparoscopy, also known as keyhole surgeries, e.g., Nissen fundoplication, cholecystectomy, and gastrectomy. Laparoscopy involves making a number (2-4) of small incisions on the abdomen, through which trocars are inserted into the abdominal cavity. The diameter of each trocar ranges from 5 to 15 mm, and a number of surgical tools can be inserted through the trocars to perform procedures inside the abdominal cavity. In a recent study, a recording laparoscopic probe was developed to measure slow wave from the serosal surface of the stomach [133]. The laparoscopic device contained a mini-array of electrodes on its tip, which was held in atraumatic contact with the GI serosal surface. The laparoscopic device was used to demonstrate that useful spatiotemporal detail could be achieved from as few as four electrodes, from which an approximation of slow wave propagation direction and velocity can be generated. The laparoscopic approach is significantly more invasive than endoscopic recordings, as it requires general anaesthesia and intraperitoneal access. However, the laparoscopic approach can be useful for generating valuable experimental data in selected patients. Early work has also demonstrated the possibility of taking longer-term recordings in conscious subjects via implantable electrode platforms, which also contained a mini-array of electrodes [175].

3.1.4 Magnetic Field Recordings

The inherent difficulties in the recording EGG and HR recording have led researchers to propose alternative non-invasive recording modalities such as electromagnetic recordings. In theory, the volume conduction effects caused by the abdominal wall would significantly attenuate the EGG recordings [52]. To overcome the drawback of the significant attenuation caused by the abdominal wall, attempts have been made
to record the associated magnetic fields induced by the slow waves [11]. One major theoretical advantage of magnetic recordings over EGG recordings of GI slow wave activity is that, unlike electric fields, magnetic fields are not as attenuated by the layers of the abdominal wall [12].

Recent studies have shown that the magnetic field of slow waves could be recorded using a special type of magnetometer called a Superconducting QUantum Interference Device (SQUID). The SQUID (637i, Tristan Inc., San Diego CA) used to record GI magnetic field contained 19 detection coils which convert the magnetic signals into voltage at extreme sensitivity [52]. The detection coils were arranged in a hexagonal shape over an area with a 100 mm diameter, and were placed in close proximity above the body surface over the stomach. Each coil measured the magnetic flux in the vertical direction to the body surface [52]. The SQUID was contained in a shielded room.

Despite the theoretical advantages of the SQUID recordings, interpreting magnetic data is difficult and further technological advances in SQUID design and signal processing advances are required to prove the potential of this approach. Given the relatively low SNR (-20 dB), advanced signal processing techniques such as the second-order blind identification method has been applied to the SQUID signals to separate the slow wave magnetic signals from noises induced by respiration and cardiac electrical events [52]. Due to the aforementioned reasons, magnetic recording remains an area of active research.

3.2 Epoxy-embedded Recording Platforms

The pioneering work done by Lammers et al. provided a solid foundation for HR mapping of slow waves. Recording of slow waves in the in vivo state is vital for a balanced understanding of GI electrophysiology in the human body under homoeostasis, which the cultured and cellular studies cannot definitively provide. Lammers et al. used manually assembled electrodes to record slow waves in the GI tract from multiple animal species [104, 102]. By recording from a spatially dense array of electrodes (Fig 3.2), the GI slow waves could be mapped, i.e., recorded in two dimensions, and the findings on the intricate patterns of gastric slow waves in dogs and intestinal
waves in cats were the first time slow wave propagation have been described in such high spatiotemporal details [103]. The key findings Lammers et al. made were to locate and quantify the gastric pacemaker region, from which gastric slow waves originate in the canine stomach, the subsequent propagation into the gastric corpus and antrum, and the existence of up to five simultaneous slow wave events in the canine stomach [106].

![32-channel array](image1.png) ![48-channel array](image2.png)

Figure 3.2: Examples of epoxy-embedded electrodes constructed by Lammers et al. (a) A 4×8 electrode array (inter-electrode spacing: 7 mm); (b) A 4×12 electrode array (inter-electrode spacing: 9 mm). The leads and cables were shielded.

Construction of these manually assembled electrode platform required considerable skill and time as it involved embedding each silver-tipped lead into dense electrode arrays within a base plate, followed by soldering, shield installation, and encasing in silicone and epoxy resin (Fig 3.2). The advantage of a manually assembled approach is that the adequately shielded setup and careful assembly achieves considerable signal quality. However, there are also certain drawbacks with this approach. The manufacturing generally takes a long time and manual assembly could also increase the chance of potential defects during the assembly process. The flexibility is limited by the hardened epoxy-resin. There is also the potential for trapping of contaminated matter in the depressions and/or seams on the casing; due to this reason, it is difficult for the epoxy-embedded recording platforms to meet the sterilisation standard for repetitive use in the human operating rooms at Auckland City Hospital. Nevertheless, previous HR mapping studies using the epoxy-embedded recording platforms have provided detailed descriptions of slow wave activities in the stomach.
and small intestine in animal subjects [103, 104, 102, 106].

### 3.3 Printed-circuit-board Recording Platforms

Spatially dense electrode arrays machine-manufactured on flexible PCB base material have been used for HR mapping of cardiac electrical activity [119]. These arrays could be mass-produced with relative ease and low cost. However, through a series of tests, the existing cardiac PCBs were found not to be suitable for recording GI slow wave activity. This was due to the inappropriate shape and/or size of their recording tips, their large electrode diameter size, and their high electrode density, all of which have been customised to suit the substantially higher propagation velocity and amplitude of cardiac electrical activity. Therefore, one of the foremost aims of this thesis was to investigate and develop an improved PCB electrode design that would be suitable for the HR mapping of GI slow wave activity under sterile environments in the operating room at hospitals. This new PCB electrode array would allow HR mapping of gastric slow waves and potentially intestinal slow waves in human subjects; and permit improved contact during mapping of curved serosa, such as the greater curvature of the stomach and intestine.

The design and validation of the first generation PCB arrays, hereafter referred to as the generation 1 PCB (G1-PCB), is presented in Section 3.3.1. In the second generation of the PCB design (G2-PCB), the configuration of the electrode head was improved to allow easier access through smaller abdominal incisions and better contact with the intestine (Section 3.3.2). The manufacturing of both generations of PCB arrays was outsourced to a contractor in Australia (Sourcem an Ltd). The manufacturing specifications of G1-PCB were mostly identical to that of G2-PCB, except for G2-PCB we also tested the difference between silver and gold electrode plating materials.

#### 3.3.1 Generation One PCB

A single-layer flexible PCB template was designed using Protel (version 99SE) software (Altium, Australia). The electrode design consisted of: (i) a head section (30×61 mm), which contained an array of 32 electrodes; (ii) a midsection (28×339 mm),
3. Recording Gastric Slow Waves

which contained connecting tracks to the electrodes; and (iii) a plug section (50×25 mm),
which contained the foot-prints (pin-holes) for a standard 68 straight-pin Small Com-
puter System Interface (SCSI) plug (Fig 3.3c). The conduction tracks of the mid-
section were routed to meet 32 of the 68 foot-prints in the plug section. The number
of electrodes per array was chosen because the electrodes connections are grouped
into blocks of 32 channels (one channel corresponded to one electrode connection) in
the signal acquisition system (the ActiveTwo Biosemi recording system, the details
of which are presented in Section 3.4.1).

The electrodes in the head section were arranged in a 4×8 configuration (Fig 3.3(a)).
An inter-electrode distance of 7.62 mm was chosen, which covered an area of approxi-
mately 19 cm² per PCB. The choice of the 4×8 configuration was chosen because
a regular configuration of electrodes was desired to easily tessellate multiple PCB
arrays together. There were two configurations available, a 4×8 configuration, and a
2×16 configuration. For the 4×8 configuration the middle two rows of electrodes had
16 tracks laid out towards the centre of the PCB (Fig 3.3(a)). The track width was
0.25 mm, and the gap between two tracks was at least 0.25 mm, which meant the
inter-electrode distance between the electrodes in the middle row should be at least
7.5 mm. A different configuration could be used to cater for a different inter-electrode
distance, as demonstrated in Section 3.3.2. The specified track width was chosen to
lower the cost of production. Halving the track width to 0.1 mm could further reduce
the inter-electrode distance between the electrodes, however, such track width would
increase the cost substantially and there were only a limited number of manufactur-
ers with the capability of manufacturing PCBs at this finer resolution. Circular pads
of diameter 0.28 mm were used as electrode contacts, based on previous experience
regarding the optimal size for slow wave recording [103]. Drills holes (0.20 mm drill
size), enclosed by annular rings (0.25 mm) were plated in the plug section to gener-
ate foot-prints to the SCSI plug. The board thickness was 0.08 mm and the entire
length of the electrode assembly was designed to be flexible. The edges of the head
section were extended by half an inter-electrode distance, so that adjacently placed
platforms could be tessellated while maintaining an equal inter-electrode distance
across separate platforms.
Figure 3.3: The design of the first generation printed circuit board electrode arrays (G1-PCB). (a) The G1-PCB head included a 4×8 array with an inter-electrode spacing of 7.62 mm coated with gold contact material. The G1-PCB design specified a track-width of 0.254 mm and an electrode diameter of 0.3 mm. (b) Whole G1-PCB design. (c) A photo of an assembled G1-PCB, with the 68 SCSI pins soldered on the end of the PCB array.

The base material of G1-PCB (and G2-PCB) was polyamide. The inlaid tracks and connectors were manufactured in copper (Type RA Cu 14.79 mL), and the connectors were surface-plated with gold. Standard solder mask colour was applied to the board. The manufacture cost was (USD) $216.00 for tooling, and $7.46 per unit for per a hundred units. Larger quantities could be ordered at a decrease in the per-unit price. The manufacture lead-time was around 15 days. A 32 straight-pin female SCSI plug was soldered to each PCB electrode, requiring an average assembly
3. Recording Gastric Slow Waves

Time per PCB of less than 15 minutes. The PCBs were tested to be suitable for sterilisation by Ethylene Oxide or Sterrad, for single or repeated use in a human under intra-operative settings.

3.3.2 Generation Two PCB

The key improvement made in the G2-PCB was to reduce the inter-electrode pacing to 4 mm, from 7.62 mm. A number of other aspects were also considered. The initial animal and humans trials of the G1-PCBs identified a number of performance issues and aspects in the G1-PCB design that could be improved. First, the lack of shielding did not appear to have a significant effect on the quality of signal [137], and filtering could be employed to remove high-frequency noises and baseline drift (See Chapter 4). However, the recorded signals were prone to movement artefacts possibly due to the gold electrode contact material, particularly when compared to the recordings obtained when using the epoxy-embedded arrays, which contained silver contacts. One explanation was that silver is a more preferred material than gold for bioelectrical recording because silver possesses a more ideal electrode potential, a lower level of intrinsic noise, and a smaller value of charge transfer resistance and a small interface impedance [137]. Furthermore, there were concerns that the 7.62 mm inter-electrode spacing was insufficient in capturing the possible re-entrant activity with tight propagation loops as described in canine animal models by Lammers et al. [105]. Finally, the placement of G1-PCBs often required large incisions in animal subjects, and it was sometimes difficult to manoeuvre G1-PCBs through a smaller incision in human subjects because of orientation of the electrode placement relative to the incision.

In order to address the aforementioned issues with the G1-PCB design, the existing G1-PCB design was modified. To address the silver contact issue, detailed specifications were arranged with the contractor to overlay the PCBs contact points with silver. The reduction in inter-electrode spacing was partly influenced by the track-width (0.25 mm) and the configuration of electrodes was changed into a ‘hammer-head’ design (2×16 configuration). The new configuration would allow easier tessellation and minimised the incision spacing required. The dimension of the G2-PCB head was 8×64 mm². The total length of the G2-PCB was also extended to allow...
easier positioning of the plug-end from the incision. In addition, to further reduce any chance of damages to the stomach and/or intestine serosal surface, the corners of the G2-PCBs were modified not to contain any sharp corners (Fig 3.4(a)).

Figure 3.4: The design of the second generation printed circuit board electrode arrays (G2-PCB). (a) The G2-PCB head included a 2×8 array with an inter-electrode spacing of 4 mm coated with silver contact material. The G2-PCB design also specified a track-width of 0.254 mm and an electrode diameter of 0.3 mm. The corners of the head of G2 PCBs were also rounded to further reduce the risk of any potential damages to the gastric serosal surface. (b) Whole G2-PCB design. (c) A photo of an assembled G2-PCB, with the 68 SCSI pins soldered on the end of the PCB array.

The base material of G2-PCB was identical to that of G1-PCB. The inlaid tracks and connectors were manufactured in copper (Type RA Cu 14.79 mL), and the connectors (both electrodes and SCSI footprints) were plated with immersion silver with a thickness of 0.2 mm. Standard solder mark colour was applied to the board.
The manufacture cost was $537.00 for tooling, and $16.83 per unit for per 100 units.

3.4 Experiment Setup

The main purpose of the following sections (3.4.2, 3.4.3) is to provide a brief account of the experimental protocols and procedures in animal and human studies [132, 50].

3.4.1 Recording Hardware and Software

Gastric slow waves were recorded using a passive unipolar bioelectrical signals acquisition system (ActiveTwo, Biosemi, Amsterdam). The Biosemi system setup is shown in Fig 3.5(a). The Biosemi system initially contained 96 recording channels, and was later upgraded to 256 recording channels, which allowed recordings from up to eight G1-PCBs or G2-PCBs (256 electrodes) simultaneously. The Biosemi system is capable of sampling rate of up to 2048 Hz, with a 24 bit analogue-to-digital converter per channel, and other advanced properties such as the 31 nV digital resolution and ±256 mV input range [8]. The reference system employed by the Biosemi system is unique - instead of a single ground electrode, Biosemi employed a common-mode-sense (CMS) coupled with a driven-right-leg (DRL) reference configuration. The CMS and DRL form a feedback loop, which maintains the average potential of the recording subject as close as possible to the reference voltage of the analogue-to-digital converter in the Biosemi system. The first advantage of this setup is that the effective impedance of the DRL electrode could be reduced by a factor of up to 100 at 50 Hz, which results in an extra 40 dB in the common-mode-rejection-ratio compared to the conventional single electrode ground setup [8]. The increase in the common-mode-rejection-ratio is important because it indicates how much the amplifier is affected by ‘noise’ that is the common-mode signals between the body and the amplifiers. The second advantage of this setup is that the DRL electrode is the only pathway for current return between the subject and the recording system, which is always limited to 50 µA, further protecting the subject from excessive current flow [8].
Figure 3.5: Experimental setups of high-resolution (HR) recording data acquisition system. (a) The Biosemi data acquisition system was used; (b) The human operating room setup at the Auckland City Hospital; (c) The front-panel view of the recording software written in Labview 8.2.

The full setup also involved connecting the plug-end of each PCB array to a 1.5 m 68-way SCSI ribbon cable and to the top-face of the Biosemi acquisition box. The acquisition box was in turn connected to a Dell M1450 notebook computer via a fibre-optic cable. For the animal experiments (Section 3.4.2), the CMS electrode was usually placed on the body surface of the lower abdomen, below the incision for all recording sessions. The DRL electrode was placed on the lower right leg [50]. For the human experiments (Section 3.4.3), the CMS electrode was usually placed the left upper torso and the DRL was placed on the right torso [132]. The placement was kept consistent for all human and animal subjects.

An acquisition software was written in Labview 8.2 (National Instruments). A
number of modifications were made to the original software recording package to provide a more intuitive view of the recordings (Fig 3.5(c)). The default setup of the software were re-initialised to contain a set of values that were determined to be suitable for gastric slow wave recordings. For example, 512/128 Hz sampling frequency, 32 seconds window ‘viewing’ length, and 2 Hz low-pass filtering frequency (the unfiltered signals were saved to the hard disk). A time counter was included to indicate the duration of the saved signals. Finally, a subroutine was written to load a configuration file that reads in the arrangements of the channels in accordance with the physical arrangements of the electrodes on the PCB arrays. This is particularly important because it provided a view of the signals in the same order as the physical arrangements of the electrodes. Either a row or a column of electrodes in the recording array could be chosen for viewing while the software saves data from all of the channels to a laptop computer.

3.4.2 Porcine Experiments

Ethical approval for porcine experiments was granted by the University of Auckland Animal Ethics Committee. The International Guiding Principles for Biomedical Research Involving Animals and Human Beings were followed. Validation of G1-PCB was performed against a 48 channel (4×12) epoxy-embedded recording array (E48; inter-electrode spacing: 9 mm) constructed by Lammers et al. Two female weaner cross-breed pigs of mean weight 37.3 kg were used. Induction anaesthesia was achieved with Zoletil (Tileamine HCl 50 mg mL\(^{-1}\) and Zolazepam HCl 50 mg mL\(^{-1}\)), followed by intubation and maintenance anaesthesia with isoflurane (2.5-5%), with oxygen flow of 400 mL within a closed-circuit anaesthetic system). Pigs were placed supine on a heating pad, with continuous monitoring undertaken to ensure that arterial blood pressure, temperature and oxygen saturation were maintained within normal physiological ranges. The pigs were euthanised at the completion of the experiments by a bolus intravenous injection of 50 mL magnesium sulphate, while they were still under anaesthesia [46].

It is probable that induction of anaesthesia may have effects on gastric slow waves. In a previous study, rats were used as an animal to test the effects of Sodium Pentobarbital on gastric slow waves [39]. It has been demonstrated that slow wave frequency
decreased by about 40% in 180 minutes by a marked decrease of core temperature after induction of the anaesthesia. MMC was only observed during the initial period of the anaesthesia at 50 mg/kg, and throughout the anaesthesia at 25 mg/kg [39]. It is unclear whether the anaesthesia used for the pig study would have the same effect on gastric slow waves, but no visible movements were ever observed during the recording under maintained conditions.

The standard animal experiment began with a bilateral subcostal laparotomy on the anaesthetised animal. The anterior gastric serosal surface was then exposed with minimal gastric handling. The epoxy-embedded electrode or PCBs were placed on the GI serosal surface (Fig 3.6b). The contact of the electrode platforms was maintained with gentle overlying pressure using warm saline-soaked gauze. The PCBs were held together using the 3M Tegaderm adhesive dressings on the non-recording surface of the PCBs. The wound edges were approximated, and a ten minute period of stabilisation was allowed prior to a recording session. The animal could be maintained under anaesthesia for up to six or more hours.

![Porcine experiment (E32)](image) ![Porcine experiment (G1-PCB)](image)

Figure 3.6: Animal gastric slow wave recording experiment setup. The electrodes were placed through a midline incision in the abdominal area. (a) Epoxy-embedded electrode array (E32) placement in a pig subject. (b) Generation one printed-circuit-board (G1-PCB) placement.

**PCB Validation Study**

The validation of G1-PCB was conducted by comparing the signals recorded via the E48 and three G1-PCBs in two pigs [46]. The amplitudes and velocities over the same
3. Recording Gastric Slow Waves

recording area covered by the E48 electrode array and G1-PCBs were compared. The method of velocity calculation is described in Chapter 5. The two data samples were tested using a Student’s t-test and a p-value of 0.05 was considered significant.

Three G1-PCBs were used for the validation study in order to cover a similar area to that covered by the E48 array described below (48 vs 39 cm$^2$). The G1-PCBs were placed in the same orientation for each animal, and between animals. This was achieved by registering the arrays on the longitudinal axis of the organ at approximately 30 mm (one PCB height) above the fundal line, which runs transversely from the upper border of the gastro-oesophageal junction to the greater curvature (An example of the fundal line is shown in Fig 5.2). The arrays were registered in the transverse axis of the organ by placing their upper-left corner against the mid-curvature line, which runs from the tip of the fundus to the pylorus at the midpoint between the curvatures. The two pigs used in the validation study had stomachs of similar size, allowing consistent placement of the arrays between animals. The E48 array was placed in a similar position on the gastric corpus where the three G1-PCBs had been. A ten minute period of stabilisation was allowed prior to a 15 minute recording period using the E48 array.

3.4.3 Human Experiments

Ethical approval of the human experiments in Auckland City Hospital was granted by the New Zealand Northern Regional Ethics Committee. Adult patients of either gender who were undergoing upper abdominal surgery at Auckland City Hospital were invited to participate, and all participating patients provided their informed consent. The human experiment setup was similar to the porcine setup in terms of recording platforms and software. The patients received the following routine combinations of perioperative and anesthetic agents prior to the onset of mapping: 1) prophylactic antibiotics (typically cefoxitin); (2) a benzodiazepine premedication (midazolam); (3) an epidural anaesthetic (ropivacaine or bupivacaine); (4) a short-acting intravenous opiate (fentanyl); (5) a muscle relaxant (suxamethonium or atracurium); (6) an anesthetic induction agent (propofol); (7) an inhalational anesthetic agent (isoflurane or sevoflurane); and other commonly administered medications included dexamethasone and metaraminol [132].
Experiment Setup

Figure 3.7: Human gastric slow wave recording experiment setup at the Auckland City Hospital. (a) The cables connected to the generation one printed-circuit-board (G1-PCB) arrays were secured to a surgical ring retractor. (b) The G3-PCB arrays were placed a middle/lateral incision in the abdominal area. The G3-PCB arrays readily confirmed to the curvature of the stomach.

All experiments were performed on patients in the operating room, after the abdominal incision had been made and prior to undertaking the planned elective surgery. The PCB electrodes were sterilised using a gas (Ethylene Oxide/Sterrad) treatment, and SCSI cables were sterilised with a pressurised heat (autoclave) treatment. The SCSI cables were long enough to extend the SCSI cables out of the sterile zone around the incision (Fig 3.7a). The PCB electrodes and SCSI cables were assembled on a sterile trolley five minutes prior to the incision. Once the PCB electrodes entered the sterile zone, they had to stay inside until the end of the recording.

HR mapping was undertaken immediately after opening the abdomen (Fig 3.7a). In general, the left portion of liver needed to be gently elevated and sometimes the omentum had to be held to one side by a swab to allow full exposure to the stomach. The PCBs were laid directly on the anterior surface of the stomach. The fundal line in the human stomach was defined by an imaginary parallel line drawing from the oesophageal gastric junction (defined by the angle of His) to the greater curvature of
the stomach. Other anatomical marks used to position the PCBs were the apex of the fundus, the junction between the corpus and antrum (defined by the nerves of Latarjet), and the pylorus (defined by the vein of Mayo) [132].

Warm moist gauze packs were laid on top of the PCBs to ensure gastric contact was maintained. Care was taken to allow the PCBs to move freely with the respiratory excursion, and traction by the PCB cables was avoided by tied a band around the cables and clipping the band to the surgical ring retractor. The total duration of recording per patient was typically 15 min, during which two to three adjacent areas of stomach surface were mapped in each patient [132].

The standard recording frequency and storage of human experiment were identical to those for the porcine experiments in Section 3.4.1.
To date, one of main methods of characterising slow wave activity is to identify the dominant frequency component in the slow wave signal. The dominant frequency of a slow wave signal can be determined using a Fast Fourier transform of the signal [13, 48]. Alteration in the dominant frequency is important because abnormalities in the frequency of the gastric slow wave have been reported in a number of clinical settings and have been associated with gastric motility disorders [23, 95]. However, HR mapping of slow waves has revealed intricate and complex underlying activation patterns far beyond what a few sparsely distributed electrodes could have shown [102, 132, 105], and relying on frequency-dependent analysis alone is insufficient to accurately interpret the information generated by HR mapping.

One technique of representing the information from a HR recording is through activation (times) maps [46, 103]. An activation map is a topographical representation of the times at which a slow wave passes through each electrode. An activation time is commonly determined by the point of the most negative first derivative in a slow wave event [46, 48, 103]. A commonly used procedure to determine activation times is to visually assess the recorded slow wave signals from every channel, and manually place a digital marker on each slow wave event [46, 106]. However, HR recordings often yield a large amount of data in a short recording period, e.g., a 256 channels recording could record up to 768 gastric slow wave events per minute; therefore there
is a need to automate the identification of slow wave activation times. Lammers et al. have proposed an automated algorithm for detection of slow wave events in unipolar HR recordings [101], however the algorithm was less effective when applied to identify activation times in the slow wave signals recorded using the PCBs, due to the lower SNR of the PCB signals [46]. Improved methods for the automated detection of slow wave activation times were therefore required.

The velocity field is another key parameter that can be quantified from activation maps. The velocity field can provide information such as the direction of propagation and anisotropy of the gastric conduction pathway [102, 132, 50]. Traditionally, investigators have defined velocity by either simply dividing the distance between two electrodes by the difference in the activation times between the slow waves, or via a finite-difference based derivative estimation from the neighbouring four electrodes [46, 104]. The drawback with the finite-difference based approach, as Bayly et al. have pointed out for calculation of velocity of cardiac electrical events, is the difficulty of determining the direction of propagation prior to velocity calculation [6]. For example, if the wavefront is not perpendicular to the line connecting two electrodes, but instead is approximately parallel, the two electrodes will activate nearly simultaneously; and since velocity is computed from the inter-electrode distance divided by a very small difference in time, it leads to a severe overestimation of the velocity [6]. Furthermore, velocity estimates from finite-difference based methods are prone to noises in the slow wave signal, particularly in HR mapping as velocity estimations are more sensitive to noise due to the finer inter-electrode spacing.

With the advent of the G1-PCB and G2-PCB mapping arrays, the focus now shifts to establish an initial set of methods for slow wave activation times detection, velocity calculation, and activation map construction. The main issues discussed in this chapter include methods of manually and automatically identifying gastric slow wave activation times (Section 4.1), and a set of more advanced methods for identification of activation times identification and grouping (Section 4.1.3). A method of calculating the velocity field based on the activation times field is introduced in Section 4.2. An outline of the algorithm used to plot the activation times maps is introduced at the end of this chapter in Section 4.3. It is important to note that the signal processing techniques outlined in this chapter were specifically designed for
gastric slow wave signals, the HR PCB recording platform, and the recording setup. Retuning will be required in the future to apply the same set of techniques to slow wave signals recorded in other areas of the GI tract.

4.1 Identification of Activation Times

The first processing step of HR mapping signals is to filter the raw recording signals, which were an ensemble of slow wave, cardiac electrical activity, and respiratory movement artefacts. The purpose of filtering is to attenuate the interference signals, such as cardiac and respiratory signals, while retaining the slow wave signals. No visible contractions were observed during recording, possibly due to the effects of fasting of the animals and the anaesthesia applied. For gastric slow wave signal filtering, a number of issues need to be considered. First, the dominant frequencies of the major physiological events in a typical slow wave recording generally consisted of: gastric slow waves occurring at approximately 3 cpm; cardiac electrical activity occurring at approximately 70 cpm; respiratory facilitated by a ventilator occurring at 15 cpm; and the main AC power frequency at 50 Hz. Second, there is a relatively steep down-stroke slope in the baseline in each slow wave event (Fig 5.1). Given the mixture of low frequency and high frequency components in the recorded slow wave signals, a bandpass filter was needed. The Butterworth filter was suited for this role because it contains a flat pass-band which ensures equal sensitivity to the slow wave signals in the pass-band. A second-order Butterworth filter was used to increase the gradient of the roll off to -12 dB per Octave. The low cutoff frequency was 0.01 Hz (0.6 cpm) and the high cutoff frequency was set to be 1 Hz (60 cpm).

Identifying slow wave activation times accurately and consistently is a non-trivial matter, particularly in low SNR signals. From these activation times, a number of further characteristics of slow waves can be quantified, such as amplitudes and velocity field of slow waves. Therefore, a number of methods were proposed and implemented to automatically identify slow wave events, and quantify the velocity fields from the resulting activation times field [48, 53, 55].
4. Signal Processing and Data Analysis

4.1.1 Manual Identification of Activation Times

Manual identification was undertaken first to develop a baseline standard to compare with the results of the automated detection methods. Manual identification was implemented by importing the data segment into the SmoothMap software developed by Lammers (Fig 4.1) [162]. Because the Biosemi recording software saved the recorded data in the Biosemi Data Format (BDF), conversion was necessary to import the recorded data into SmoothMap (version 305).

![Figure 4.1: The SmoothMap (version 305) manual slow wave identification software. (a) A 48 channel epoxy-embedded electrode array was placed on the corpus of a porcine stomach. (b) The activation time markers (red vertical lines) were manually placed as shown in the left panel, and the corresponding activation map is shown in the right panel. Each click on the recorded signals would generate a manual marker and a corresponding colour-coded ‘square’ on the activation map.](image)

Manual identification of slow waves was performed by manually clicking on the slow wave recordings on a graphical-user-interface in SmoothMap. A marker (the red line in Fig 4.1(b)) was placed on the recording trace where a slow wave event was visually identified. A slow wave event was identified by the typical biphasic shape of the recorded voltage. The time location of the marker, i.e., the time point which corresponded to the most negative slope, was the activation time of the identified slow wave event.

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To test the consistency of the manually marked activation times, three individuals were asked to independently identify the activation times in the same 180 s segment of recording (Fig 4.1(a)). Three separate lists of activation times were compiled. The ‘test bench’ was compiled by averaging the marked times of the slow wave events that were agreed by all three markers only, and a list of slow wave activation times was generated using this conservative approach. The root-mean-square (RMS) of the manually marked activation times by each individual was calculated by comparing the activation times in each list to the mean of the activation times, using the following expression,

$$
\epsilon = \sqrt{\frac{1}{m} \sum_{j=1}^{m} (t_{ij} - \frac{1}{n} \sum_{i=1}^{n=3} t_{ij})^2},
$$

where $i$ is the index of the number of people who identified slow waves ($n = 3$), and $j$ is index of the identified activation times at an electrode. The index of the total number of commonly identified slow wave events is denoted by $m$. At each electrode, the activation times within ±500 ms of each other were deemed as the same slow wave event. If one or more people did not identify the same slow wave event, i.e., if one or more people missed a slow wave event, then that slow wave event was rejected from the calculation, and the RMS was not computed for that slow wave event. A list of commonly identified slow wave events and their averaged activation times were used as the baseline for quantifying the efficacy of the automated detection methods. There was some variation in the accuracy of the manually marked slow wave events between markers, as shown in Table 4.1.

<table>
<thead>
<tr>
<th>Measures</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time taken (min)</td>
<td>10</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Events (n)</td>
<td>287</td>
<td>257</td>
<td>255</td>
</tr>
<tr>
<td>RMS error (ms)</td>
<td>85</td>
<td>111</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 4.1: Variation between the manually marked slow wave events of three individuals (M1-3).

The maximum RMS error was 111 ms and the minimum RMS error was 82 ms. The markers spent an average of 11.2 min on marking slow waves in a 180 s segment. However, this time was a conservative measure because the markers paid close
4.1.2 Derivative-based Identification

Manual identification of slow waves is time consuming and prone to human errors. The manually marked slow wave activation times were deemed accurate because extensive care was taken to mark and review the slow wave activation times. However, manual identification is not an efficient way of identifying slow wave activation times. Therefore, an automatic slow wave identification method was developed. We took advantage of the fact that the serosal recording of slow waves contained a negative slope of the baseline potential (Fig 4.1(b)), and devised a derivative-based method to identify this negative slope as the activation time of a slow wave event. The maximum negative slope of the signals recorded from the extracellular tissue has been correlated to the maximum upstroke of the $V_m$ in a previous cardiac study [62], and serosal recording of gastric slow wave was assumed to have the same correlation with the intracellular recording of the same slow wave event. The setup of the detection algorithm itself is illustrated in Fig 4.2.

![Diagram of detection algorithm](image)

Figure 4.2: Derivative-based activation times detection. (a) The detection algorithm: in each channel, the threshold was calculated as $4.5\times$ the average of the negative derivatives ($\frac{dv}{dt}$) of the slow wave signal ($v(t)$). (b) An example of detected slow wave activation times. (top) Slow wave recording; (bottom) the threshold (dashed line) was computed from the first derivative. Three activation times (dot) were detected.
The average of the negative first derivatives was first calculated, and then the first derivative of the signal at every time point was compared to the averaged value. If the first derivative was greater than 4.5× the average value of the negative first derivative, then that time point was considered a candidate for an activation time. The 4.5×-threshold was chosen empirically to obtain the best performing outcome using the algorithm. Different threshold values were used and the resulting identified times were compared to the manual markers in terms of RMS error and other criteria until the minimal RMS error was achieved. The constant threshold approach was later revised to be a variable threshold based method (Section 4.1.3).

To test the accuracy of the derivative-based identification method, the automatically identified activation times were compared to the average of the manually marked activation times, as prescribed previously in Section 4.1.1. The performance of the derivative-based algorithm was quantified using the following measures: (i) True positive (TP), was defined as the number of correctly identified activation times by the derivative-based method as aforementioned; (ii) False positive (FP), was defined as the number of automatically identified slow wave events that were not marked manually; (iii) False negative (FN), was defined as the number of manually marked slow wave events that were not identified by the algorithm; (iv) Positive predictive value (PPV), was calculated using the following expression,

$$PPV = \frac{TP}{TP + FP},$$

(4.2)

where PPV is the ratio of TP and the total number of automatically identified slow wave activation times. The performance of the derivative-based method is summarised in Table 4.2.

<table>
<thead>
<tr>
<th>Derivative-based method</th>
<th>Accuracy measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>230</td>
</tr>
<tr>
<td>FP</td>
<td>94</td>
</tr>
<tr>
<td>FN</td>
<td>22</td>
</tr>
<tr>
<td>PPV</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Table 4.2: Accuracy of the derivative-based identification method. The derivative-based algorithm identified 230 true positive activation times out of the potential 324 automatically identified slow wave activation times (71%).
The 71% PPV achieved by the derivative-based method presented opportunities for further improvements. Even though 230 out of 324 activation times were correctly identified, there were still 94 falsely identified activation times. Further manual editing was still necessary to correct the automatically identified slow wave activation times. Furthermore, the performance of the method was not consistent, particularly in low SNR recording with high noise interferences.

4.1.3 More Advanced Methods of Slow Wave Analysis

A limitation of the derivative-based identification method was the simplistic constant negative derivative threshold for each channel. The example recording in Fig 4.2(b) demonstrates variation in the signal over time, which was typical in both animal and human experiments. The derivative-based constant threshold method was improved to increase the accuracy of the identification algorithm. Therefore a more advanced and robust detection method was required to further improve the PPV of automatically detected slow wave events. Through our collaboration with Dr Jonathan Erickson at the Washington and Lee University, two more advanced algorithms for detecting slow wave events were developed, one for identifying slow wave events, and one for grouping the identified activation times into the separate cycles [53, 55]. Here, a brief description of the algorithms is given.

**FEVT Detection Method**

The variation in the amplitude and slope of the slow waves over time can lead to missed detection of the smaller amplitude events or FP marks. Thus, instead of using a constant threshold for slow wave event detection, a Falling-Edge, Variable-Threshold (FEVT) method was developed [53]. The FVET method was built on the aforementioned derivative-based scheme [54], and contains two parts: (i) a falling-edge detector; (ii) a time-varying threshold which changes based on a statistical measure of the signal.

The negative slope in the slow wave signal was amplified by using a falling-edge kernel [160]. The kernel acts as an edge-detector to accentuate the maximum negative slope while suppress the positive slope in the process [54]. The kernel is expressed
as,

\[ E(t) = V(t) * d_{N_{edge}}, \]

(4.3)

where \( E(t) \) is the convolution between the slow wave signal \( (V(t)) \), and an edge-detector \( (d_{N_{edge}}) \) (see [160] for full details of this kernel). The time width of the edge-detector was defined as a 1 s wide kernel at 30 Hz, to correspond to the time scale of a typical large, negative transient in the slow wave signal (Fig 4.3(a)) [54]. A negative slope in \( V(t) \) would produce a positive deflection in \( E(t) \), and vice versa. When \( V(t) \) remained constant, \( E(t) \) was approximately 0. Thus, \( E(t) \) was large and positive if \( V(t) \) contained a falling edge, and vice versa.

In order to keep the focus on the falling edge in \( V(t) \) only, a smoothed \( V(t) \) \( (S(t)) \) was first calculated by a 4 s rolling-average filter, and a dot-product was performed between \( S(t) \) and \( E(t) \), as follows,

\[
F(t) = \begin{cases} 
S(t)E(t) & \text{if } S(t)E(t) \geq 0, \\
0 & \text{if } S(t)E(t) < 0,
\end{cases} 
\]

(4.4)

where \( F(t) \) was termed the ‘FEVT signal’ [54]. All the negative values in \( F(t) \) were set to 0 (Fig 4.3(b)).
Figure 4.3: The detection method used in the FEVT method [53]. (a) Peak detector kernel employed. The kernel contains a collection of 30 points corresponding to 1 s. (b) The convolved signal between the peak detector kernel and an example of recorded slow wave signal (c). The high frequency, large amplitude of the downward slope in (c) is accentuated by convolving the peak detector.

The second component of the FEVT algorithm was a time-varying threshold ($\hat{\sigma}$), which was calculated as follows,

$$\hat{\sigma} = \frac{M\{|F(t - \tau_{HW}) - \bar{F}(t)|, ..., |F(t + \tau_{HW}) - \bar{F}(t)|\}}{0.6745}, \quad (4.5)$$

where $\bar{F}(t)$ is the sample mean $F(t)$ in the time range $[t - \tau_{HW}, t + \tau_{HW}]$ and $M\{\cdot\}$ denotes the sample median. The variable threshold was then defined as: $F_{\text{threshold}} = \eta \times \hat{\sigma}$, where $\eta$ is a tunable parameter. The moving median window ($\tau_{HW}$) must be long enough to include the quiescent period in $F(t)$ in between two consecutive slow wave events, but not so long that one slow wave event can unduly influence the threshold defined for an event occurring much earlier or later. In Eqn 4.5, 0.675 was used to approximate $\hat{\sigma}$ given a sample median [128, 54]. The significance of the factor is that the median of $F(t)$ was used to estimate its standard deviation. Since the median is much less susceptible to the presence of noise, it provides a more robust estimate of $\sigma$ [128].
The outline of the full FEVT algorithm is summarised as follows,

1. Calculate $S(t)$, the smoothed signal is calculated using a window width of up to 4 s.

2. Compute $E(t)$ by convolving $d_{\text{Edge}}$ with $S(t)$ (Eqn 4.3).

3. Compute $F(t)$, by convolving $E(t)$ with $S(t)$ and setting all negative values to 0 (Eqn 4.4).

4. Compute the time varying threshold $\hat{\sigma}$ (Eqn 4.5).

5. Identify times in $F(t)$ that exceed the $\hat{\sigma}$.

6. Identify individual slow wave events and save the corresponding activation time.

The performance of the FEVT method was found to be superior to the aforementioned derivative-based method (Section 4.1.2), particularly in low SNR recordings. For example, the PPV of the FEVT algorithm demonstrated an improved PPV to 0.87 over the derivative-based identification method on the data presented in Fig 4.1(a) [54]. When compared to the manually marked activation times, the manual markers consistently marked approximately 0.2-0.3 s before the FEVT identified times.

**REGROUPS Grouping Method**

An automated cycle grouping algorithm termed the Region Growing Using Polynomial-surface-estimate Stabilisation (REGROUPS) method was developed [55]. Each cycle was defined as the slow wave activation times that belonged to the same propagating wave. One challenge facing slow wave activation times grouping was the possibility overlapping of cycles at a specific time, i.e., one slow wave emerged before the previous exited the recording field, and this created a spatial over-lap between two or more consecutive cycles of activation times.

The REGROUPS method employed a predictor step by fitting a polynomial activation to a number (typically around 12) of seeding points, and the first seeding point ($T_{\text{master}}$) in each cycle of slow waves was selected as a three-step process [55],
1. For each electrode site, the number of slow wave activation times identified at that electrode are grouped in a variable called $N_{x,y}$.

2. Compute the centre of mass $(x_c, y_c)$ of $N_{x,y}$,

$$x_c = \frac{\sum_i N_{x_i,y_i} x_i}{\sum_i N_{x_i,y_i}},$$  \hspace{1cm} (4.6)

where the sum is taken over all electrode sites $(x_i, y_i)$, indexed by $i$. The $y_c$ is similarly computed.

3. Check if $(x_c, y_c)$ corresponds to the coordinates of an electrode with a marked activation time. If yes, then the activation time is selected as the $T_{\text{master}}$. If not, the next closest electrode site meeting this condition is selected. In practice, the $T_{\text{master}}$ was usually selected to be at the centre of mass.

An additional number (typically around 11) of seeding points ($T_{\text{refs}}$) were then added to the list of seeding points. The remaining $T_{\text{refs}}$ were selected from the master $T_{\text{master}}$ by checking the activation times at a nearby electrode site. A nearby electrode site was defined to be within $\sqrt{2}d_{\text{min}}$ from the $T_{\text{master}}$, where $d_{\text{min}}$ was defined to be the closest distance between the seeding $T_{\text{ref}}$ and the closest electrode site containing at least one identified activation time. If a nearby activation time was within a certain time threshold, then that activation time and electrode site became the next seeding $T_{\text{ref}}$, and another $T_{\text{ref}}$ would be selected based on the same selection criterion, until the required number of $T_{\text{refs}}$ for the predictor step were reached.

A second-order polynomial (Eqn 4.7) function was fitted to the $T_{\text{refs}}$ as the predictor step to calculate a set of estimated activation time ($T_{\text{est}}$) at each electrode site. $T_{\text{est}}$ was calculated using the following expression,

$$T_{\text{est}} = p(1)x^2 + p(2)y^2 + p(3)xy + p(4)x + p(5)y + p(6),$$  \hspace{1cm} (4.7)

where the array of $p$ contained six coefficients. A least-square-fitting algorithm
(Eqns 4.8 and 4.9) was used to calculate the polynomial coefficients [6].

\[
Ap = \begin{bmatrix}
T_{ref1} & x_1^2 & y_1^2 & x_1y_1 & x_1 & y_1 & 1 & p_1 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & p_2 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & p_3 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & p_4 \\
T_{refn} & x_n^2 & y_n^2 & x_ny_n & x_n & y_n & 1 & p_6
\end{bmatrix}
\] (4.8)

The polynomial coefficients array, \( p \), was solved by,

\[
p = VS^{-1}U^T T_{ref},
\] (4.9)

where matrix \( A \), where \( A = VSU^T \), contains evaluated terms using the \( x \) and \( y \) coordinates of the corresponding \( T_{ref} \).

The grouping of activation from the \( T_{est} \) was achieved via an iterative process. Each identified activation time was consecutively compared to \( T_{est} \) at each \((x, y)\) location, and if the difference between \( T_{est}(x, y) \) and the activation time at that location was less than a time threshold, then that activation time was grouped to the cycle belonging to the \( T_{ref} \). The grouping procedure was performed iteratively to group all of the cycles in a segment of processed slow wave data.

### 4.2 Velocity Calculations

#### 4.2.1 A Finite-difference Method

A finite-difference based method was developed to calculate the velocity of slow waves [46]. The method was based on the approach previously reported by Lammers et al. [104]. The local conduction velocity was calculated based on the gradient of averaged activation times in the \( x \) and \( y \) coordinates of the recording array, from the four neighbouring electrode sites (Fig 4.4).
Figure 4.4: Local velocity is calculated based on the activation times \((t_1-4)\) at the immediate four neighbouring electrodes. \(d\) is the inter-electrode spacing. The location of the velocity is situated in the centre of the four neighbouring electrodes. Adapted from [46].

The local velocity vector was calculated using the following equation,

\[
\begin{bmatrix}
V_x \\
V_y
\end{bmatrix} = \begin{bmatrix}
\frac{d}{t_{3,4} - t_{1,2}} \\
\frac{d}{t_{2,4} - t_{1,3}}
\end{bmatrix}
\]  

(4.10)

and,

\[
t_{1,2} = \frac{t_1 + t_2}{2}, \quad (4.11)
\]

\[
t_{1,3} = \frac{t_1 + t_3}{2}, \quad (4.12)
\]

\[
t_{2,4} = \frac{t_2 + t_4}{2}, \quad (4.13)
\]

\[
t_{3,4} = \frac{t_3 + t_4}{2}, \quad (4.14)
\]

where, \(t_{1-4}\) are the activation times in the four neighbouring electrodes (Fig 4.4), and \(d\) is the inter-electrode spacing.

The angle of propagation \((\theta)\) was calculated using the following expression,

\[
\theta = \tan^{-1} \frac{V_y}{V_x}
\]  

(4.15)

The finite-difference based method was repeated for all the electrode sites in the recording field. However, a potential drawback of the finite-difference based method was that each velocity vector required all four neighbouring electrodes to have recorded activation times. In actual experimental recordings, there were often
electrodes with bad contacts and the loss of data would translate to a significant reduction in the number of velocity vectors calculated using the finite-difference based method. Therefore, an activation times interpolation scheme was later applied to increase the number of locations that a velocity vector could be calculated.

### 4.2.2 A Polynomial Interpolation Method

Another velocity calculation method was adapted from the algorithms developed by Bayly et al. [6] The method developed by Bayly et al. employed the aforementioned second-order polynomial, least-square-fitting method (Section 4.1.3). The velocity calculation method improved upon Bayly’s approach by adopting a single $n^{th}$-order polynomial function fitted over an entire cycle of slow waves. The same least-square-fitting method (Eqns 4.8 and 4.9) was used to fit the $n_{th}$-order polynomial. Whereas the FEVT employed the least-square-fitting method as a predictor step using only a limited number of activation times, the velocity calculation fitted the polynomial function to all the identified activation times thus ensuring a much more accurate representation of the activation times field.

The RMS error of the fitted activation times field was used to measure the accuracy of fitting. In general, the higher order of the polynomial, the lower the RMS error (Table 4.3).

<table>
<thead>
<tr>
<th>Poly order ($n$)</th>
<th>Activation times RMS error ($s$)</th>
<th>1-norm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.51</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>0.39</td>
<td>$5.57 \times 10^3$</td>
</tr>
<tr>
<td>3</td>
<td>0.32</td>
<td>$1.45 \times 10^5$</td>
</tr>
<tr>
<td>4</td>
<td>0.28</td>
<td>$0.71 \times 10^7$</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
<td>$0.82 \times 10^9$</td>
</tr>
<tr>
<td>6</td>
<td>0.21</td>
<td>$0.82 \times 10^{23}$</td>
</tr>
</tbody>
</table>

Table 4.3: Root-mean-squared (RMS) error of the fitted polynomial activation times field compared to the original data set, in terms of the RMS error of activation time, and 1-norm condition number.

To prevent over-fitting, the 1-norm condition estimator of $V$ in Eqn 4.9 was calculated [68, 72]. The 1-norm condition estimator was invoked using the `condest`
function in Matlab, and if the condition number was greater than $1 \times 10^{10}$ then the activation times field had been over-fitted. The velocity field was calculated from the fitted activation times field using the following equation,

$$V(x, y) = \begin{bmatrix} \frac{T_x}{T_x^2 + T_y^2} \\ \frac{T_y}{T_x^2 + T_y^2} \\ \frac{T_x}{T_x^2 + T_y^2} \end{bmatrix}$$

where,

$$T_x = \frac{\partial T}{\partial x},$$

$$T_y = \frac{\partial T}{\partial y},$$

where $V(x, y)$ is the velocity vector evaluated at the $x$ and $y$ coordinates of the electrode array. The accuracy of the velocity field was directly related to the accuracy of the fitted activation times field. Therefore, in general the polynomial interpolation method was used when the activation times field could be accurately fitted, and the finite difference based method was used for activation times field with excellent coverage and large concentrated patch of activation times.

### 4.3 Gastric Electrical Mapping System

Activation times maps have been successfully employed as a graphical representation of electrical conduction for decades in cardiac research, and later in GI research [103]. The generation of the earlier GI activation maps relied on manual registration of the identified slow wave events [103]. However, as the number of recordings increased, especially with the advent of automatic slow wave detection algorithms such as the FEVT and REGROUPS [53, 54], it was necessary to generate the activation maps automatically.

An activation map graphing package was programmed in Matlab (Mathworks) called Gastric Electrical Mapping Suite (GEMS) to generate activation maps automatically based on a configuration file and marked activation times file [185]. The FEVT and the REGROUPS methods were also implemented in the GEMS. A screenshot of the GEMS graphical user interface is illustrated in Fig 4.5. A slow wave activation time was marked by a red dot. The configuration file was also loaded and the electrograms (left traces in Fig 4.5) could be viewed in the same physical configuration as the electrode arrays (green in Fig 4.5).
Figure 4.5: The Gastric Electrical Mapping Suite (GEMS) was written in Matlab. The program automatically identifies the slow wave events using the FEVT algorithm [54]. The activation maps (three examples are given) were automatically generated after the slow wave events had been grouped into individual cycles using the REGROUPS algorithm [55]. More information can be found at (https://sites.google.com/site/gimappingsuite/).
Chapter 5

Experimental Results

Gastric slow waves were recorded from both pigs and human patients. The animal experiments served as a validation step for the PCB electrodes, and an opportunity to refine the experimental protocols. The PCB electrodes and refined experimental protocols were then applied in a number of human subjects who underwent upper GI related surgeries at Auckland City Hospital. In addition, we also gained useful insights to the inter-species differences in gastric slow waves between pig and human. Together, those information helped us gain a more coherent view of the physiological significance of gastric slow waves in different species.

As of December 2010, gastric slow waves had been recorded from 24 pigs. A combination of the epoxy-embedded and PCB electrode platforms were used for the initial six pigs, and only the PCBs for the remaining 18 pigs. Gastric slow waves had also been recorded from 22 human patients with conditions that were not known to affect human gastric slow wave activity. The human results were used to establish a baseline of the human gastric slow wave activity.

The main purpose of this chapter is to present the PCB validation study (Section 5.1). A brief outline of the gastric slow wave conduction pathways in pig is given in Section 5.2. Previous animal and human studies have only used a few sparsely distributed electrodes and therefore the intricate regional variations of gastric slow waves were not sufficiently quantified [150, 182, 184]. An improved baseline via HR mapping would be highly valuable to inform and facilitate future experimental and mathematical modelling studies using the pig model. A brief outline of the human
5. Experimental Results

gastric slow waves is given in Section 5.3. Again, the human gastric slow waves have been recorded using sparse electrodes in a number of studies previously [73, 184], and there were a similar insufficiency in the descriptions of the regional variations of slow waves in the human stomach. Nevertheless, from these previous human studies, it is understood that human gastric slow wave originates from a pacemaker region along the greater curvature of the mid to upper corpus, and propagates in the antegrade direction into the corpus and antrum [73]. It is however important to note that due to intrinsics slow waves generated in the ICCs, most regions of the stomach could act a potential pacemaker; so the normal pacemaker region in the stomach should be technically termed the leading pacemaker region. In this study, we have conformed to the general standard of terming the normal pacemaker region as the pacemaker region. The fundus of human stomach is typically considered to be electrically quiescent [73]. One of the key assumptions of the human gastric slow waves was that there is a single wave at any instance in time in the human stomach, such that a new cycle of slow wave begins in the pacemaker region as the previous cycle of slow waves terminates in the pylorus [13, 95]. The assumption of a single slow wave in the human stomach was significant because it had implications to the interpretation of validation of mathematical modelling and interpretation of EGG [13, 95]. The human results would be used as an input parameter to the whole-organ modelling study in Section 7.6.

5.1 PCB validation

The PCB validation study was conducted by recording gastric slow waves with three G1-PCB arrays and the E48 array. Recordings were conducted in two pigs and for both subjects a minimum of 82% of adequate contact between electrodes and tissues was achieved, i.e., electrodes registered discernible slow wave activities. The arrays were placed in the same orientation for each animal, and between animals. This was achieved by registering the arrays in the longitudinal axis of the organ at approximately 30 mm above the fundal line, which runs transversely from the upper border of the gastro-oesophageal junction to the greater curvature (Fig 5.2(a)). The two pigs had stomachs of similar size, allowing consistent placement of the arrays.
between animals. A further 10 min period of stabilisation was allowed prior to a 15 min recording period using the 48E array. Sample slow wave electrograms from the G1-PCB arrays and E48 array on the porcine stomach are presented in Fig 5.1.

Figure 5.1: An example comparison trace of slow wave recording between the 48-channel epoxy-embedded electrode (E48) and the generation one printed-circuit-board (G1-PCB) electrode. The gastric slow waves were identified by the negative slope from the baseline and the subsequent recovery to the baseline. The amplitude was defined as the difference between the peak and trough voltage values within ±500 ms of the activation time.

The recorded results from each electrode platform were analysed separately and no significant differences in terms of amplitudes and velocities were found between the two pigs, and were deemed suitable to be averaged together for comparison. The comparison between G1-PCB and E48 is shown in Table 5.1 [46].
## 5. Experimental Results

<table>
<thead>
<tr>
<th></th>
<th>G1-PCB</th>
<th>E48</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNR (dB)</td>
<td>9.71</td>
<td>18.67</td>
<td>N/A</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>0.17±0.01</td>
<td>0.52±0.04</td>
<td>1.05×10^{-7}</td>
</tr>
<tr>
<td>X-velocity (mm s(^{-1}))</td>
<td>12.2±0.8</td>
<td>11.1±3.8</td>
<td>0.0648</td>
</tr>
<tr>
<td>Y-velocity (mm s(^{-1}))</td>
<td>8.2±0.5</td>
<td>7.7±1.5</td>
<td>0.4250</td>
</tr>
<tr>
<td>Net-velocity (mm s(^{-1}))</td>
<td>16.0±0.3</td>
<td>13.8±2.9</td>
<td>0.1473</td>
</tr>
<tr>
<td>Direction (°)</td>
<td>63.0±2.1</td>
<td>60.6±4.1</td>
<td>0.3060</td>
</tr>
</tbody>
</table>

Table 5.1: Comparisons between slow wave recordings using the 48 channel epoxy-embedded electrode arrays (E48) and generation one printed-circuit-board electrode arrays (G1-PCB). The velocity and amplitudes values are expressed in mean±SEM. The table was adapted from [46].

The main difference in performance between the two types of electrode platforms was in the recorded slow wave amplitude, which was approximately three times lower in the G1-PCBs; and in the SNR, which was approximately two times lower in the G1-PCBs [46]. Nevertheless, the slow waves recorded via the G1-PCBs were readily characterised by the methods described in Section 4.1. There was no statistically significant difference in the velocity measures in the slow wave recorded between the two types of platforms. However, slow wave characteristics recorded from the E48 platform generally showed greater variability than those recorded by the G1-PCBs. The PCBs therefore provided an alternative to the epoxy-embedded electrodes for the HR mapping of gastric slow waves [100, 103].

The greatest benefit of the PCB array was their potential for use in the HR mapping of slow wave activity in human subjects which had not previously been attempted. Previously described epoxy-embedded platforms were suitable for work on animal models [103], but they were prone to retaining contaminated matter such as dried blood and tissue fluid in small depressions in the recording head, or in recesses around joints. Trapped contaminated matter would be a significant safety concern, as it could lead to transmission of infected diseases, despite conscientious attention to sterilisation practices [109]. By contrast, the seamless PCBs were without depressions or manufacturing defects. Repeat sterilisation and usage in pigs had shown that with careful attention to cleaning, they could be safely repeatedly sterilised by standard gas methods such as Ethylene Oxide or Sterrad. The PCBs also provided a potentially
disposable option because they could be economically mass-produced, with relatively little additional time required for assembly.

5.2 Porcine Studies

The results from a number of experiments are presented here to demonstrate the capability of the PCB electrodes, and quantify the basic information required for future modelling studies. Information such as the variations in slow wave amplitudes and velocities in the porcine stomach needed to be quantified in order to validate the mathematical model. Three examples of typical porcine gastric slow wave activities in different regions of the porcine stomach are presented in this section.
5. Experimental Results

The first example shows the gastric pacemaker, i.e., the origin of gastric slow wave activities in the porcine stomach (Fig 5.2).

![Diagram](image)

Figure 5.2: Porcine pacemaker slow wave activity. (a) Five generation one printed-circuit-boards (G1-PCBs; 20×8 electrodes) were placed on the fundus of the stomach. (b) An activation map of a single cycle of slow waves in the pacemaker region (marked by the arrows in (c)). The ‘boundary’ of the slow wave activity is marked by the double-line in (c). Each contour line represented a time interval of 1 s, and the velocity field (shown by the arrows) was superimposed on the activation map. (c) The slow wave activities in the fifth column of the electrodes in the recording array (indicated by the vertical arrow in (b)).
The second example shows the gastric slow wave activities in the corpus and antrum of the porcine stomach (Fig 5.3).

Figure 5.3: Porcine corpus and antrum slow wave activity. (a) Six generation one printed-circuit-boards (G1-PCBs; 12×16 electrodes) were placed on the distal corpus and antrum of the stomach. (b) An activation map of a single cycle of slow waves in the pacemaker region (marked by the arrow in (c)). The layout of the activation map and the velocity field is the same as in Fig 5.2. (c) The slow wave activities in the 13th column of the electrodes in the recording array (indicated by the vertical arrow in (b)).
The third example shows the slow wave activities simultaneously recorded from the anterior and posterior serosal surfaces of the porcine stomach (Fig 5.4).

Figure 5.4: Porcine anterior and posterior slow wave activity. (a) Six generation one printed-circuit-boards (G1-PCBs; 16x12 electrodes) were wrapped around the greater curvature of the stomach. (b) An activation map of a single cycle of slow waves in the pacemaker region (marked by the arrow in (c)). The layout of the activation map and the velocity field is the same as in Fig 5.2. (c) The slow wave activities in the ninth column of the electrodes in the recording array (indicated by the vertical arrow in (b)).
A summary of the amplitudes and velocities of gastric slow waves is given in Table 5.2.

<table>
<thead>
<tr>
<th>Region</th>
<th>Amplitude (mV)</th>
<th>Velocity (mm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal fundus</td>
<td>0.8±0.1</td>
<td>8.1±0.5</td>
</tr>
<tr>
<td>Distal fundus</td>
<td>0.9±0.1</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>Pacemaker region</td>
<td>1.3±0.2</td>
<td>13.3±1.0</td>
</tr>
<tr>
<td>Proximal corpus</td>
<td>1.0±0.1</td>
<td>8.4±0.8</td>
</tr>
<tr>
<td>Distal corpus</td>
<td>0.9±0.2</td>
<td>8.3±0.8</td>
</tr>
<tr>
<td>Antrum</td>
<td>1.1±0.2</td>
<td>6.8±0.6</td>
</tr>
</tbody>
</table>

Table 5.2: Regional variations in porcine gastric amplitude and velocity (all values are expressed in mean±SEM). The table was adapted from [50].

A number of findings regarding the porcine gastric slow waves warranted a more detailed analysis here because of their relevance to modelling studies. The first finding that would be relevant to modelling studies is the description of the gastric pacemaker, which is located in the mid-fundus of the stomach, along the greater curvature (Fig 5.2). The gastric pacemaker is the origin of gastric slow waves in the stomach, i.e., the initial site of electrical activation in the stomach. Thus, the location of the gastric pacemaker must be considered when constructing a mathematical model of the stomach. For the porcine stomach, the location of the gastric pacemaker was measured to approximately two-fifth of the distance from the fundal line to the apex of the fundus (labelled in Fig 5.2(a)) [50]. The slow wave activity originates from the centre of the pacemaker and propagates in the retrograde (33±6 mm into the fundus) as well as the antegrade directions (Fig 5.2) [50].

The second finding that would be relevant to modelling studies is the heterogeneity in the slow wave amplitudes and velocities in the porcine stomach. The heterogeneity in the slow waves is important because of its contribution to an accurate interpretation of EGG [43]. The corpus of the porcine stomach is also electrically active (Fig 5.3). After exiting the pacemaker, the slow wave activity propagates continuously from the distal fundus into corpus, and then forms a broad wave in the proximal corpus. The amplitude of slow waves in the corpus is approximately 0.95 mA and the velocity in the corpus is approximately 8.4 mm s\(^{-1}\) (Table 5.2). The velocity of slow wave activity appears to decrease slightly (6.8 mm s\(^{-1}\)) towards
the pylorus. A possible physiological explanation for the heterogeneity in the slow waves is the distribution of ICC-MY in the stomach. Hirst et al. previously reported a regional variation in ICC-MY distribution, which correlated with the variation of slow wave amplitude and velocity in the mouse stomach [75]. It might be possible that the ICC-MY distribution is the highest in the corpus of the porcine stomach and diminishes towards the lesser curvature and antrum of the porcine stomach. However, more histological studies would be required to validate the relationship between sub-class ICC distributions and slow wave activity in the porcine stomach.

The third finding that would be relevant to modelling studies is the symmetrical slow wave activation in anterior and posterior serosal surfaces of the porcine stomach (Fig 5.4). The symmetrical activation would be important for modelling studies in which the slow waves over the entire stomach model are simulated. The porcine animal model offered an opportunity to place the G1-PCBs in the anterior surface of the stomach. The G1-PCBs were wrapped around the greater curvature, contacting both the anterior and posterior surfaces simultaneously. The activation map in Fig 5.4 clearly demonstrated a circumferential band of slow wave activity that propagates down both gastric serosal surfaces in synchrony [50]. Again, based on results in Fig 5.4, it is evident that the slow waves form a broad wavefront in the corpus and propagate distally towards the pylorus.

5.3 Human Studies

An outline of the human gastric conduction pathway is provided to demonstrate the capability of the PCB electrodes, and quantify the basic information required for the whole-organ modelling study section (Section 7.6). Three examples of typical human gastric slow waves activity are presented in this section. The examples recordings were taken from three separate human patients at Auckland City Hospital. The G1-PCBs were used in all of the human recordings. The exclusion criteria involved patients who had previous gastric surgery, or had taken prokinetic agents or medications, e.g., progesterone, erythromycin, domperidone, metaclopramide, and imatinib, that were known to affect gastric slow waves [132]. In general, recordings were performed from patients who were undergoing pancreatic surgery, oesophageal surgery, liver
resections, or other non-gastric related surgeries [132].

The first example shows the slow wave activities in the gastric pacemaker of the human stomach (Fig 5.5).

Figure 5.5: Human pacemaker slow wave activity. (a) Three generation one printed-circuit-boards (G1-PCBs; 12×8 electrodes) were placed on the upper corpus of the stomach. (b) An activation map (9×8 electrodes were shown) of a single cycle of slow waves in the pacemaker region (marked by the arrows in (c)). Each contour line represented a time interval of 1 s, and the velocity field (shown as the arrows) was superimposed on the activation map. (c) The slow wave activities in the seventh column of the electrodes in the recording array (indicated by the vertical arrow in (b)).
The second example shows the slow wave activities in the corpus of the human stomach (Fig 5.6).

Figure 5.6: Human corpus slow wave activity. (a) Six generation one printed-circuit-boards (G1-PCBs; 24×8 electrodes) were placed on the corpus of the stomach. (b) The layout of the activation map and the velocity field (22×6 electrodes were shown) is the same as in Fig 5.5, except the each contour line represented a time interval of 2 s. (c) The slow wave activities in the fifth column of the electrodes in the recording array (indicated by the vertical arrow in (b)). The arrow indicates the direction of slow wave propagation in the corpus, and the vertical line with three circles indicates the three simultaneous waves.
The third example shows the slow wave activities in the antrum of the human stomach (Fig 5.7).

Figure 5.7: Human antral slow wave activity. (a) Six generation one printed-circuit-boards (G1-PCBs; 12×16 electrodes) were placed on the antrum of the stomach. (b) An activation map of a single cycle of slow waves in the pacemaker region (marked by the arrow in (c)). The layout of the activation map and the velocity field is the same as in Fig 5.5, except the each contour line represented a time interval of 0.5 s. (c) The slow wave activities in the fifth column of the electrodes in the recording array (indicated by the vertical arrow in (b)). There was an increase of amplitude from the corpus to the antrum (the top two traces compared to the bottom two traces in (c)).
5. Experimental Results

A summary of the amplitudes and velocities of gastric slow waves is given in Table 5.3.

<table>
<thead>
<tr>
<th>Location</th>
<th>Amplitude (mV)</th>
<th>Velocity (mm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundus</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pacemaker region</td>
<td>0.57±0.1</td>
<td>8.0±1.9</td>
</tr>
<tr>
<td>Corpus</td>
<td>0.25±0.03</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.52±0.07</td>
<td>5.7±1.0</td>
</tr>
</tbody>
</table>

Table 5.3: Regional variations in human gastric amplitude and velocity (all values are expressed in mean±SEM). The table was adapted from [132].

We expected the slow wave activity in the human stomach would be similar to those in the porcine stomach (Section 5.2). However, a number of discrepancies between the human and porcine gastric slow wave activities were identified. Therefore, the relevance of the human gastric slow wave activity to whole-organ modelling studies are discussed here in more detail.

The human gastric pacemaker is located in the proximal corpus of the stomach (Fig 5.5) [132]. The location of the G1-PCBs was determined by measurements relative to a number of anatomical landmarks on the human stomach (labelled in Fig 5.5a). Gastric slow waves propagates for only a limited distance in the retrograde direction toward the fundus, cardia and upper lesser curvature, often becoming slow and low amplitude prior to terminating in the fundus (Fig 5.5(b)) [132]. The slow wave activities in the pacemaker region are of higher amplitude (0.57±0.1 mA) and faster velocity (8.0±1.9 mm s\(^{-1}\)) compared to the slow wave activities amplitude (0.25±0.03 mA) and velocity (3.0±0.5 mm s\(^{-1}\)) in the corpus. Furthermore, slow waves were observed to be organised in all the patients studied, therefore the anaesthesia used were unlikely to have induced any slow wave dysrhythmias.

The slow wave activity in the human corpus is continuous with the slow wave activity in the pacemaker region (Fig 5.6(b)). However, whereas the pig gastric corpus contains high amplitude slow waves, the human gastric corpus contains low amplitude and velocity slow wave activity (Table 5.3). Similar to the pig stomach, the slow wave activity in the human corpus organises into a broad wave in the organ-axial direction [132]. The gastric slow waves can take up to 40 s to propagate across
the corpus, resulting in multiple simultaneous wave fronts (Fig 5.6(c)); this finding is very significant because it proves that there exists at least three or more simultaneous wavefronts of gastric slow waves in the human stomach.

Reproducing a realistic number of wavefronts in the stomach would be important for an accurate interpretation of EGG in modelling studies [13]. It has been speculated that there can be at least three or more wavefronts traversing the corpus at a given time [176]. However, the direct evidence of this multiple wavefronts in the human stomach could not be presented, even though multiple wavefronts has been shown to exist in the canine stomach [106], as well as in the rat stomach [108]. Here, the flexibility, coverage, and the improved spatial resolutions of the PCBs demonstrated the existence of up to three simultaneous slow wave events in the stomach.

From the results presented in this section, we have proven that assumption of a single slow wave front was erroneous and the interpretation of EGG could be much more complex than previously thought. However, simultaneous recordings of slow waves and EGG in human subjects were difficult due to the sterilisation field (Fig 3.7), where a thin film was placed on surface of the body, and the type of incision made on the abdominal could disrupt the conduction of the EGG signals. The problem of relating gastric slow waves to EGG is explored using forward modelling techniques in Section 7.7.

The human antrum contains high amplitude (0.52±0.07 mA) and fast velocity slow wave events (5.7±1.0 mm s$^{-1}$) (Table 5.3). Almost the entire human antrum is active and terminates at the pylorus sphincter (Fig 5.7) with an electrically quiescent gap, which has also been demonstrated in animals [106]. Interestingly, there appeared to be a relatively sharp electrophysiological transition of slow wave amplitude and velocity from the low amplitude and slow propagating events in the corpus to the high amplitude and fast propagating event in the antrum. The electrophysiological transition occurs over a physical distance of $\sim 15$ mm as shown in Fig 5.7. It is possible that this transition is due to the variation in the underlying ICC-MY and/or ICC-IM networks [76]. Interestingly, ICC-IM have been found to be absent in mouse and guinea-pig antrum, where the slow wave amplitudes fell in a manner that was consistent with the fall in density of ICC-MY [76]; this finding is consistent with the pig antral data (Fig 5.4), but is the opposite in the human antrum (Fig 5.7), which
suggest that there might be a difference in ICC-IM and/or ICC-MY distribution between the animal and human stomach.

The human stomachs were observed to be held in place by omentum, and surgical access to the posterior surface of the stomach was difficult (or prohibitive because of the extra incisions on the omentum required to gain access to the posterior stomach); therefore slow waves could not be recorded from the posterior surface of the human stomach. However, based on the evidence of the symmetrical slow wave activation in the anterior and posterior surfaces of the porcine stomach study (Fig 5.4) [50], we assumed that this would also be the case in the human stomach. However, the assumption of symmetrical activation in the human stomach would need to be validated in future experiments.
Chapter 6

Biophysical Cell Models of Gastric Slow Waves

Gastric slow waves are mediated by a dynamic system of physiological processes in the cytoplasm of ICCs and SMCs, e.g., ion channels, calcium dynamics, and other chemical transmitters. Some intracellular processes are also sensitive to extracellular electrochemical stimuli, e.g., electrical current and neurotransmitters, which integrate the physiological activities of ICCs and SMCs to an overall coordinated function in the body. Mathematical equations can be used to model these physiological processes, e.g., using an ordinary differential equation (ODE) to describe the rate of formation/decomposition of a chemical compound in the cell. The parameter values in an equation can be adjusted to match the output of the model to experimental data or be directly measured from experiments, which provide the equation with a biophysical basis specific to the type of reaction it describes. In general, a system of equations which describe the key physiological processes required in the generation of slow waves form the basis of a mathematical ICC and/or SMC model.

The main purpose of this chapter is to introduce the key cell models. In particular, there are two recently developed biophysical cell models of interest, the Corrias and Buist (C&B) SMC model (Section 6.1) model and the C&B ICC model (Section 6.2) [32, 33]. For each cell model, further modelling studies of cellular components were conducted, and applied to simulate slow wave activity under experimentally realistic conditions. The SMC model is modified to simulate the responses of rat antral...
SMC to GES protocols (Section 6.1.1); An IP_3-dependent component is added to the ICC model to simulate entrainment of slow waves (Section 6.2.1). An alternative ICC model by Faville et al. [61, 60] is also discussed (Section 6.3). Finally, two coupling mechanisms between ICC and SMC are proposed (Section 6.4).

### 6.1 Corrias and Buist SMC Model

The C&B SMC model was modelled by integrating models of ion conductances which were modelled using the classical H&H gating equations [32]. The parameter values in the C&B SMC model were tuned to reproduce canine gastric slow wave activity. There were nine types of ion conductance models in the SMC model.

The cell membrane equation of the C&B SMC model is,

\[-C_m \frac{dV_m}{dt} = I_{CaL} + I_{LVA} + I_{Kr} + I_{KA} + I_{BK} + I_{Na} + I_{NSCC} - I_{ICC},\]

where $I_{CaL}$ denotes the long-type (long activation time) $Ca^{2+}$ conductance in the SMC model; $I_{LVA}$ denotes a $Ca^{2+}$ conductance that is activated by small depolarisation and is also fast-inactivating; $I_{Kr}$ denotes a commonly expressed $K^+$ conductance in excitable cells; $I_{KA}$ denotes a fast-inactivating $K^+$ conductance; $I_{BK}$ serves as a mediator between the nervous system and its inhibitory signals to the digestive system; $I_{Kb}$ denotes a background $K^+$ conductance prescribed in order to yield a stable resting membrane potential; $I_{Na}$ denotes the $Na^+$ current in SMCs; $I_{NSCC}$ denotes the non-selective conductance, which is also $Ca^{2+}$-dependent. The membrane capacitance ($C_m$) was estimated to be 77 pF [33]. One of the key features of the C&B SMC model was the depolarisation response of the SMC model to a slow wave current ($I_{ICC}$) from an ICC. Without the $I_{ICC}$ term, the SMC model would stay at a resting membrane potential of -68 mV, with the parameters specific in the original article [32]. The components of the C&B SMC model are illustrated in Fig 6.1.
Figure 6.1: A schematic diagram of the Corrias and Buist smooth muscle cell model [32]. The meanings of the ion conductances, e.g., $I_{CaL}$, are as defined in Eqn 6.1. The solid arrows represent the contribution of the $Ca^{2+}$ conductances to the intracellular calcium concentration ($[Ca^{2+}]_i$). The dashed line arrows represent either a positive feedback (annotated by ‘+’), or a negative feedback (annotated by ‘−’) of the $[Ca^{2+}]_i$ on the ion conductances. SR: sarcoplasmic reticulum. The schematic was modified from the CellML database [19].

Control of $[Ca^{2+}]_i$ is one of the essential mediums through which the SMC model regulates several types of ion conductances, e.g., $I_{CaL}$ and $I_{NSCC}$, in addition to the cell membrane equation the C&B SMC model also linked $[Ca^{2+}]_i$ to $Ca^{2+}$-related ion conductances using the following expression,

$$\frac{d[Ca^{2+}]_i}{dt} = -\frac{I_{CaL} + I_{LVA}}{2FV_c} - I_{CaExt},$$

(6.2)

where $F$ denotes the Faraday’s constant, and $V_c$ denotes the total cytoplasmic volume. Eqn 6.2 related the rate of change of $[Ca^{2+}]_i$ to the rate of $Ca^{2+}$ flux through the cell membrane via $I_{CaL}$, $I_{LVA}$ and $I_{CaExt}$. Here, $I_{CaExt}$ denotes an approximated and combined total rate of $Ca^{2+}$ uptake by the endosarcoplasmic reticulum (ER), the
mitochondria, and \( Ca^{2+} \) extrusion via a plasma membrane \( Ca^{2+} \)-pump [32]. Simulated traces of the gastric slow wave and the ion conductances in Eqn 6.1 are shown in Fig 6.2.

Figure 6.2: A simulated trace of canine gastric slow wave activity, \([Ca^{2+}]_i\), and ion conductances using the Corrias and Buist smooth muscle cell model (Eqn 6.1). \( I_{CaL} \) and \( I_{LVA} \) are calcium conductances; \( I_{BK} \), \( I_{kr} \), \( I_{ka} \), \( I_{kb} \) are potassium conductances; \( I_{Na} \) is a sodium conductance; \( I_{NSCC} \) is a non-selective conductance, which is also \( Ca^{2+} \)-dependent; the \( I_{ICC} \) term was fitted to an experimental data of an ICC slow wave (not shown here).

### 6.1.1 Modification of C&B SMC Model for GES

The C&B SMC model was modified to provide an example of its potential applications in \textit{in silico} hypothesis testing [41]. In this study, the C&B SMC model
was parametrised to match the $V_m$ response of an initial set of experimental recordings, in which gastric pacing protocols were applied to rat antral SMCs to provide an initial baseline parameter identification and validation of the SMC model. The parametrised SMC model was then used to predict the effects of different gastric pacing/GES protocols.

**Cell Current-clamp Setup**

The experimental study was approved by the Institutional Animal Care and Use Committee at the VA Medical Center, Oklahoma City, Oklahoma, USA. The International Guiding Principles for Biomedical Research Involving Animals were followed. All chemicals were purchased from Sigma (St Louis, MO) unless otherwise indicated. Seven male rats (250 to 350 g) were used in this study. Following cervical dislocation under anesthesia, the stomach of each rat was dissected along the lesser curvature near the antrum and pinned to a dissecting plate with the mucosal side down. Strips of gastric smooth muscle were removed by sharp dissection under anatomical microscope vision, and were then finely sectioned. The antral SMCs were isolated via enzymes using modified procedures as previously described [97]. Briefly, the isolation procedure began with incubation of the sectioned muscle for 30 min at 4°C in a low $Ca^{2+}$ solution (in mM: HEPES 10; NaCl 140; KCl 5; CaCl$_2$ 0.05; MgCl$_2$ 2; Glucose 10) with the pH adjusted to 7.4 with NaOH. The muscle sections were then transferred to a low $Ca^{2+}$ physiological solution containing enzymes (in mg mL$^{-1}$: Collagenase IA 2; Papain 2; BSA 2; Trypsin inhibitor 2), and incubated for a further 15 minutes at 35°C. Following the second period of incubation, the sectioned muscle strips were washed several times in an enzyme-free low $Ca^{2+}$ physiological solution and gently agitated to create a cell suspension. Isolated cells were stored at 4°C for whole-cell current-clamp recording within eight hours of the tissue preparation.

The whole-cell current-clamp stimulation and recording were performed on relaxed single antral SMC with smooth appearance and spindle shape using the conventional whole-cell configuration of the current-clamp technique [69]. The myocytes were first superfused with HEPES solution (in mM: HEPES 10, NaCl 140, KCl 5, CaCl$_2$ 2, MgCl$_2$ 2, Glucose 10), with the pH adjusted to 7.4 with NaOH. The patch pipettes (borosilicate glass, WPI) were pulled with a Flaming Brown P-97
micropipette puller (Sutter Instruments, USA) and their tips fire polished. The resistances of the pipettes were on average $5 \, M\Omega$ when filled with internal high $K^+$ HEPES solution (in mM: KCl 30; potassium gluconate 100; EGTA 5; CaCl$_2$ 1; Na$_2$ATP 1; Na$_3$GTP 2; Na$_3$GTP 0.5; MgCl$_2$ 1) with the pH adjusted to 7.2 with KOH. The temperature of the preparation was maintained at 20°C. The $V_m$ of the SMCs dialysed with potassium gluconate ranged between -40 to -60 mV. Cells were hyperpolarised to -60 to -70 mV by a constant negative current injection to stabilise the resting membrane potential. The $V_m$ of the single SMC was recorded using an Axopatch amplifier (Axopatch 200B, Axon Instruments Inc, USA). Data acquisition and analysis was performed with pClamp software (Version 9.0, Axon Instruments Inc, USA).

The stimulation current was generated via DiDdata1200 analog/digital interface (Axon Instruments Inc, USA). The recording pipette was also used to administrate the stimulation current. The stimulation protocols were characterised in terms of amplitude in pA, pulse-width in ms, frequency in Hz, on/off-time of a ‘train’ of pulses in s. The following stimulation protocols were applied,

- Baseline: 250 pA stimulation amplitude, a single 1000 ms pulse-width.
- Single-pulse, 250 pA stimulation amplitude, in 10, 50 to 300 ms in 50 ms increments pulse-width.
- Single-pulse, 50 to 200 pA in 25 pA increments stimulation amplitude, 1000 ms pulse-width.
- 250 pA stimulation amplitude, 10 ms pulse-width, 40 Hz frequency, 2000 ms on-time.

**Parameter Determination**

The biophysical basis of the C&B SMC model made it straightforward to update the values of parameters that could be readily measured experimentally, such as temperature and $C_m$. However, there were still a number of parameters, such as the maximum ion conductance, that were specific to the rat gastric SMCs which needed to be determine. Identifying these parameter values that were specific to this experiment
would require a specialised chemical treatment and setup of ion conductance recording experiments which were not readily available for this study. Therefore, the remaining parameters that could not be directly identified were computationally optimised to match the output of the modified SMC model to an initial set of baseline recordings.

To determine parameter values for the unknown SMC model parameters, the $V_m$ responses to the baseline stimulation protocol were compared between simulation and an initial set of intracellular recordings from rat antral SMCs (Fig 6.3).

![Figure 6.3](image)

**Figure 6.3:** (a) Top: Simulated membrane potential ($V_m$) measured in mV, in response to a virtual stimulus of 250 pA and 1 s duration (below). The resting membrane potential was -79 mV; the peak potential was 28 mV; the plateau potential was 0.7 mV. The mechanical threshold was defined to be -30 mV, based on a previous smooth muscle cell study on mongrel dogs [141]. (b) The experimental recording (top), in response to the stimulus (below). Reproduced from [41].

The parameter values were selected based on the following rationale. In a previous study, sodium channels were not identified in rat SMCs [58], whereas sodium channels have been determined in the gastric SMCs of guinea pigs [83]. Since rats were used for this study, the maximum conductance of $I_{Na}$ was set to zero to eliminate the $Na^+$ conductance from the modified SMC model. The $C_m$, $[K^+]_o$, $[Ca^{2+}]_o$, $[K^+]_i$, and experimental temperature ($T_{exp}$) were adjusted to match one set of preliminary experimental measurements of the $V_m$ of a rat SMC. The corresponding equations
that contained the parameters of $[K^+]_o$, $[Ca^{2+}]_o$, $[K^+]_i$, and $T_{exp}$ are included in Appendix B.2. To determine the values of the parameters that could not be determined by direct electrophysiological experimentation, the RMS error between the simulated and measured $V_m$ was minimised as a function of: the maximum conductance values of the L-type $Ca^{2+}$ current ($G_{Ltype}$), the background potassium current ($G_{bk}$), the A-type $K^+$ current ($G_{ka}$), the $Ca^{2+}$-activated $K^+$ current ($G_{BK}$), and the delayed-rectifier current ($G_{kr}$). The Matlab minimisation function $f_{minsearch}$ was used to perform the parametrisation. The combination of $G_{Ltype}$, $G_{BK}$, $G_{LVA}$, $G_{ka}$, $G_{kr}$, and $G_{NSCC}$ that resulted in the minimal RMS was chosen as the new values for those conductances as shown in Table 6.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Default value</th>
<th>Adjusted value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$</td>
<td>Membrane capacitance</td>
<td>77 pF</td>
<td>52 pF</td>
</tr>
<tr>
<td>$K_o$</td>
<td>$[K^+]_o$</td>
<td>5.9 mM</td>
<td>5 mM</td>
</tr>
<tr>
<td>$Ca_o$</td>
<td>$[Ca^{2+}]_o$</td>
<td>2.5 mM</td>
<td>2 mM</td>
</tr>
<tr>
<td>$K_i$</td>
<td>$[K^+]_i$</td>
<td>164 mM</td>
<td>140 mM</td>
</tr>
<tr>
<td>$T_{exp}$</td>
<td>Temperature</td>
<td>310 K</td>
<td>300 K</td>
</tr>
<tr>
<td>$G_{Na}$</td>
<td>Maximum $Na^+$ conductance</td>
<td>9 nS</td>
<td>0 nS</td>
</tr>
<tr>
<td>$G_{L-type}$</td>
<td>Maximum $G_{L-type}$ conductance</td>
<td>65 nS</td>
<td>5.2 nS</td>
</tr>
<tr>
<td>$G_{bk}$</td>
<td>Maximum $G_{bk}$ conductance</td>
<td>0.014 nS</td>
<td>0.021 nS</td>
</tr>
<tr>
<td>$G_{kr}$</td>
<td>Maximum $G_{kr}$ conductance</td>
<td>35 nS</td>
<td>3.5 nS</td>
</tr>
<tr>
<td>$G_{BK}$</td>
<td>Maximum $G_{BK}$ conductance</td>
<td>45.7 nS</td>
<td>0.05 nS</td>
</tr>
<tr>
<td>$G_{ka}$</td>
<td>Maximum $G_{ka}$ conductance</td>
<td>9 nS</td>
<td>4.95 nS</td>
</tr>
<tr>
<td>$G_{NSCC}$</td>
<td>Maximum $G_{NSCC}$ conductance</td>
<td>50 nS</td>
<td>30 nS</td>
</tr>
</tbody>
</table>

Table 6.1: The adjusted parameters of the smooth muscle cell model to replicate the rat antral smooth muscle membrane potential. The details of the experiments are specified in Section 6.1.1.

Using these parameters, the following values were obtained from simulation,
<table>
<thead>
<tr>
<th>Measure</th>
<th>Simulation</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>-79</td>
<td>-77.4±3.2</td>
</tr>
<tr>
<td>Peak potential (mV)</td>
<td>28</td>
<td>28.25±7.5</td>
</tr>
<tr>
<td>Plateau potential (mV)</td>
<td>0.73</td>
<td>3.1±3.3</td>
</tr>
<tr>
<td>Upstroke (mV ms(^{-1}))</td>
<td>3.26</td>
<td>4.1±1.2</td>
</tr>
</tbody>
</table>

Table 6.2: Comparison between simulated and experimentally recorded \( V_m \) of rat antral smooth muscle cell. Note that the simulated plateau potential produced the least accurate match to the experimental data.

The updated SMC model was used as a virtual platform to quantitatively determine the \( V_m \) responses of rodent gastric SMCs to a range of stimulation protocols. Animal experiments were performed at selected combinations of GES/pacing protocols to validate the simulated results, thereby significantly reducing the time and resources required.

**Methods of Stimulation Studies and Validation**

The re-parametrised C&B SMC model was applied in a series of virtual stimulation studies to evaluate its response to a range of pertinent stimulation protocols. The \( V_m \)-threshold for a successful SMC activation (i.e., the mechanical threshold) used for this study was based on the work of Ozaki et al. [141], who suggested the SMC mechanical threshold to be between -40 to -30 mV during the plateau phase of depolarisation. The upper limit of this range (-30 mV) was chosen as the \( V_m \) threshold value for achieving significance mechanical activation of SMC [141] (Fig 6.3). The \( V_m \) above this threshold has been shown to achieve significant \( Ca^{2+} \) entry, in particular, sustained depolarisation above this threshold is thought to lead to the sustained entry of \( Ca^{2+} \) and the accumulation of \([Ca^{2+}]_i\), which then activates the contractile intracellular elements [141]. It is important to note here that the use of a single mechanical threshold is relatively simplistic, because the contractile apparatus within \textit{in vivo} SMCs is understood to be regulated by factors that may substantially alter the force generated for a given change in \([Ca^{2+}]_i\), and the relationship between the area under the slow wave plateau, \( Ca^{2+} \) flux and tension generated is unlikely to be linear [155, 141].
The modified SMC model was subjected to a number of virtual stimulation protocols to mimic the range of actual GES/pacing protocols that were applied experimentally. The following virtual simulation protocols were used (Fig 6.4),

- Varying pulse-widths: 250 $pA$ amplitude; 0 to 400 $ms$ in 25 $ms$ increments pulse-width (Fig 6.4(a)).

- Varying amplitudes: 0 to 200 $pA$ in 25 $pA$ increments amplitude; 1000 $ms$ pulse-width (Fig 6.4(b)).

- Varying frequencies: 250 $pA$ amplitude; 10 $ms$ pulse-width; 0 to 100 $Hz$ in 10 $Hz$ increments frequency (Fig 6.4(c)).

![Simulated stimulation protocols](image)

Figure 6.4: Simulated stimulation protocols. (a) 250 $pA$, 0 to 400 $ms$ in 25 $ms$ increments; (b) 1000 $ms$, 0 to 250 $pA$ in 25 $pA$ increments; (c) A pulse-train, 250 $pA$, 10 $ms$ pulse-width, at 40 $Hz$, 2000 $ms$ duration.

The rate of upstroke (in $mV$ $ms^{-1}$), peak potential ($mV$), plateau potential ($mV$), resting membrane potential ($mV$), and duration ($ms$) were calculated over five events for each stimulation protocol and expressed in mean±standard error mean (SEM).
The measured values were then compared to the simulated values. Power consumption was calculated and expressed in \((mA ms)\). The \([Ca^{2+}]_i\) was also quantified for a single pulse stimulation protocol (amplitude 145 pA; pulse-width 2000 ms) and compared to a frequency train protocol (amplitude 145 pA; frequency 40 Hz; on-time 2000 ms). The results are presented in Section 8.1.

### 6.2 C&B ICC Model

The C&B ICC model integrates descriptions of ion conductances which were modelled using the classical H&H gating equations [33]. The parameter values in the C&B SMC model were tuned to reproduce canine gastric slow wave activity. There are nine types of ion conductance models in the ICC model.

The cell membrane equation of the C&B ICC model is,

\[-C_m \frac{dV_m}{dt} = I_{ion} + 2F V_c J_{PMCA},\]

where,

\[I_{ion} = I_{VDDR} + I_{L-type} + I_{Kv1.1} + I_{ERG} + I_{BK} + I_{Kb} + I_{Na} + I_{NSCC} + I_{CaCl},\]

where both \(I_{VDDR}\) and \(I_{L-type}\) are \(Ca^{2+}\) conductances; \(I_{Kv1.1}\) denotes a \(K^+\) conductance; the name \(Kv1.1\) denotes a code name of a gene that has been identified in cell cultures of murine ICC. ERG (Ether-a-go-go) denotes another type of \(K^+\) conductance, which has been identified as an important contributor to ICC slow wave activity. \(I_{BK}\) denotes a type of \(K^+\) conductance that is also \([Ca^{2+}]_i\) dependent; \(I_{Kb}\) denotes the background \(K^+\) conductance; \(I_{Na}\) denotes a \(Na^+\) conductance; \(I_{NSCC}\) denotes a non-selective ion conductance that allows passage of cations, and its primary role being to allow influx of \(Ca^{2+}\) into the sub-space compartment; \(I_{CaCl}\) denotes a \(Ca^{2+}\) activated \(Cl^-\) conductance. In addition to the nine ion channels, a \(Ca^{2+}\) extrusion mechanism \((J_{PMCA})\) was also included in the model, allowing \(Ca^{2+}\) to be removed from the cytoplasm. \(V_c\) denotes the average cell volume of an ICC. The components of the ICC model are illustrated in Fig 6.5.
Figure 6.5: A schematic diagram of the Corrias and Buist interstitial cell of Cajal (ICC) model [33]. The meanings of the ion conductances, e.g., $I_{Ka}$, are as defined in Eqn 6.3. There are four compartments in the ICC model, the cytoplasm, mitochondria, endoplasmic reticulum (ER), and a sub-space (SS) compartment. The schematic was modified from the CellML database [19].

The C&B ICC model adopted an extensive description of $[Ca^{2+}]_i$ dynamics from a previously developed $[Ca^{2+}]_i$ model by Fall et al. [56], because a detailed model of $[Ca^{2+}]_i$ was required in order for the C&B ICC model to autonomously generate slow waves. The $[Ca^{2+}]_i$ equation of the C&B ICC model is,

$$
\frac{d[Ca^{2+}]_i}{dt} = -f_c \left( \frac{I_{L-type} + I_{VDDR}}{2FV_c} \right) - J_{leak} + J_{PMCA},
$$

(6.5)

where $J_{leak}$ acts as the ‘link’ between the subspace compartments: mitochondria, ER, and the bulk cytoplasm in the C&B ICC model; $f_c$ denotes the fraction of the cytoplasm the $[Ca^{2+}]_i$-related intracellular organelles occupied. The $Ca^{2+}$ dynamics in the mitochondria and ER induces changes of $[Ca^{2+}]_i$ in the subspace compartment, from which $Ca^{2+}$ leaks to the bulk cytoplasm through $J_{leak}$ [56], and this leads to a localised increase in $[Ca^{2+}]_i$, which in turn leads to activation of a slow wave activity in the C&B ICC model [33].

Simulated traces of gastric slow wave and the ion conductances in Eqn 6.3 are shown in Fig 6.6. The simulated traces were reproduced using the original C&B ICC.
Figure 6.6: A simulated trace of canine gastric slow wave activity and ion conductances using the Corrias and Buist interstitial cells of Cajal model (Eqn 6.1). $I_{VDDR}$ and $I_{L-type}$ are calcium conductances; $I_{BK}$, $I_{ERG}$, $I_{KB}$, $I_{Kv1.1}$ are potassium conductances; $I_{Na}$ is a chloride conductance; $I_{Na}$ is a sodium conductance; $I_{NSCC}$ is a non-selective conductance.
6. Biophysical Cell Models of Gastric Slow Waves

6.2.1 IP$_3$-dependent Entrainment Model

Previous studies have shown that acute reduction in adenosine triphosphate (ATP) production did not affect the generation of slow waves [96]. Therefore, the components and equations of the C&B ICC model associated with ATP production in the sub-component representing the mitochondria were simplified. Specifically, equations representing ATP hydrolysis were eliminated from the modified ICC model, and the processes of mitochondrial respiration and $H^+$ diffusion were approximated by flux constants. Although experimental evidence has shown that ICCs contain an ATP-sensitive $K^+$ conductance, this type of conductance was not included in the original C&B ICC model, so simplifying the ATP components has no effect on the generation of slow waves by the C&B ICC model [33, 45].

The original C&B ICC model was unresponsive to injected current sources and therefore was unsuitable for simulations of entrainment in a voltage-dependent manner [45]. A number of studies have demonstrated that an IP$_3$ mediated $[Ca^{2+}]_i$ mechanism is likely to be responsible for slow wave entrainment [155, 96]. Therefore, the C&B ICC model was further modified by introducing a voltage-dependent IP$_3$ related mechanism based on the equations previously published by Imtiaz et al. [90]. The effect of this modification was to invoke an active slow wave in the C&B ICC model in response to an appropriate extracellular and/or intracellular current sources, such as from another depolarising ICC. The IP$_3$ component in the cell model by Imtiaz et al. assumed a positive feedback of IP$_3$ kinetics in slow wave generation using the following equations,

$$\frac{d[IP_3]}{dt} = \beta - \eta [IP_3] - V_{m4} \frac{[IP_3]^4}{K_4^4 + [IP_3]^4} + P_{MV}(1 - \frac{V_m^8}{K_8^8 + V_m^8}),$$  \hspace{1cm} (6.6)

where $\beta$ is a physiological stimulus agent (such as acetylcholine) that modulates the sensitivity of IP$_3$ to the changes in $V_m$. The temporary surge of IP$_3$ due to an increase in $V_m$ allows the slow wave activity simulated using the modified C&B ICC model to respond to a current source by producing a phase shift relative to its intrinsic slow wave activity. The full descriptions and values of the parameters in Eqn 6.6 are listed in Table 6.3. The parameter values were redimensionalised to be consistent with the units of C&B ICC model.

The response of simulated slow wave frequency to $\beta$ is as shown in Fig 6.7. The
### Table 6.3: Redimensionalised parameter values of the IP₃ component of the slow wave model by Imtiaz *et al.* [90]. Reproduced from [45].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>Physiological stimulus</td>
<td>$2.7 \times 10^{-5} \text{ mM s}^{-1}$</td>
</tr>
<tr>
<td>η</td>
<td>Rate constant of IP₃ degradation</td>
<td>0.015 s⁻¹</td>
</tr>
<tr>
<td>u</td>
<td>Hill coefficient</td>
<td>4</td>
</tr>
<tr>
<td>$V_{m4}$</td>
<td>Maximal rate of IP₃ synthesis</td>
<td>$3.33 \times 10^{-5} \text{ mM s}^{-1}$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>Half saturation constant of IP₃ degradation</td>
<td>0.0005 mM</td>
</tr>
<tr>
<td>$P_{mV}$</td>
<td>Maximal rate of IP₃ synthesis</td>
<td>$1.33 \times 10^{-5} \text{ mM s}^{-1}$</td>
</tr>
<tr>
<td>$K_v$</td>
<td>Half saturation constant of IP₃ synthesis</td>
<td>$-58 \text{ mV}$</td>
</tr>
<tr>
<td>R</td>
<td>Hill coefficient</td>
<td>8</td>
</tr>
</tbody>
</table>

Relative range of frequencies $\beta$ could control was relatively narrow, ranging from 1.8 to 4.3 $\text{cpm}$; this range was a sufficient range for the simulations of the normal gastric slow waves ($\sim 3 \text{cpm}$), but not for the simulations of the normal intestinal slow waves ($\sim 15 \text{cpm}$). To simulate slow wave at frequencies beyond the range shown in Fig 6.7, one could scale the time-dependent variables and equations in the modified ICC model to match the desired frequency. This is because the ODE’s in the cell model would become unstable for $\beta$ values outside the range specified in Fig 6.7.

**Figure 6.7:** Frequency dependence on $\beta$ in the modified Corrias and Buist ICC model. A higher value of $\beta$ leads to a higher intrinsic frequency. Reproduced from [45].
The response of concentration of IP$_3$ to $V_m$ is shown in Fig 6.8. The relationship between IP$_3$ and $V_m$ could be described as a sigmoid shape. Over the range of the $V_m$ during a slow wave event (-70 to -30 mV), the general trend was that as the ICC depolarised, there was an increase in the IP$_3$, which levelled off at approximately 13.1 pM. There was also a baseline IP$_3$ of approximately 2.1 pM in the ICC model.

Figure 6.8: Response of IP$_3$ to $V_m$. A more depolarised $V_m$ leads to a higher concentration of IP$_3$. Reproduced from [45].

### 6.3 Faville ICC Model as a Possible Alternative Cell Model

Faville et al. modelled intestinal slow waves based on the physiological evidence of unitary potentials. This ICC model consisted of two co-dependent components, a unitary potential model [61], and a bulk cytoplasm model that incorporated multiple unitary potential pacemakers and ion channel conductances to simulate whole cell slow wave activity in the intestine [60].

The hypothesis of unitary potentials stated that a slow wave is initiated by the release of Ca$^{2+}$ from IP$_3$-receptor operated stores that are in close physical proximity to the plasma membrane and to the mitochondria [61]. The local increase in [Ca$^{2+}$], invokes a Ca$^{2+}$ update mechanism in mitochondria that results in a transient
reduction in $[Ca^{2+}]_i$ in the localised space of a pacemaker unit. The reduction of $[Ca^{2+}]_i$ in the micro-domain of the pacemaker unit activates the primary pacemaker conductance. Experiments on isolated ICC have shown this to be a $Ca^{2+}$-inhibited, non-selective cation conductance, and a number of other types of ion conductances. The first component of the ICC model was a pacemaker unit (PU) model which simulated the ion conductances and intracellular processes that contribute to unitary potentials [61].

The equation of the PU model is,

$$-C_m \frac{dV_m}{dt} = I_{Ca} + I_{PM} + I_{Kv1.1} + I_{NSCC(Ca)} + I_{Na}, \quad (6.7)$$

where $I_{Ca}$ denotes an inward $Ca^{2+}$ current; $I_{PM}$ denotes a plasma membrane $Ca^{2+}$-ATPase; $I_{Kv1.1}$ denotes the same $K^+$ conductance as the conductance in the C&B ICC model (Eqn 6.4); $I_{SNCC}$ denotes the non-selective cation conductances for $Ca^{2+}$ and $Na^+$; $I_{Na}$ denotes an outward $Na^+$ pump. The combined activities of those four types of ion conductances result in autonomous small fluctuations in the membrane potentials of ICCs.

The second component of the ICC model by Faville et al. was a whole cell conductance model that incorporated a number of pacemaker unit models and other types of membrane conductances to simulate whole cell slow wave activity [96]. The whole cell conductance model is,

$$-C_m \frac{dV_m}{dt} = I_{Ca(T)} + I_{Ca(Ext)} + I_{Kv1.1} + I_{KB} + I_L + \sum_{i=1}^{nP_U} I_{ionPU}, \quad (6.8)$$

where $I_{Ca(T)}$ denotes the T-type $Ca^{2+}$ conductance; $I_{Ca(Ext)}$ denotes a plasma membrane $Ca^{2+}$-ATPase; $I_{Kv1.1}$ denotes a voltage-dependent $K^+$ conductance; $I_{KB}$ denotes a background $K^+$ conductance; $I_L$ denotes the non-selective cation conductance; the sum of $I_{Ca(T)}$, $I_{Ca(Ext)}$, $I_{K(ERG)}$, $I_{K(v1.1)}$, $I_{K(B)}$, $I_L$ represents the bulk cytoplasm conductance. The ICC model is ‘driven’ by the summation of a number ($nP_U$) of pacemaker unit currents ($I_{ion(PU)}$) which is represented by the summation of the conductances in Eqn 6.7.

The major omission from the ICC model by Faville et al. was the $Ca^{2+}$-activated $Cl^-$ conductance ($I_{ClCa}$), which has been identified to have a critical role in the generation of slow wave activity [89]. However, the underlying mechanism of the unitary
potentials mechanism in the ICC model by Faville et al. has come into question, i.e., a reduction in $[Ca^{2+}]$, close to the pacemaker unit conductances would result in slow wave generation in the ICC - in a recent study, a set of mathematical simulations on the spatially-dependent $[Ca^{2+}]$, dynamics have shown that reduction of $[Ca^{2+}]$, below the baseline concentration was impossible within the normal physiological ranges of the unitary potential conductances [121]. The ICC model nevertheless still holds merit in its multiple intracellular compartments approach, and the models of the individual types of ion conductances.

While the ICC model by Faville et al. has the potential to simulate entrainment, in its current state it does not support cell to cell entrainment. A proposed mechanism of entrainment, based on unitary potentials, is that the elevated voltage-dependant activation of unitary potentials induces a small but highly localised $Ca^{2+}$ influx, which in turn activates a $Ca^{2+}$-induced-$Ca^{2+}$-release involving IP$_3$ receptors [155]. Although there is a consensus that $Ca^{2+}$ release is mediated via IP$_3$ receptors, there remains a controversy over whether the IP$_3$ mediated release is $V_m$ dependent or $Ca^{2+}$ dependent [61]. The implementation of the cell models in this thesis favoured the hypothesis of a $V_m$-dependent IP$_3$ model, because the IP$_3$ component of the model by Imtiaz was mediated through $V_m$ [90], therefore providing a direct way of coupling ICC cell models at a continuum level in tissue models [45]. In future studies a $Ca^{2+}$-dependent mechanism could be included in the ICC model of Faville et al. to simulate entrainment. Nevertheless, the $V_m$-dependent entrainment mechanism adopted for the C&B ICC model in this thesis remains a valid strategy.

6.4 C&B ICC-SMC Coupling

A slow wave starts in an ICC and then depolarises the SMCs coupled to the ICC [155, 77]. In areas of the stomach other than the gastric antrum, the main pacemakers of SMC slow waves are the ICC-IM. In the gastric antrums of $W/W^+$ mice, the main pacemakers of SMC slow waves are the ICC-MY [77]. It has been proposed that the slow wave coupling with ICC and SMC is through a type of gap junction (Fig 2.1(c)) [77, 98]. However, the description of this gap junction is unclear as most studies in the literature have focused on the properties of the gastric ICCs
rather than exactly how ICCs influence SMCs [155, 77]. In this section, two SMC activation mechanisms are introduced. First, a $V_m$-threshold activation method was incorporated in the SMC model to allow generation of a slow wave activity in the presence of a small amplitude electrical stimulus (Section 6.4.1). Second, a gap junction conductance was incorporated in a combined ICC-SMC model, in which the ICC slow wave activity directly drive the SMC slow wave activity (Section 6.4).

### 6.4.1 Threshold Activation Model

The C&B SMC model was updated to incorporate a $V_m$-threshold activation of slow wave activity in the SMC. In the original implementation of the SMC model, the $I_{ICC}$ term in Eqn 6.1 was fitted to a prescribed term with fixed onset times [33], which would not be applicable in a multiscale model. A $V_m$-threshold activation mechanism was proposed to allow the SMC model to respond to a current source that could represent the passive voltage of electrical activities in the surrounding tissue matrix. Furthermore, the $V_m$-threshold activation mechanism would also allow the slow waves to be regenerated and actively propagated. The following piece-wise function was used to model the $V_m$-threshold activation,

$$\frac{dL_t}{dt} = \begin{cases} 
1 & \text{for } V_m > V_{\text{threshold}}, \\
-L_t & \text{for } V_m < V_{\text{threshold}} \& L_t > 0, \\
0 & \text{otherwise}, 
\end{cases} \tag{6.9}$$

where $L_t$ is an activation variable of SMC that is also $V_m$-dependent. The activation variable functions in a piece-wise manner: $L_t$ increases linearly with respect to time when $L_t$ exceeds a $V_m$ threshold ($V_{\text{threshold}}$: -65 mV); in this case, the $L_t$ acts as a ‘time counter’ for the original $I_{ICC}$ function [32] (Fig 6.9).
Figure 6.9: Function of the time counter function. (a) A stimulus of 1 pA amplitude, 1.5 s pulse-width was applied to the modified smooth muscle cell (SMC) model at 10 s. (b) Equation 6.9, a $V_{\text{threshold}}$ of -65 mV was used. (c) Solution of Eqn 6.9. (d) Fitted $I_{\text{ICC}}$ current as a function of the time counter values in (c).

The $I_{\text{ICC}}$ was fitted as a function of $L_t$,

$$I_{\text{ICC}} = \begin{cases} 
60 & \text{for } 0 < L_t < t_{\text{peak}}, \\
\frac{60}{1+e^{L_t-8}} & \text{for } t_{\text{peak}} < L_t < t_{\text{plateau}}, \\
\frac{78}{1+e^{L_t-8}} & \text{for } t_{\text{plateau}} < L_t < t_{\text{stim}}, \\
0 & \text{otherwise}, 
\end{cases} \quad \text{(6.10)}$$

where $t_{\text{peak}}$ is the time to peak, which was set to 0.098 s; $t_{\text{plateau}}$ is the duration of the plateau of the slow wave activity, which was set to 7.56 s; $t_{\text{stim}}$ is the duration of the slow wave activity, which was set to 10 s. The parameter values in Eqn 6.10 were obtained from the original values used by Corrias et al to fit an experimental
recording of ICC $V_m$. [32]. After the activation of the slow wave activity, $L_t$ decreases by $-L_t$, *i.e.*, the ‘time counter’ resets back to 0, and then the whole cycle would repeat again when $V_m$ of the SMC exceeds the $V_{\text{threshold}}$ again. The simulated slow wave activity using the modified C&B SMC with the inclusion of Eqn 6.9 is presented in Section 8.2.1.

### 6.4.2 Direct Coupling Activation Model

An important drawback of $V_m$-threshold activation mechanism described in Section 6.4.1 is that it was not strictly biophysically-based. Therefore, a gap junction conductance mechanism was proposed to directly coupled the $V_m$ of the ICC to SMC, which better resembles the coupling slow waves between ICC and SMC in the GI tract [98]. The coupled ICC and SMC models contained two components, an ICC component that generated autonomous slow waves at a set intrinsic frequency, and a SMC component that responded to the slow wave activity in the ICC component. The gap conductance was modelled using the following equation,

$$I_{ICC} = G_{\text{couple}}(V_{m(ICC)} - V_{m(SMC)}) ,$$

(6.11)

where $V_{m(ICC)}$ and $V_{m(SMC)}$ are the $V_m$ of ICC and SMC, respectively. The gap junction was modelled as a passive-resistor of the difference between the $V_m$ of the two cell components, which was then multiplied by a gap channel coupling conductance ($G_{\text{couple}}$: 60 nS). The choice of the coupling conductance was scaled to reproduce the amplitude of the slow wave activity reported by Hirst *et al.* [78].

Experimental studies have demonstrated different morphological features of gastric slow waves, in terms of the resting membrane potentials and amplitude [78]. It was important for the ICC-SMC model to be capable of reproducing those values, especially in the whole-organ model (Section 7.6.2). Experimental studies have also demonstrated a gradient of resting membrane potentials (-45 to -70 mV), and an increasing gradient of amplitude (15 to 20 mV) of slow waves from corpus to antrum in the guinea-pig stomach [155, 78]. The amplitude of the slow wave activity in the SMC compartment of the ICC-SMC model could be modulated by $G_{\text{couple}}$ as shown in Fig 6.10(a), whereas the resting membrane potential of the SMC compartment
in the ICC-SMC model was dependent on both $G_{\text{couple}}$ and the Nernst potential of Potassium ($E_K$) as shown in Fig 6.10(b).

There are many other cellular parameters which could be changed to vary the resting membrane potential, such as the maximum conductance of the $I_{Kb}$ term in Eqn 6.1. Since the ICC-SMC model contains mostly $K^+$-type conductances, changing $E_K$ offered the most direct means of adjusting the resting membrane potential. While there was also experimental evidence of carbon monoxide being a modulator of SMC and ICC resting membrane potentials [65], the ICC-SMC did not include a parameter for adjusting the concentrations of carbon monoxide, and the effects of carbon monoxide were not modelled. Furthermore, in some parts of the GI tract, the relative density of SMCs relative to each ICC is high, e.g., up to 100 [14], in some areas of the GI tract, so that the contribution of other parameters in the ICC compartment to the resting membrane potential of SMC was ignored in this case. Simulated slow wave activity using the modified C&B SMC with the inclusion of Eqn 6.11 is presented in Section 8.2.1.
Figure 6.10: Coupled ICC-SMC resting membrane potential and amplitude calibration curves. (a) The resting membrane potential (RMP) of $V_{m(SMC)}$ as a function of potassium Nernst potential ($E_k$) at different levels of ICC-SMC coupling conductance ($G_{couple}$). (b) The amplitude of SMC slow wave as a function of $G_{couple}$. Both graphs were reproduced from [43].
6.5 Standard Encoding and Distribution of Cell Models

Published biophysical cell models often encompass a system of many ODEs and it is difficult to accurately reproduce the published results from printed paper publications. For example, the modified C&B ICC model contained 32 ODEs and as many as 96 cellular parameters, all of which would need to be specified in order to reproduce the published results in a paper [33]. Furthermore, cell models are often programmed in different programming languages, such as Matlab, C, or Fortran, and solved using different numerical solvers, which further impedes their reproducibility. For these reasons, there has been a recognised need for a standard coding environment and online database for published cell models.

CellML is a standardised XML markup language designed to store and exchange computer-based mathematical models [115]. CellML allows scientists to share and reuse models even if they are using different model building software, thus ensuring the reproducibility of the model and accelerating model building. More importantly the CellML language also provides a direct integration of the cell models to the continuum modelling software, CMISS, used to simulate tissue and organ level slow waves in this study. To date, all of the aforementioned cell models, C&B SMC model, C&B ICC model, and both components of the Faville ICC models are available from the CellML database [19]. The modified ICC, SMC, and combined ICC-SMC model were also encoded using the CellML standard. The models were solved using the CVODE integrator with the Adams-Moulton method in the Cellular Open Resources (COR) environment [31].
Chapter 7

Multiscale Models of Gastric Slow Waves

Propagation of slow waves is mediated by a large number of connected ICCs in the GI tract. As the role of ICCs in the underlying electrophysiology of gastric slow waves is better understood, attention has turned to propagation of slow waves in a multicellular setting, e.g., tissue (ICC networks), organ (stomach), and body (EGG). The mathematical cell models described in Chapter 6 can be incorporated into the larger scale multicellular models, either as a discrete set of ICCs and/or SMCs or as a continuum representation of a volume-averaged block of tissue [148]. In both approaches, multiple governing equations are employed to model the slow wave activity in a multiscale model. The GI multiscale models are under the umbrella of the International Union of Physiological Sciences World Physiome Project, which aims to develop computational models of the entire human body in health and disease [88]. One of the key aims envisaged by the Physiome project is to provide a comprehensive framework for modelling the human body using mathematical and computational modelling techniques that incorporate details of physiological relevance. Major developments in science and medicine occurring are being conducted at the cellular levels, and also imaging and recording modalities such as MRI, CT, ultrasound, electrical mapping occurring at the organ levels. It becomes more important than ever to be able to integrate the understanding of this information across a multitude of biophysical scales. The GI tract modelling has been variously termed the Digestive Physiome.
or the GI-ome by current investigators, although neither term is yet in widespread use [25]. The key challenge of the Physiome project, in particular the aspect of the project dealing the digestive system, is the ability to bridge the gaps of knowledge in the current understanding of GI electrophysiology while relying on experimental validation to consolidate our knowledge of the digestive system.

In the discrete approach, each cell model acts as the basic unit of the coupled model, with the ICC-ICC, ICC-SMC, and SMC-SMC coupling modelled as networks composed of individual conductances. Previous cardiac modelling studies have used the discrete approach to simulate propagation of cardiac electrical events in a network of coupled cells that represented a small volume of tissue (0.5×1.5 mm) with a large number of individual ionic and coupling conductances [92]. However, modelling propagation of gastric slow waves using the discrete approach would require much computational power, as the fine spatial information is required. Even with the increase of the power and widespread use of high performance computers (HPC), it remains infeasible to simulate propagation of slow waves in large blocks of tissue at discrete cell levels.

Another approach to model propagation of slow waves is the continuum modelling approach. The basic unit of a continuum model is modelled in a volume-averaged sense, i.e., the conductive medium is modelled as a continuum of unit (rather than being made up of discrete cells) [147]. The volume-averaged treatment of the conductive medium reduces the size of the model and thereby reduces the computational time required to solve the model. In Chapter 6, ODEs were used to model the ion conductances in ICCs and SMCs. To relate the conduction of slow waves at the cellular scale to higher physical scales, e.g., from cell to tissue, another set of governing equations would be needed. Furthermore, structural information such as conductivity of the tissue matrix also need to be incorporated into the tissue models.

The main purpose of this chapter is to introduce a series of models used to study slow wave propagation. The equations governing continuum modelling are introduced first (Section 7.1). The simulation setup of the continuum models is briefly described in Section 7.2. The modified ICC model is incorporated in a 1D model (Section 7.4). An automata model is coupled to the C&B SMC model and incorporated in a 2D model (Section 7.5.1). Two sets of real ICC network geometries and the modified
C&B ICC model are incorporated into 2D models (Section 7.5.2). The method used to simulate the propagation of gastric slow waves in a geometric model of the human stomach is described in Section 7.6.2. The dipoles of the simulated slow wave activity in the human stomach model are used to calculate the resultant EGG in a geometric model of the human torso (Section 7.7).

7.1 Monodomain and Bidomain Models

There are a number of continuum modelling approaches that can be used to model the propagation of biological electrical potentials [13, 25]. One approach is to model the interactions of electrical potentials in the intracellular and/or extracellular domain(s). For example, the monodomain model assumes that the biological tissue behaves as an excitable domain, with diffusion and local excitation of cellular electrical activity [27]. For example, in a network of ICC, there is a component of passive flow of current due to the slow wave activity of one ICC on the surrounding ICCs in the tissue. The current depolarises the $V_m$ of neighbouring tissues slightly, which then entrains the slow wave activities in the surrounding ICCs. The monodomain models the passive current flow as a diffusion-reaction process,

$$\nabla \cdot (\sigma \nabla V_m) = A_m \left( C_m \frac{\partial V_m}{\partial t} + I_{ion} \right), \quad (7.1)$$

where $\nabla$ is the gradient operator, $\sigma$ donates the passive conductance tensor of the tissue medium surrounding the ICCs/SMCs, and $A_m$ denotes the surface-to-volume ratio of the cell membrane to the tissue volume. The current term $I_{ion}$ denotes the current flow though the cell membrane, e.g., as described in Eqns 6.1, 6.3, and 6.8.

The bidomain model is another major continuum modelling approach that has previously been employed to simulate the propagation of cardiac electrical events, and more recently adapted for slow wave simulations [147]. In contrast to the monodomain described above, the bidomain model (Eqns 7.2 and 7.3) conceptualises two interpenetrating cellular domains, i.e., the cytoplasm and the extracellular tissue matrix, and represents the flow of electrical currents between these two domains. The key advantage of the bidomain equations over the monodomain equation is that the bidomain considers the extracellular potential ($\phi_e$), which is often measured experi-
mentally at the tissue and organ levels [103, 132]. A system of two equations comprise the bidomain model,

\[
\nabla \cdot (\sigma_i \nabla V_m) = -\nabla \cdot ((\sigma_i + \sigma_e) \nabla \phi_e),
\]

(7.2)

\[
A_m(C_m \frac{\partial V_m}{\partial t} + I_{ion}) - \nabla (\sigma_i \phi_e) = \nabla \cdot (\sigma_i \nabla V_m),
\]

(7.3)

where the \(\sigma\) terms denote tissue conductivity tensors, with subscript \(i\) denoting the intracellular domain and subscript \(e\) denoting the extracellular domain. The \(I_{ion}\) denotes the current flow through the cell membrane, as described in the cell models, e.g., Eqn 6.1 or Eqn 6.3. Equation 7.2 describes the relationship between \(V_m\) and \(\phi_e\). Equation 7.3 is a reaction diffusion equation in terms of the \(V_m\), where the sum of ionic conductances from cell models provides the non-linear reaction term [147].

The bidomain model presented here is a voltage dependent system, in line with the standard of previous simulation studies of both cardiac and GI electrical activity [147]. However one difference between the bidomain model adopted here and the general cardiac studies is the absence of a stimulus current, generally termed \(I_{stim}\), due to the self-excitatory behaviours of ICCs.

There are two reasons why a bidomain model may be preferred over a monodomain model in GI slow wave simulation. First, the bidomain model contains two classes of conductivities, e.g., \(\sigma_i\) and \(\sigma_e\), which better represent the heterogeneity in the underlying tissue structure. Second, GES are generally applied to the extracellular tissue matrix rather than directly via an intracellular electrode (Section 6.1.1), which can be readily applied using the bidomain model.

The bidomain model has previously been applied to simulate the propagation of slow waves in a multi-layered section (LM and CM) of the gastric wall [25], and the smooth muscle layers were separated by layers of ICCs (ICC-MY and ICC-SEP). The multi-layered gastric wall model combined structural information with the electrically active tissues. The Aliev model was used to simulate slow wave activity in the tissue model [24]. The simulated slow wave using the Aliev model activation began in the ICC-MY and ICC-SEP layers, and subsequently activated the LM and CM layers [24]. There were a number of drawbacks with the Aliev model as aforementioned in Chapter 6, and with the advent of the biophysical cell models of slow wave activity, the multiscale framework could be improved by incorporating these


7.2 Grid-based Finite Element Method

The rapid increase in high performance computing power and applications of numerical solution techniques have seen an increase in both the scale and efficiency of simulations of biological processes. There are a number of simulation techniques that have been used to solve the monodomain and bidomain equations in anatomically realistic models [25, 44]. These simulation techniques include finite difference methods, finite volume methods, and finite element methods [44]. The finite difference methods model the strong second derivative form of the bidomain equations, whereas the finite volume and finite element method (FEM) model the weak weighted integral of the bidomain equations [173]. The approaches based on the weak form of the bidomain equations are more suitable for irregular and non-orthogonal computational meshes whereas the finite difference methods are more suitable for regular and orthogonal meshes.

In biological simulations, the FEM has been used to approximate the solutions of mathematical equations that represent physiological processes over a complex geometry, by discretising the physical shape of a biological organ into smaller local elements (Fig 7.2) [44]. The anatomy of the organ of interest, e.g., the stomach, was generally digitalised from CT, MRI, or other imaging modalities [24]. For longer GI organs, such as the small intestine, a one-dimensional curve representing the centre line down the length of the small intestine was digitalised from an image source and a radius $10 \ mm$ was then extruded from the centre line to form a volumetric mesh of the intestine [24, 110, 111].

In particular, the GI mathematical models have benefited from the application of the grid-based FEM technique. A numerically convergent solution of the propagation of slow waves generally needed to be simulated at finer spatial resolutions within each local element, which was achieved by allocating a number of solution points (grid points) to each local element (Fig 7.2(b)) [44]. The bidomain model could then be solved to simulate propagation of slow waves within and across the local elements in the tissue/organ model [24, 110]. Numerical solvers, such as the Euler method, were
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Figure 7.1: Grid-based finite element model setup. (a) a geometric element model with Cubic Hermite basis functions. (b) finer grid points (dots; $10 \times 10 \times 2$) overlaid over the geometric element. (c) slow wave potentials were simulated over the grid points and displayed as colours over the geometric element.

applied to solve the equations represented by $I_{ion}$, e.g., Eqn 6.4. Unless otherwise specified, the models described in Section 7.4, 7.5.2, and 7.6 were solved using the following numerical methods. Equation 7.2 was solved using the Euler method with LU decomposition; Equation 7.3 was solved using the LSODA solver [74] with the Conjugate Gradient linear solution procedure. The Point Jacobi method was used as a preconditioning step; All of the simulation steps were advanced in 1 ms time steps.

7.3 Boundary Element Method

The boundary element method (BEM) seeks to divide the large problems of solving for the extracellular and the torso cavity outside the stomach into smaller zone in which the BEM can be applied individually, while maintaining the coupling across the gastric serosal surface. In this sense, BEM is similar to FEM, but with one important distinction - the dimensionality of the problem is reduced by one by formulating the governing equation as a boundary integral equation.

The general BEM solution process is started by incrementing solution time and solving for the updated cellular parameters and membrane potential at each new time step [148]. In the case where linear elements are used to model the regions surrounding the stomach then the current potential values are exchanged between the gastric boundaries, i.e.,
\[ \phi_e \iff \phi_o, \quad (7.4) \]
\[ (\sigma_e \nabla \phi_e) \cdot n_e \iff (\sigma_o \nabla \phi_o) \cdot n_o, \quad (7.5) \]

The boundary condition values are updated iteratively on the torso and the serosal surfaces. The potential value at each node is directly evaluated from the extracellular potential, and the normal current can be evaluated using a numerical approximation of the Neumann (current) boundary condition [148]. Once all of the passive region boundary values have been updated, each of the passive problem is resolved in a new iteration [148].

Theoretically, once the passive problems have been solved, a ‘feedback’ step in which nodal interpolation is applied to update the extracellular serosal boundary condition is implementation [148]. However, this would require the FEM model to be solved simultaneously with the BEM model in a fully coupled system, which would be very computationally demanding. Furthermore, due to the passive nature of the torso model, it is unlikely that the resultant torso potential would have too much influence on the serosal potential itself. So in practice this final step was not implemented in the BEM models.

### 7.4 One-dimensional Model Setup

A continuum modelling approach was used to simulate the entrainment of slow waves in a 1D model, over a simulated period of 120 s. A single 1D geometric model with a linear Lagrangian basis function was used to construct a line of 3 mm in length (Fig 7.4). Grid points were distributed at even spacing in the coordinates of the basis function, in which the spatial variable is denoted as \( \xi_1 \). A linear gradient of intrinsic frequencies were assigned to the 1D model by varying the parameter \( \beta \) in Eqn 6.6 over the 1D geometric element to obtain an intrinsic frequency gradient between 3.0 to 2.8 cpm, as shown in Fig 7.4.
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Figure 7.2: One-dimensional (1D) model setup. The length of the 1D model is 3 mm, and a number of grid points were allocated at equal spacing (Δx = 0.3 mm in this example). A linear gradient of ICC slow wave intrinsic frequencies (3-2.8 cpm) were assigned across the 1D model, with the highest intrinsic frequency on the left-most grid point and the lowest intrinsic frequency on the right-most grid point. A zero flux boundary condition was applied to the two boundary grid points in the 1D model.

To test the spatial resolutions required for the numerical convergence of the 1D model, the number of grid points the 1D model was varied between 2 points (Δx = 3 mm) and 101 grid points (Δx = 0.03 mm). The velocity was calculated as the difference in time between the maximums of the simulated slow waves at the end boundary grid points, and was used as a measure of numerical convergence (Fig 7.4). Simulations were solved using the Continuum Mechanics, Image analysis, Signal processing and System Identification (CMISS) software [28].

The solution times were tested by measuring the CPU times took to solve 60 s, 120 s, and 180 s of slow wave propagation in the 1D model at each of the aforementioned spatial resolutions (Fig 7.4). The model was solved on a single CPU of an IBM p595 HPC.
Two-dimensional Model Setup

Two different two-dimensional (2D) models are introduced in this section. As an initial step, a rule-based automata model was used to model the entrainment and propagation of slow waves in a regular grid of ICCs coupled to a smooth muscle layer. A corresponding regular grid of SMCs was coupled to the ICC layer to simulate the slow wave activity in the smooth muscle layer (Section 7.5.1). The automata model allowed two aspects of GI slow waves to be investigated: (i) it is a useful tool to visualise the formation of a broad wavefront from a single activation source, i.e., pacemaker; (ii) the gradual entrainment of an ectopic pacemaker over successive cycles of slow waves and stimulation. A more advanced 2D model was created from incorporating the modified C&B ICC model (Section 6.2.1) in 2D models with real geometries of ICC networks obtained from confocal images of ICC-MY from a normal mouse and a mouse with depleted ICC-MY count.

Figure 7.3: Convergence test of ICC slow wave entrainment. A one-dimensional geometric element of length 3 mm and the modified C&B ICC model were used to simulate active propagation of entrained slow waves. Velocity was used to test for the spatial resolution required to achieve numerical convergence. The converged velocity was 3.73 mm s$^{-1}$ and the minimum converged spatial resolution was $\sim$4 grid points per mm or grid point spacing of $\sim$0.25 mm.
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Figure 7.4: The computational times of the one-dimensional model at varying simulated times of 60 s, 120 s, and 180 s of simulated time.

7.5.1 Idealised Tissue Model Setup

A 2D model of gastric slow wave activity was developed by modelling the slow waves originating in an idealised ICC network and the subsequent depolarisation of a smooth muscle layer. The ICC network and SMC layer were modelled as two interconnected tissue layers. A number of experiments have shown that ICCs act as pacemakers and mediators of slow wave propagation through the entrainment of slow waves [155]. Furthermore, smooth muscle layers cannot actively propagate slow waves without the presence of ICCs [156]. In previous simulations of gastric slow waves, multiple ICC and smooth muscle layers have been incorporated [24, 149]. In the model presented here, two interconnected layers were incorporated to represent the averaged effects of all electrically active layers measured from the serosal surface. While this approach did not allow microscopic details of gastric slow waves to be investigated, it presented a computationally efficient way of reliably comparing simulated data to experimental measurements.

Each layer was represented as a 2D continuum tissue model, in which mathematical descriptions of the ICCs and SMCs were incorporated. An automata algorithm was used to simulate the response of ICC to the slow wave activity of a surrounding ICC in the ICC layer. A similar concept of algorithm-based modelling has been suc-
cessfully used to simulate electrical activation of canine ventricles in earlier cardiac modelling work [64]. The algorithm of the automata model is illustrated in Fig 7.5. Briefly, the algorithm involved assigning a set of conditions for which a continuum unit in the ICC layer (hereafter named a continuum cell) was to generate a slow wave activity; the overall frequency of slow waves in the surrounding continuum cells would be entrained to the continuum cell containing the highest intrinsic frequency in the ICC layer.

Once a continuum cell in the ICC layer entered the refractory period in accordance with the automata algorithm, a predefined ICC membrane potential trace was used to represent the current of ICC slow wave activity (Fig 7.5(a)). The predefined trace was a fitted curve of the membrane potential of a single excitation of an isolated ICC that was experimentally obtained from guinea-pigs [32]. This trace has been previously shown to adequately depolarise the C&B SMC model [32]. Each ICC continuum cell was assigned an intrinsic frequency at which it produced slow waves. Based on previous experimental recordings of decoupled canine gastric slow wave frequencies [181], a gradient of the intrinsic frequency was set linearly ranging from 3.65 to 2 cpm from the proximal to the distal end of the tissue model. A regenerated/new slow wave could only be invoked in each ICC cell during the non-refractory period (Fig 7.5(b)). Conversely, during the refractory period, the ICC continuum cell was insensitive to the depolarising currents from another ICC continuum cell.
Figure 7.5: Summary of slow wave entrainment automata algorithm. (a) The refractory period and non-refractory period of a slow wave was defined as the period during which the slow wave occurs and the rest potential, respectively. A continuum cell in a non-refractory state (b; grey square) was capable of either generating a new slow wave due its own intrinsic frequency, or being entrained by one of the eight neighbouring continuum cells, according to the flowchart of the cellular automata algorithm outlined in (c).

A time counter was assigned to each ICC continuum cell for recording the time since the onset of a slow wave. The counter was reset to 0 s each time that a continuum cell produced a slow wave. Once an ICC continuum cell entered the non-refractory period (i.e., counter > refractory period), the automata algorithm determined whether any of the eight surrounding continuum cells produced a slow wave that could entrain the resting ICC continuum cell (Fig 7.5b). The automata algorithm also checked whether entrainment occurred in advance of a slow wave arising due to the intrinsic frequency in the resting ICC continuum cell (Fig 7.5c). The counter of the resting ICC was then assigned a ‘delay-time’ before the next slow wave.
wave occurred. The time delay ($n$) was calculated using Eqn 7.6.

$$n = \frac{d}{V_{\text{circ}}} \sqrt{1 - \left(1 - \frac{V_{\text{circ}}^2}{V_{\text{long}}^2}\right) \cos^2(\theta)}, \quad (7.6)$$

where $d$ and $\theta$ are the distance and angle between the resting and depolarising ICC, respectively; and $V_{\text{circ}}$ and $V_{\text{long}}$ are the conduction velocities in the circular and longitudinal directions, respectively. The assigned slow wave conduction velocity in the circular direction ($V_{\text{circ}} = 8.51 \text{ mm s}^{-1}$) and in the longitudinal direction ($V_{\text{long}} = 4.58 \text{ mm s}^{-1}$), were based on an analysis of an initial set of porcine slow wave recordings. The anisotropy of the assigned velocity components represented a possible micro-structural heterogeneities in the underlying tissue structure, particularly ICC-IM, ICC-MY, and other mechanical junctions [78, 170], which were not modelled in the automata model. Eqn 7.6 was used in conjunction with the algorithm outlined in Fig 7.5(c) to assign the timing of the entrained slow wave activity in the ICC layer.

**Smooth Muscle Layer**

The smooth muscle layer was modelled as a passive conduction layer in which the C&B SMC model was incorporated [32]. The smooth muscle layer was coupled to the ICC layer via the $I_{\text{ICC}}$ term in Eqn 6.1. The monodomain equation (Eqn 7.1) was used to simulate the passive conduction of the slow waves in the smooth muscle layer. The monodomain equation was used because propagation of slow waves was modelled using the automata algorithm and $\phi_e$ in the smooth muscle layer was not needed to actively propagate slow waves. The conductivity in the smooth muscle layer was set to 0.005 $\text{ mS mm}^{-1}$. The monodomain equation was solved using the Euler method with a five-point finite difference method. The second derivatives of the smooth muscle $V_m$ were scaled to represent the serosal slow waves, based on a previous study on the relationship between $\phi_e$ and second derivative of $V_m$ in neurons [29]. The amplitudes of the second derivatives were scaled to match experimental data.

**Gastric Pacing Mapping Model**

The automata model was first used to simulate normal porcine propagation (Section 5.2), and then used to simulate the effects of a set of gastric pacing protocols.
mapped by using the G1-PCB electrodes. The setup of the gastric pacing experimental recording was similar to that the standard pig experiment (Section 3.4.2), with the exception of inserting a pair of cardiac pacing needles into the gastric corpus [134]. Two 23 g hollow-bore stainless steel pacing electrodes were inserted 11 mm below the fundal line as shown in Fig 7.6a. The electrodes were 8 mm apart, providing an average tissue resistance of 16.5 kΩ. The pacing wires leading out of the wound were connected via an electrical isolator to a DS8000 multichannel stimulator (World Precision Instruments, Saresota, Florida). A gastric pacing protocol (Fig 7.6b) was administered for 20 min.

Figure 7.6: Gastric pacing mapping setup. (a) The placement of six generation 1 printed-circuit-board (G1-PCB) electrodes. The ‘+’ and ‘−’ represent the location of the positive and negative leads of the stimulator, respectively. (b) Pacing protocol (amplitude 2 mA; pulse-width 400 ms; frequency 3.53 cpm (17 s)).

The results of the automata model are presented in Section 8.4.1.

7.5.2 Tissue Models With Real ICC Network Structures

The structural integrity of the network of ICCs in the GI tract is an important factor for normal slow wave entrainment and GI motility. As described in Section 7.5.1, simulations of GI slow waves at the tissue level involving a large physical network of ICCs and SMCs have generally adopted an idealised layered setup [42]. Similarly, the geometric structure of the gastric wall has also been divided into regular layers of ICCs and SMCs [24, 25]. With the recently developed of ICC and SMC models [32, 33], incorporation of a physiologically realistic description of the tissue structure has
become a priority, to enable multiscale simulations that provide informative outputs of realistic physiological data.

**WT and 5-HT$_{2B}$ Tissue Imaging**

The mouse has long been used as an animal model for ICC physiology [67, 169]. For the biophysical tissue model, slow wave entrainment was modelled over two ICC network geometries obtained from mouse jejunum. The first geometry was derived from a wild-type (WT) mouse intestine with an intact ICC network (Fig 7.7(a)), and the second geometry was obtained from a mouse with a germline deletion of the 5-HT$_{2B}$ serotonin receptor (Fig 7.7(b)). The 5-HT$_{2B}$ receptors are expressed on ICCs and their stimulation by serotonin induces proliferation of ICC populations. Thus these receptors are an important regulator of ICC network density [169]. There are two types of ICCs in the small intestine, the ICC-MY and ICC-DMP [155]. Since ICC-MY is the primary ICC structure responsible for the generation and propagation of slow waves its geometry was used as the network model, and ICC-DMP was not considered in this study [45].

A series of 2D bitmap images of the Kit-positive ICC structures were obtained from mice as previously described in an experimental study [169]. Briefly, the whole mount preparations of the muscularis propria from the jejunum of four week old mice were used. ICC were labelled by incubation with a primary monoclonal antibody against Kit (ACK2, eBiosciences, San Diego CA) and a secondary polyclonal antibody conjugated to Cy3 and raised against rat IgG (Jackson Immunoresearch, West Grove PA). Images of the labelled structures were collected by an Olympus FV1000 laser scanning confocal microscope using 40x water, 1.2 numerical aperture; and 40x oil, 1.3 numerical aperture objectives (Olympus America Inc., Center Valley, PA, USA). Images were collected using a z-axis step size (0.46 to 0.54 µm) that matched an Airy number of 1 for the objective and the excitation wavelength for the fluorophore. Voxel dimensions were corrected for aberrations in the vertical axis using calibration beads to determine the actual pixel dimensions. Stacks of confocal image slices were volume rendered in three dimensions and bitmaps of the positively labelled structures were obtained by using Analyze™ running on a PC operating Microsoft Windows, as previously described in a separate study [122]. The volume quantification was done
using unbiased thresholding algorithms to segment the images and determine the volume of Kit positive structures thereby minimising any human influence in assessing the differences.

Figure 7.7: Image block of myenteric interstitial cells of Cajal (ICC-MY) networks. Three-dimensional anatomical image blocks of (a) a wild-type (WT) mouse intestinal sample, and (b) a 5-HT$_{2B}$ knockout mouse intestinal sample. The physical size of the WT tissue block was 316 × 316 × 7 μm, and the physical size of the 5-HT$_{2B}$ knockout tissue block was 316 × 316 × 8 μm. Two-dimensional representation of the depth averaged (c) WT model and, (d) 5-HT$_{2B}$ knockout model. Reproduced from [45].

The segments of images of the WT and 5-HT$_{2B}$ knockout mouse tissues containing only the ICC-MY networks were rendered into three-dimensional (3D) stacks of images sequentially taken at many transmural depths. In the samples obtained there were 43 slices in the myenteric plexus region of the WT tissue image stack...
Two-dimensional Model Setup

and 41 slices in the 5-HT$_2B$ knockout tissue image stack. The physical dimensions were 316×316×7 µm$^3$ for the WT image stack and 316×316×8 µm$^3$ for the 5-HT$_2B$ knockout image stack. Each in-plane image was sampled at 512×512 pixels$^2$ for both tissue blocks. The in-plane resolution was 0.62 µm in both tissue blocks, and the transmural resolution was 0.17 µm in the WT image stack and 0.20 µm in the 5-HT$_2B$ knockout image stack.

Due to the relatively thin depth of the transmural direction of the myenteric plexus ICC networks, and because the majority of the network geometry lay in the in-plane directions, the image stacks were depth-averaged into 2D images as shown in Fig 7.7(b). A similar 2D tissue modelling strategy was also recently employed in a tissue-specific model of atrial function, where a comparison study between thin-3D and 2D modelling showed no significant quantitative differences in the functional outcome [191].

WT and 5-HT$_2B$ Mathematical Models

The ICC network geometries were obtained from the 2D images of the WT and 5-HT$_2B$ knockout mouse samples at the original in-plane resolutions. Each pixel in the image was represented by a grid point in the tissue models, such that each resultant tissue model consisted of four 2D linear finite elements with a total of 262,144 grid points. More importantly, this allows the tissue models to be investigated at the same spatial discretisations as the original tissue images.

The grid points in the tissue models were grouped into either a continuum ICC node (green in Fig 7.7) or a non-ICC tissue (black in Fig 7.7). Eqns 7.2 and 7.3 were solved to simulate propagation of slow waves. The non-ICC tissue nodes were represented using an invariant $V_m$ term with $I_{ion} = 0$ pA. The purpose of the invariant $V_m$ term is to eliminate any active responses of the background tissue to the slow waves in the simulations (by reducing the conductivities in the non-ICC tissue nodes). With the appropriate conductivity values, the invariant term could act as a ‘current sink’ to the slow wave generated in the ICC network.

The ICC network in each tissue model was assigned a gradient of intrinsic frequencies by linearly varying $\beta$ between $2.55 \times 10^{-5}$ mm s$^{-1}$ and $2.54 \times 10^{-5}$ mm s$^{-1}$, which correlated to a frequency gradient from 3.0 to 2.9 cpm (Fig 6.7). The gradient
of intrinsic frequency was very small due to the limited physical dimensions of the tissue models. Frequencies close to the intrinsic frequency of gastric slow waves were applied to the intestinal tissue geometries for two practical reasons. First, the C&B ICC model was originally designed to reproduce gastric slow waves and it is most stable between 1 to 5 cpm. Second, the simulations in this study focused only on the activation phase of the first 400 ms of slow wave activity, whereas the duration of the slow wave is in the order of seconds. Therefore, the absolute value of the intrinsic slow wave frequencies was not an important factor for the simulation outcomes. In both the WT and 5-HT$_2B$ knockout tissue models, the highest intrinsic frequencies were assigned to ICCs in the top left corner of the tissue domain, where the density of ICCs was qualitatively the highest (Fig 7.7).

Slow waves were simulated in the anatomically-realistic 2D ICC networks over a much shorter temporal scale (400 ms) compared to the 1D model (120 s). The activation period of 400 ms was deemed sufficient time to capture a complete depolarisation phase of the tissue model assuming a propagation velocity of approximately 2 mm s$^{-1}$ [144].

To relate the simulation results of the tissue models to physiologically meaningful quantities, the functional outcomes of the simulated slow wave activity across each tissue model were quantified by calculating [$Ca^{2+}$], density and current density during activation across the tissue model. The [$Ca^{2+}$] density was calculated by averaging the [$Ca^{2+}$] from the C&B ICC model at each node in the intracellular domain in the tissue model at each solution time step. A dipole vector was used as the source term ($J$), i.e., the current density of the simulated slow waves using the following equation [4],

$$J = -\sigma \nabla V_m,$$  \hspace{1cm} (7.7)

where $\sigma$ is the conductivity tensor. The main procedure of computing $J$ is the computation of the $\nabla V_m$, which was calculated through the use of the local coordinate system and the global coordinate system via a mapping matrix [4]. The $\sigma$ represents a homogenised set of conductivity values to obtain $J$. Normally $\sigma$ is a tensor and may contain discontinuities as the micro-structural heterogeneity between SMCs and ICCs. A single $J$ was computed per grid point in the 2D tissue model. The simulated slow wave propagation over the two ICC network geometries and the quantified results
7.6 Whole-organ Model Setup

Until recently, an accurate representation of electrical activation in a whole-organ model of the GI tract remained an elusive goal, due to several significant technical challenges. First, to represent the geometry of the stomach accurately, a large number of linear elements would be needed. Second, biophysical models of gastric SMCs and ICCs were not available. Third, the experimental data regarding GI slow waves propagation was derived mainly from sparse-electrode studies, whereby recordings were obtained from few distributed electrodes (typically four) [22, 21, 114]. These sparse electrode studies provided limited information of the global gastric slow wave propagation in the whole-organ, and therefore lack sufficient spatiotemporal detail to inform a reliable whole-organ model description. However, recent work has addressed and resolved most of these challenges, enabling significant progress to be made in GI whole-organ level modelling.

To create an anatomically-realistic geometry of a GI organ, a finite element technique was used fit a geometric mesh to a data cloud identifying the organ of interest outline, e.g., the stomach, from a HR image source, such as the Visible Human data or from clinical imaging systems such as MRI or CT [24, 165]. The surface mesh of the organ was then created from the data cloud by an iterative linear fitting technique, and a volumetric mesh of the organ wall was subsequently constructed in order to better represent known anatomical features. The wall of the organ can be further refined to incorporate the layered structure of the tissue model, for more detailed multiscale representations [25].

Slow wave activation over the whole-organ models has been modelled using the continuum approach [25, 44]. To date, the phenomenological Aliev model was the only cell model that had been integrated into a whole-organ model to solve for slow wave activation [24]. The resultant simulations have generally shown a reasonable approximation of sequential activation of gastric or intestinal slow waves across the many layers of the organ models. For example, normal gastric slow waves have been simulated in a human stomach model, with outcomes successfully matching the
conduction velocity and entrained frequency observed from sparse-electrode experiments [42]. Recent studies have also investigated the effects of functional uncoupling of slow waves, where the stomach model contained more than one entrainment frequencies [13]. To model the uncoupled slow wave activity, the parameters in the Aliev model were updated by adjusting the excitability parameter, for the region that represents the gastric antrum in the stomach model. By altering the excitability profile of ICCs in the antrum, a condition which allowed decoupled slow wave generation at the cellular level was imposed on the organ model under the multiscale framework [13].

Two improvements were made to improve the current model of gastric slow wave activation in the whole-organ. The first improvement was to incorporate the coupled C&B ICC and SMC models into a multiscale framework and to reproduce the regional variation in membrane potentials and slow wave amplitudes. The second improvement was to simulate slow wave propagation over the stomach in direct accordance with recent human gastric HR mapping data presented in Section 5.3.

7.6.1 Subject-specific Anatomical Model

A subject-specific virtual anatomical model of the stomach was constructed from preoperative CT images taken from a human patient (Fig 7.8a). This particular subject was chosen as the reference model because a complete description of gastric slow wave times were obtained by intra-operative HR mapping and this patient was representative of typical normal activity [132]. The 3D outline of the stomach was manually digitised from the CT images. A cubic Hermite finite element mesh was fitted to the digital outline of the stomach, from which the anatomical model was constructed using a finite element fitting method [24]. The surface of the initial stomach mesh was then projected towards the centre-line of the mesh to obtain a muscle layer thickness of 2.66 mm [84]. The final anatomical model of the stomach is as shown in Fig 7.8(b). The idealised layered structure of the gastric wall adopted by Buist et al in their previous study [13], was not employed in this study because the ICC-SMC model cell model conceptually represents a ‘continuum unit’ of a mixture of ICC and SMC (Section 6.4). Therefore, artificially establishing distinct layers of these cells in the simulation was not required.
Figure 7.8: Construction of a subject-specific virtual anatomical stomach model and torso model. (a) An axial view of the virtual stomach model embedded in the CT images taken of the patient prior to surgery; (b) The virtual stomach model that was constructed from the CT images. Nine virtual electrodes (G<sub>1</sub>-G<sub>9</sub>) were placed on the stomach model. G<sub>1</sub> was placed in the fundus, G<sub>2</sub> was placed in the pacemaker region, G<sub>5</sub> was placed in the corpus and G<sub>7</sub> was placed in the antrum. (c) A 10 mV membrane potential gradient was imposed across the gastric wall (site of shown cross-section of the stomach indicated by the dashed-line in b). (d) The virtual torso in which the stomach model was embedded to match the approximate orientation in the CT images. Reproduced from [43].
7.6.2 Whole-organ Activation Model

The initial activation times of gastric slow waves were established according to recent evidence from the human gastric HR mapping study presented in Section 5.3. The locations of the recording arrays used in the human study were manually registered onto the virtual stomach based on a set of anatomical landmarks (Fig 5.5) as previously described in Section 5.3 [132]. The activation times of slow waves in the anterior and posterior stomach were assumed to be symmetrical, based on experimental evidence from the simultaneous mapping of slow waves in the anterior and posterior surfaces of a porcine stomach (Fig 5.4) [50], and direct observations of contraction dynamics from MRI studies [143]. The average activation times obtained from multiple human subjects and recordings were averaged into a single generic propagation pattern, and the initial activation times of slow waves were then interpolated and mapped to each elemental node in the stomach model as one of the initial conditions of the cell model (Table 7.1). Only the initial activation times of the first event were prescribed; the subsequent slow waves were generated autonomously by the ICC-SMC component of the multiscale model.

<table>
<thead>
<tr>
<th>Location</th>
<th>Activation time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundus</td>
<td>NA</td>
</tr>
<tr>
<td>Pacemaker Region(^1) (G(_1)-G(_3))</td>
<td>3 to 0 (origin) to 3.86</td>
</tr>
<tr>
<td>Corpus (G(_3)-G(_6))</td>
<td>3.86 to 35.50</td>
</tr>
<tr>
<td>Antrum(^2) (G(_6)-G(_9))</td>
<td>35.50 to 55.48</td>
</tr>
</tbody>
</table>

Table 7.1: Initial activation times at the nodes along the greater curvature of the whole-organ model. The node locations are marked by electrodes (G\(_1\)–G\(_9\)) in Fig 7.8(b). \(^1\) G\(_2\) marked the centre of the pacemaker region. \(^2\) G\(_9\) was not placed on the pylorus of the whole-organ model, i.e., the edge of the whole-organ model. The total time slow waves took to propagate from G\(_2\) to the pylorus was 58 s.

A gradient of resting membrane potentials was also set in the whole-organ model (Table 7.2). The gradient of potentials in the fundus was set to -30 to -43 mV from the cardia to the proximal edge of the pacemaker region along the greater curvature; The gradient of potentials in the pacemaker and corpus was set to -43 to -58 mV from the proximal edge of the pacemaker region to the corpus and antrum border.
The gradient of potentials in the pacemaker and corpus was set to -58 to -67 mV from the corpus and antrum border to the pylorus of the whole-organ model. A 1.04× variation in the resting potentials from the greater curvature to the lesser curvature of the stomach, e.g., a gradient of -43 to -44.72 mV was assigned to the membrane potentials in the fundus. A gradient of -10 mV was also assigned across the stomach wall (Fig 7.8(c)). The $E_k$ value was adjusted to set the resting membrane potentials according to the calibration curves as shown in Fig 6.10(a). The 0 mS calibration curve was used to set the resting membrane potentials in the fundus. The 30 mS calibration curve was used to set the resting membrane potentials in the corpus. The 60 mS calibration curve was used to set the resting membrane potentials in the pacemaker region and antrum.

<table>
<thead>
<tr>
<th>Location</th>
<th>RMP (mV)</th>
<th>$E_k$ (along GC) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundus</td>
<td>-30 to -43</td>
<td>-41 to -42</td>
</tr>
<tr>
<td>Corpus</td>
<td>-43 to -58</td>
<td>-42 to -66</td>
</tr>
<tr>
<td>Antrum</td>
<td>-58 to -67</td>
<td>-66 to -88</td>
</tr>
</tbody>
</table>

Table 7.2: Resting membrane potentials of differential areas along the greater curvature (GC) of the whole-organ model.

Prescribing the initial activation times in this way allowed computational efficiency in the model. In the modelling study of slow wave entrainment over realistic ICC network geometries, we previously simulated slow wave propagation at spatial resolutions of ~0.25 mm (Fig 7.4), and this was computationally feasible due to the small physical dimensions of the tissue geometries employed (<0.4×0.4 mm) and the short duration of simulation times (<500 ms) (Section 7.7) [45]. However, the whole-organ model was many centimetres in size, and the required simulation time was also an order of magnitude longer than the previous tissue scale simulations (e.g., 120 versus 0.5 s). In combination, these two computational challenges made whole-organ simulations using the ICC-SMC model at a fine spatial resolution (<0.3 mm) a computationally demanding problem. The prescription of initial activation times reduced the number of grid points required to achieve a stable gastric slow wave propagation pattern, as well as providing a convenient framework by which the HR
mapping data could be directly applied to enforce experimentally-derived behaviours on the whole-organ model.

The simulation results of the whole-organ model are presented in Section 8.5.

### 7.7 Torso Model

At the body scale, the resultant electrical potential (EGG) and magnetic field (MGG), on the torso surface from the underlying whole-organ scale slow wave propagation was modelled. Applications of torso model simulations include informing far-field techniques such as EGG (Section 2.4.2) and MGG (Section 3.1.4), by relating the underlying slow wave activity to the resultant torso surface electrical or magnetic activities [13]. Although these far field recording techniques have the advantage of being non-invasive, one of the key issues is that the recorded signals are difficult to interpret, partly due to the complex signal morphology and relatively low SNR of the transmitted slow waves [13, 95]. Therefore, a torso model with an integrated whole-organ model provides a virtual medium to help interpret the signals recorded from the far field recordings, in order to relate them to pathological states.

It is also important to note that while certain classes of dysrhythmias are associated with either tachygastria or bradygastria, recent studies have revealed that complex dysrhythmic patterns could occur at the normal frequency of gastric slow waves [134, 136]. So in this a frequency-based analysis would miss the dysrhythmias.

The modelling problem that relates the simulated slow waves at the organ level to the resultant torso surface is known as the forward problem in multiscale modelling. A previous investigation of EGG attempted to use a conoidal dipole model of the stomach as the underlying source of activation and relating it to EGG [124, 125], and it could be improved by incorporating the gradient of resting membrane potentials and an accurate slow wave activation pattern, both of which might have a significant impact the orientation of the dipole.

In this study, the whole-organ model described in Section 7.6.2 was applied to determine the relationship between the gastric slow wave activity and the EGG. The EGG was calculated as a secondary step from the simulation of the whole-organ model, mainly due to computational requirements of calculating slow waves and EGG
simultaneously [147]. The stomach model (Section 7.6.2) was used to calculate the
equivalent dipole of lumped slow wave activation, which could be presented as either
a single slow wave source or a series of slow wave sources [148]. The equivalent dipole,
\( \rho \), was calculated by,

\[
\rho = - \frac{\sigma_i \sigma_e}{\sigma_i + \sigma_e} \nabla V_m,
\]  

(7.8)

The dipoles were calculated at each continuum unit, i.e., per grid point, in the
whole-organ model, and were then vectorially summed to calculate the desired num-
ber of dipoles. In the simplest case, a single dipole could be used to represent a
lumped representation of the whole-organ slow wave activity in line with the tra-
ditional clinical representation of ECG [148]. However, a single dipole may not be
sufficient to accurately represent the overall orientation of the whole-organ model
due to the existence of multiple simultaneous wavefronts in the stomach. Therefore,
a dipole was calculated per geometric element of the stomach model to improve the
resolution of the dipoles. A total of 88 dipoles were calculated per solution step.

A volume conductor torso model (Fig 7.8(d)), consisting of an upper torso and
the stomach model was used to calculate the EGG on the torso surface. A generalised
Laplace equation was used as the governing equation for the passive torso regions,

\[
\nabla \cdot (\sigma_o \nabla \phi_o) = 0,
\]  

(7.9)

where \( \phi_o \) is the EGG on the torso surface, and \( \sigma_o \) is the conductivity of the torso.
Here, the torso was assumed to be a homogeneous volume conductor. The equation
demonstrates an inverse relationship between \( \sigma_o \) and \( \phi_o \). The torso model was solved
using the boundary element method (BEM) [147]. The BEM was more suitable for
the torso model than the FEM because only the potential values on the boundary of
the torso model were of interest. By applying the BEM, the dimensionality of the
problem was reduced by one, which would in theory lead to an improved computa-
tional efficiency [130].

Even though it was theoretically possible to simulate the EGG over the entire
torso model, in reality the EGG was generally sampled from a selected number of
points on the abdomen, typically in areas close to the stomach [95]. However, there
is not a standard for the configuration of electrodes placement in EGG recording.
In order to mimic an experimental setup of EGG recording, the EGG signals were
sampled from 16 points, in a $4 \times 4$ array on the torso model (Fig 7.9(a)). In addition, we also adapted the Einthoven’s triangle approach to derive a single EGG trace from the torso model (Fig 7.9(b)).

![Virtual body surface electrogastrogram (EGG) setup](image)

Figure 7.9: Virtual body surface electrogastrogram (EGG) setup. (a) A $4 \times 4$ array of virtual electrodes were placed on the abdominal region over the stomach. (b) A single EGG trace ($V_{EGG}$) was calculated by choosing three electrodes from the virtual array: the right electrode ($R$), the left electrode ($L$), and the abdominal electrode ($Ab$).

A single EGG trace was calculated using the following equations,

$$V_I = V \cos(\theta), \quad (7.10)$$
$$V_{II} = V \cos(\theta - 60^\circ), \quad (7.11)$$

where $V_I$ is the potential in Lead I, $V_{II}$ is the potential in Lead II, and $V$ is the net trace. The system of equations could be solved simultaneously to obtain the following solutions,

$$\theta = \tan^{-1}\left(\frac{2V_{II}}{V_I} - \frac{1}{\sqrt{3}}\right), \quad (7.12)$$
$$V_{EGG} = \frac{V_I}{\cos(\theta)}, \quad (7.13)$$

The timing of events in the simulated single EGG trace, i.e., $V_{EGG}$ was compared to the activations of gastric slow wave events over electrodes $G_{1-9}$ (Fig7.8(b)) in the stomach model. The simulated results are presented in Section 8.6.
Chapter 8

Modelling Results

This chapter presents the simulation results of the models described in Chapters 6 and 7. At the cellular scale, the results of simulations and validations of the effects of gastric pacing on SMC are presented in Section 8.1, and the coupled ICC-SMC model results in Section 8.2.1. At the tissue scale, the entrainment of slow waves in a 1D model is presented in Section 8.3, the propagation and response of slow waves to gastric pacing in an automata 2D model in Section 8.4.1, and the slow wave propagation in WT and 5-HT$_{2B}$ ICC networks geometries are presented in Section 8.4. At the whole-organ scale, the simulated slow wave propagation in a geometric stomach model is presented in Section 8.5, and their relevance and relationship to the EGG is presented in Section 8.6.

8.1 SMC Stimulation

8.1.1 Effects of Pulse-width

The simulated SMC responses to a range of pulse-widths (10 ms, and 0 to 400 ms in 25 ms increments), at constant amplitude of 250 pA, are as shown in Fig 8.1. For a stimulation amplitude of 250 pA, 65 ms was the minimum pulse-width for a single pulse stimulation protocol necessary to reach the mechanical threshold, which was defined to be -30 mV [141, 41] (Fig 8.1(a)). For pulse durations shorter than 65 ms, a distinct plateau-phase could not be identified, and these were identified as...
inactivating protocols. Fig 8.1(b), for example, demonstrates that there is a lower $V_{\text{peak}}$ response and gradual repolarisation for a short pulse-width (10 ms). For pulse-widths $>65$ ms, the potential of the plateau phase was 27.5 to 30.8 mV above the threshold (-30 mV). The length of the plateau phase that was maintained in the SMC was directly related to the length of the pulse-width, with longer pulses causing a longer plateau phase (Fig 8.1(c)).

Figure 8.1: The effects of stimulation pulse-widths. (a) Simulated plateau potential above the mechanical threshold (a series of stimuli of 250 pA, and widths from 65 ms and 75 to 400 ms in 10 ms increments); (b) Simulated membrane potential response of the SMC model under a series of stimulation protocols (250 pA, at 10, 50 to 300 ms in 50 ms increments), compared with experimental measurements under the same simulation protocols (c). Reproduced from [41].

The simulated results were in good agreement with the validation patch-clamp studies (Fig 8.1). The highest simulated peak potential was 21.59 mV, compared to experimental value of 17.3±7.5 mV. The simulated plateau potential was -0.66 mV, compared to an experimental value of 3.1±3.3 mV. The simulated rate of upstroke was 3.3 mV ms$^{-1}$ compared to the experimental value of 5.6±0.7 mV ms$^{-1}$. The experimental results showed that pulse-widths shorter than 65 ms were unable to sustain a substantial plateau phase, and that a strong rapid upstroke response and plateau was consistently achieved at pulse-widths greater than 50 ms. Longer pulse-
widths again resulted in a longer plateau phase of the SMC response.

8.1.2 Effects of Pulse-amplitude

The simulated SMC responses to a range of pulse-amplitudes, at a constant width of 1000 ms, are as shown in Fig 8.2. With a constant pulse-width (1000 ms), an increasing pulse-amplitude (range: 25 to 250 pA in 25 pA increments) induced a graded response of the membrane potential of the SMC model (Fig 8.2(a)). Stimulation amplitudes less than 75 pA did not elicit a significant upstroke depolarisation response of the membrane potential of the SMC (Fig 8.2(b)). For pulse-amplitudes greater than 75 pA, a supra-mechanical threshold response was achieved. The $V_{peak}$ and plateau potential of the membrane potential showed a graded response to the amplitude of the simulated pulse, with higher amplitudes inducing a higher $V_{peak}$ and plateau.

![Figure 8.2: The effects of stimulation pulse-amplitudes.](image)

(a) Simulated difference between plateau potential and the mechanical threshold (stimuli of 1000 ms pulse-width, amplitude range 0 to 250 pA in 25 pA increments). (b) Simulated membrane potential of the SMC model under a series of stimulation protocols (50 to 200 pA in 25 pA increments), compared with experimental measurements under the same simulation protocols (c). Reproduced from [41].

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The simulated results were generally in agreement with the experimental validation studies (Fig 8.2(c)), with increasing pulse-amplitudes (pulse-width constant at 1000 ms, pulse-amplitude range: 50 to 250 pA) leading to a higher $V_{\text{peak}}$ in the rat SMCs. The simulated potential and plateau potential were compared to the experimental recordings in the Tables (8.1 and 8.2).

<table>
<thead>
<tr>
<th>Stimulation amplitude (mA)</th>
<th>200</th>
<th>150</th>
<th>100</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation (mV)</td>
<td>10.4</td>
<td>-4.4</td>
<td>-20.4</td>
<td>-40.4</td>
</tr>
<tr>
<td>Experiment (mV)</td>
<td>22.1±5.8</td>
<td>-13.8±2.7</td>
<td>-20.0±2.3</td>
<td>-38.8±4.7</td>
</tr>
<tr>
<td>Absolute difference (mV)</td>
<td>11.7</td>
<td>9.4</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Relative difference</td>
<td>-0.53</td>
<td>-0.68</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 8.1: Simulated peak amplitude compared to experimental measurements at different levels of stimulation amplitudes, a single 1000 ms pulse. Reproduced from [41].

<table>
<thead>
<tr>
<th>Stimulation amplitude (mA)</th>
<th>200</th>
<th>150</th>
<th>100</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation (mV)</td>
<td>-9.9</td>
<td>-16.2</td>
<td>-23.2</td>
<td>-38.2</td>
</tr>
<tr>
<td>Experiment (mV)</td>
<td>-19.5±3.6</td>
<td>-27.8±1.7</td>
<td>-32.1±2.9</td>
<td>-43.9±2.6</td>
</tr>
<tr>
<td>Absolute difference (mV)</td>
<td>9.6</td>
<td>11.6</td>
<td>8.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Relative difference</td>
<td>-0.49</td>
<td>-0.42</td>
<td>0.28</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 8.2: Simulated plateau amplitude compared to experimental measurements at different levels of stimulation amplitudes, a single 1000 ms pulse. Reproduced from [41].

A 50 pA stimulus also failed to elicit a substantial rapid upstroke depolarisation in the experimental study, whereas pulse-amplitudes of 75 pA or greater did achieve a rapid upstroke response (Fig 8.2). However, the simulated and experimental data did deviate in terms of the amplitude of the membrane potential (Table 8.1) and plateau potential (Table 8.2). For example, at 200 pA stimulation amplitude, the simulated result was on average 10 mV lower in both the peak amplitude and potential amplitude when compared to experimental data. On the other hand, at 50 pA stimulation amplitude, the simulated result produced a better match to the experimental data (matching peak amplitude and 5 mV lower in the plateau potential). The mismatches between the experimental recording and simulated results highlighted the need for more accurate and species-specific models of SMC conductances and parameter fitting.
from more recordings. Furthermore, the cell is unlikely to contain enough degrees-of-freedom to match the variations in the experimental signals over the entire range of stimulation frequencies.

### 8.1.3 Effects of Pulse-train Frequency

The simulated SMC response to a range of pulse-train-frequencies, at a constant pulse-width of 10 ms and pulse-amplitude of 250 pA, is shown in Fig 8.3. Between 10 and 100 Hz pulse-train-frequencies, the amplitude of the simulated SMC membrane potential above the mechanical threshold increased in relation to the increasing stimulation frequency (Fig 8.3(a)). Stimulation frequencies below 30 Hz could not maintain the plateau phase, and therefore were deemed unsuccessful in activating the SMC model. Simulations demonstrated that although a single pulse of width 10 ms and amplitude of 250 pA had been unable to elicit a normal $V_{peak}$ and sustain a plateau phase (as detailed above), a train of 10 ms wide stimuli at 40 Hz frequency could induce a full depolarisation of the SMC model (Fig 8.3(b)).

The experimental validation studies demonstrated a close accord with the simulation study, particularly at 40 Hz stimulation frequency (Fig 8.3(c) and Table 8.3). The simulated resting membrane potential was -79.6 mV, compared to experimental value of -77.4±3.2 mV. The peak potential was 2.2 mV, compared to experimental value of -0.8±3.2 mV. The simulated plateau potential was -20.7 mV, compared to experimental value of -21.7±2.7 mV. The simulated upstroke was 5.8 mV ms$^{-1}$ compared to experimental value of 4.1±1.2 mV ms$^{-1}$. Additional simulations and experiments were performed at 20 and 80 Hz, and the plateau potentials are listed in Table 8.3.

A series of further theoretical simulation studies were conducted to identify the effects of altering the pulse-width during constant high frequency pulse-train stimulation, as prior animal experiments used stimulation protocols with pulse-widths between 0 to 10 ms, and showed these to be effective protocols in altering gastric motility [190]. Results (Fig 8.4(a)) showed that with an increasing pulse-width, at a constant stimulation frequency of 40 Hz, pulse-widths between 4 to 6 ms approached closely to the mechanical threshold (7.9 mV below and 5.0 mV above the mechanical threshold, respectively).
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Figure 8.3: (a) Simulated plateau potential above the mechanical threshold following a series of stimuli of 10 ms pulse-width, 250 pA, 2000/3000 ms on-off time (0 to 100 Hz in 10 Hz increments). (b) Simulated membrane potential of the parameterised SMC model under a pulse train (40 Hz, 250 pA, 10 ms pulse-width, 2000/3000 ms on/off time), compared with experimental measurements under the same simulation protocols (c). Reproduced from [41].

<table>
<thead>
<tr>
<th>Stimulation frequency (Hz)</th>
<th>20</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation (mV)</td>
<td>-34.9</td>
<td>-20.7</td>
<td>-5.7</td>
</tr>
<tr>
<td>Experiment (mV)</td>
<td>-30.3±1.9</td>
<td>-21.7±2.7</td>
<td>-6.9±2.4</td>
</tr>
<tr>
<td>Absolute difference (mV)</td>
<td>4.6</td>
<td>0.98</td>
<td>1.2</td>
</tr>
<tr>
<td>Relative difference</td>
<td>0.15</td>
<td>-0.05</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

Table 8.3: Simulated plateau amplitude compared to experimental measurements at different levels of stimulation frequencies. Reproduced from [41].
SMC Stimulation

Figure 8.4: (a) Simulation identifying the necessary pulse-width required to achieve supra-mechanical threshold response at 250 $pA$, 40 $Hz$, 2000/3000 $ms$ on/off time. (b) Simulated cell model responses at selective pulse-widths (2 and 4 $ms$). Reproduced from [41].

8.1.4 Simulated Effects of Stimulation on $[Ca^{2+}]_i$

A simulated quantitative comparison between $[Ca^{2+}]_i$ following a single pulse protocol and stimulation via a frequency train is demonstrated in Fig 8.5. The $[Ca^{2+}]_i$ accumulation at the minimum amplitude required to activate the SMC model above the mechanical threshold at 40 $Hz$, 10 $ms$ pulse-width, 2000 $ms$ on-time (i.e., 150 $pA$; Fig 8.5(a)) was compared to the $[Ca^{2+}]_i$ following a single pulse stimulation protocol with equal amplitude, but a 2000 $ms$ pulse-width. The plateau phase potential of the single pulse protocol was 27.4 $mV$ higher than the plateau phase potential of the frequency train protocol. The initial upstroke of the single pulse protocol was 2.70 $mV$ $ms^{-1}$, whereas the initial upstroke of the frequency train was 0.01 $mV$ $ms^{-1}$. The resting $[Ca^{2+}]_i$ for both stimulation protocols was 0.06 $\mu M$. The single pulse protocol achieved an initial simulated $Ca^{2+}$ influx of 0.87 $\mu M$, compared to 0.55 $\mu M$ for the frequency train protocol. During the plateau phase, the $[Ca^{2+}]_i$ was 0.77 $\mu M$ for the single pulse protocol and 0.53 $\mu M$ for the frequency train protocol. The single
pulse protocol requires a power consumption of 300 mA ms, whereas the frequency train protocol requires a 60% lower power consumption of 120 mA ms.

![Image](image.png)

Figure 8.5: (a) Simulated membrane potential response to two different stimulation protocols that achieved supra-mechanical threshold (-30 mV in (a)): (i) a single-pulse stimulus of 2,000 ms pulse-width, 145 pA; and (ii) a frequency train, 10 ms pulse-width, 145 pA, 40 Hz, 2000/3000 ms on/off time. (b) Simulated intracellular calcium concentration to the same two simulation protocols in (a); the single-pulse protocol achieved a higher initial Ca\(^{2+}\) influx, as well as [Ca\(^{2+}\)]\(_i\) accumulation during the plateau phase than the frequency train. Reproduced from [41].

Since the natural duration of gastric slow waves is approximately 10 s, the simulation analysis above suggested that while pulse trains may effectively achieve a supra-mechanical threshold response at substantially lower power than single pulses, the trade-off of using a pulse-train was a reduced accrual of [Ca\(^{2+}\)]\(_i\) (Fig 8.5(b)). The implications of this are not straightforward, however, because the relationship between the area under the slow wave plateau, Ca\(^{2+}\) influx and tension generated is understood to be indirect [141].

### 8.2 Response of ICC to Stimulation

The response of the C&B ICC model to an electrical current was evaluated by comparing the responses of the original and updated C&B ICC models to the same injected current source of amplitude 25 pA and duration 400 ms (Fig 8.6). This in-
jected current source is analogous to the current arising as the result of the potential difference between the $V_m$ of the resting ICC and a neighbouring ICC undergoing depolarisation. The intrinsic frequency of both versions of the C&B ICC model was identical at $3 \text{ cpm}$ following a pulse applied after $15 \text{ s}$ of simulated time. The stimulus resulted in a depolarisation in the original C&B ICC model, as shown in Fig 8.6(b), reaching a peak $V_m$ of $-25 \text{ mV}$, which is similar to the $V_m$ peak of a normal slow wave of $-24 \text{ mV}$. However, the plateau of the stimulus-invoked activity was not maintained in the original C&B ICC model, with the $V_m$ repolarised to $-51 \text{ mV}$ at $5 \text{ s}$ after the onset of the stimulus. The subsequent onsets of slow waves at $20 \text{ s}$ and $40 \text{ s}$ in the original C&B ICC model thereafter occurred at times and rates as dictated by the intrinsic frequency.

In the updated C&B ICC model, the same stimulus successfully invoked and maintained a slow wave event, with the plateau of the invoked slow wave maintained for the nominal duration of approximately $9 \text{ s}$ as per a normal slow wave cycle, after the onset of stimulus (Fig 8.6(c)). The peak $V_m$ of the invoked slow wave in the updated C&B ICC model was also $-25 \text{ mV}$, identical to the peak of $V_m$ simulated by the original C&B ICC model. The onset times of subsequent slow waves generated by the updated C&B ICC model were at $36 \text{ s}$ and $56 \text{ s}$, which were $4 \text{ s}$ in advance of the timings by the intrinsic frequency without the stimulus. This implies that the updated C&B ICC model responded to the stimulus by phase advancing its intrinsic slow wave activity by $4 \text{ s}$ while maintaining the same intrinsic frequency, and therefore demonstrates that the updated C&B ICC model was capable of producing an active response of slow wave generation to an extracellular current source.
Figure 8.6: Comparison of response of the original and updated Corrias and Buist (C&B) ICC models to a stimulus. (a) A single pulse stimulation current of amplitude 25 pA and duration 0.8 s was applied to the original C&B ICC model (Eqn 6.3) and the modified C&B model (Section 6.2.1). (b) The original version of the C&B ICC cell model, in which a partial slow wave was invoked but plateau was not maintained. The timings of slow waves did not change after the stimulus. (c) The updated version of the C&B ICC cell model (Section 6.2.1), in which a phase shift in slow waves was invoked and the subsequent activity was entrained to an earlier onset time without changing the underlying intrinsic frequency.
8.2.1 ICC-SMC Coupling

The $V_m$-threshold SMC model (Eqn 6.9) was subjected to a series of single-pulse stimuli (Fig 8.7(b)). The pulse-width of the stimuli (3.5 s) was chosen to be shorter than the duration (8.1 s) of the simulated SMC slow wave. The SMC model produced slow waves at the designated stimuli times after 1.8 s of a slight depolarisation (∼2 mV) from the resting membrane potential.

The directly coupled ICC-SMC model (Eqn 6.9) produced simultaneous slow wave components (Fig 8.7(c)). The ICC component generated the slow wave activity intrinsically, which then activated the slow wave activity in the SMC component the ICC-SMC model. The simulated slow wave activity in the ICC model was generated autonomously at an intrinsic frequency of 3.0 cpm, and the slow wave activity in the SMC model occurred as a consequence of the ICC slow wave.
8. Modelling Results

Figure 8.7: Smooth muscle cell (SMC) and interstitial cell of Cajal (ICC) coupling model. (a) $V_m$-threshold SMC model, slow waves were invoked by the stimuli (amplitude 0.2 $pA$; pulse-width 3.5 s; at 10, 30 and 50 s) in (b). (c) The combined SMC-ICC model was innervated by the self-excitatory slow waves of the ICC model (red: ICC slow wave activity; blue: SMC slow wave activity).
8.3 One-dimensional Model

In the 1D model (Fig 8.8), slow waves in a simplified network of coupled ICCs were simulated to investigate entrainment for 120 s. Each slow wave was modelled using the updated C&B ICC model.

Slow wave entrainment in a decoupled 1D network was investigated first. When the electrical connections between the nodes were reduced by setting $\sigma_e$ to $0 mS mm^{-1}$, the simulated slow waves demonstrated out-of-phase activity, with each ICC responding according to the intrinsic frequency assigned to its node (Fig 8.8(b)). As the simulated $V_m$ of the nodes in the decoupled 1D model gradually became out-of-phase over time, the apparent velocity of propagation also decreased over time, from approximately $0.9 mm s^{-1}$ during the first cycle of slow wave activity to $0.3 mm s^{-1}$ during the last cycle of slow wave activity. However, the velocities in the decoupled 1D model reflected the timings of the intrinsic events of the ICC cell models in the network rather than the constant velocity as result of an entrained wave. In the functionally-coupled network, simulated $V_m$ using the updated C&B ICC model at the boundary nodes of the 1D network displayed a constant phase-locking of approximately $3.1 s$ (Fig 8.8(c)), demonstrating that the ICC with the highest intrinsic frequency effectively entrained the ICC with lower intrinsic frequencies into phase. The constant phase-lock also allowed the active propagation of slow wave events to occur at a stable velocity over time, in this case, approximately $0.6 mm s^{-1}$, through the functionally-coupled 1D model.
Figure 8.8: (a) The one-dimensional (1D) setup consisted of 11 coupled ICC models, with a linear intrinsic frequency gradient that was assigned to the 1D network by varying the values of $\beta$ in Eqn 6.6. (b) The connectivity between the models was decoupled by setting the conductivity values to 0 $ms$. Simulated membrane potentials ($V_m$) at the two boundary nodes demonstrate slow waves at different intrinsic frequencies. Slow waves at the ICC with the highest intrinsic frequency (solid line, the left-most cell) occurred at 3.0 $cpm$, whereas slow waves at the ICC with the lowest intrinsic frequency (dashed line, the left-most cell) occurred at 2.8 $cpm$. (c) Entrained slow waves of the two boundary ICCs. The ICC with the lowest intrinsic frequency was entrained to the ICC with the highest intrinsic frequency, with a constant phase-lock of 3.1 s.
8.4 Two-dimensional Models

8.4.1 Automata Model

Gastric slow wave recordings and activation maps from the simulated and experimental studies are compared in Fig 8.9. Overall, the simulated slow wave activity was in good agreement with the recordings of normal slow waves, in terms of frequency and propagation velocity. The frequency of recorded normal slow waves was $3.62 \pm 0.07 \text{ cpm}$ and $3.56 \pm 0.03 \text{ cpm}$ (Student’s T-test p-value = 0.52) over ten consecutive waves in the porcine trials. The overall frequency of the simulated slow waves was $3.60 \text{ cpm}$, which overrode the underlying gradient of intrinsic frequencies across the tissue model, demonstrating that successful entrainment to the dominant intrinsic frequency was achieved. The propagation velocity in the longitudinal direction was $5.05 \pm 0.04 \text{ mm s}^{-1}$ and $5.48 \pm 0.50 \text{ mm s}^{-1}$ (p-value = 0.47); the propagation velocity in the circular direction was $8.31 \pm 0.18 \text{ mm s}^{-1}$ and $8.71 \pm 0.17 \text{ mm s}^{-1}$ (p-value = 0.15). The statistical analysis was based on the number of electrodes in a recording field over consecutive cycles of activities. The simulated slow waves propagated at the designated velocity of $4.58 \text{ mm s}^{-1}$ in the antegrade direction and $8.51 \text{ mm s}^{-1}$ in the circular direction. The sample of the selected channels of experimentally-recorded slow waves (Fig 8.9(a)) showed a more gradual upstroke phase than the simulated slow waves (Fig 8.9(b)). Both activation maps of normal porcine slow wave activities (experimental and simulated) demonstrated that slow waves propagated in the antegrade direction, and in the case of the experimental recording, that they originated from the porcine gastric fundus and propagated in the organoaxial direction towards the gastric antrum.

Gastric slow wave recordings and activation maps from the simulated and experimental studies, under gastric pacing at $3.53 \text{ cpm}$ (period 17 s), are shown in Fig 8.10. The pacing frequency of $3.53 \text{ cpm}$ was chosen for the purposes of model validation, because pacing at a similar frequency to the normal slow wave activity allowed a detailed assessment of the interaction between the normal and entrained activities in both the experiment and simulation. Slow wave entrainment was consistently and reliably achieved in the experimental study, and the frequency of the invoked slow wave activity was similar to that of the baseline frequency of $3.6 \text{ cpm}$. The stimulus
effectively produced an artificially induced ectopic pacemaker of slow wave activity in addition to the normal pacemaker in the porcine stomach (Fig 5.2). The slow waves shown in Fig 8.10(a) demonstrate that the entrained slow waves propagated simultaneously in both the antegrade and retrograde directions from the point of stimulus.

Figure 8.9: Comparison between recorded and simulated normal porcine slow waves. (a) Activation map of the normal slow waves. (b) Recorded slow waves from the electrodes in the fourth column of the array (marked by the vertical arrow). (c) Activation map of the simulated normal slow waves. (d) Simulated slow waves from the electrodes in the fourth column of the array (marked by the vertical arrow).
To simulate the effects of the gastric pacing protocol in the tissue model, a virtual stimulus was placed at a location corresponding to the site of the experimental stimulus as shown in Fig 8.10. The intrinsic frequency of the ICC paced by the stimulus was set to the stimulation frequency (3.53 cpm). The frequency of the normal slow waves remained at 3.60 cpm. Both the intrinsic and paced activity began at $t = 0 \text{ s}$. The origin of the secondary pacemaker corresponded to the location of the pacing needles, which was within 11 mm distal to the fundal line in both experimental recording and simulation. Both experiment and simulation achieved an overall entrainment frequency of 3.53 cpm. The simulation demonstrated that the displacement of entrainment in the retrograde direction was 53 mm from the point of stimulation, and 77 mm in the antegrade direction from the point of stimulation (Fig 8.10(b)). The experimental data also demonstrated similar results in terms of entrainment displacements. Over ten consecutive entrained events in the trial demonstrated in Fig 8.10(b), the entrained displacement in the retrograde direction was $55\pm3 \text{ mm}$, and $71\pm1 \text{ mm}$ in the antegrade direction (to the extent of the mapped boundary), both measured relative to the location of pacing needles. It should be noted that the distance entrained in the antegrade direction was underestimated by entrainment mapping, because the slow waves would be expected to continue to propagate beyond the mapped area, until the pylorus [106]. The difficulty in achieving effective entrainment in the retrograde direction has been noted in a previous study, in which the authors believed that the ‘resistance’ was lower for antegrade entrainment than for retrograde entrainment [184]. The automata modeling study suggests an alternative plausible explanation, that the efficacy of entrainment in the retrograde direction could be strongly dependent on the phase interaction between the native and entrained activities in the proximal stomach. Traditionally, gastric pacing has been employed at frequencies somewhat higher than the native activity. This is in part because if stimulation was performed at a similar frequency to the native slow wave activity, while recording with few sparsely-placed electrodes, then it would be very difficult for the investigator to determine whether entrainment was being achieved or not. With HR entrainment mapping, as shown in this study, the precise spatiotemporal effects of gastric pacing on gastric slow waves can be determined at any stimulation frequency.
Figure 8.10: Comparison between recorded and simulated porcine slow waves under pacing. (a) Activation map of the slow waves. The location of pacing electrodes are marked by ‘+’ and ‘-’. The dashed line boundary divides the zone-of-normal (ZON) and the zone-of-entrainment (ZOE) by the pacing protocol. (b) Recorded slow waves from the electrodes in the fourth column of the array (marked by the vertical arrow). (c) Activation map of the simulated slow waves. (d) Simulated slow waves from the electrodes in the fourth column of the array (marked by the vertical arrow).
To further quantify the effects of entrainment by the gastric pacing protocol, the area of the stomach successfully entrained by gastric pacing was termed the ‘zone-of-entrainment’ (ZOE), whereas the area where the baseline slow wave activity continued to occur was termed the ‘zone-of-normal’ (ZON). Over multiple consecutive waves during pacing at 3.53 cpm, the experimental data demonstrated a ZOE of 64±10 cm². The high degree of variability in the ZOE was due to the variable phase interaction between the native and entrained activities. During some events, the majority of the mapped area could be entrained, whereas in others a native event was found to be propagating distally, clashing with the entrained event, and limiting propagation in the retrograde direction, at a variable distance down the porcine stomach.

Figure 8.11: Zone of entrainment (ZOE) is the proportion of total mapped area containing entrained slow waves following the stimulation protocol. Zone of normal (ZON) is the proportion of total mapped area containing normal slow waves. The onset of the stimulus was incrementally delayed relative to onset of the native activity, with the values being the number of seconds of the onset of stimulus behind the native activity. The ZOE of the stimulus and native activity inter-changed depending on the length of the delay. Reproduced from [42].

An additional theoretical study was conducted using the automata model to define
8. Modelling Results

the relationship of the phase interaction between the native and entrained slow wave activities on the ZON and ZOE achieved during pacing. The onset of stimulation was delayed from 0 to 17 s in 1 s increments relative to the onset of the native slow waves, and the results are presented in Fig 8.11. As the delay time between the onsets of stimuli relative to the normal slow wave activity lengthened, the stable ZOE of the stimulus decreased. The highest ZOE was 78%, as shown in Fig 8.11).

8.4.2 Tissue Models With Realistic ICC Network Geometries

Slow wave propagation was simulated in the tissue models containing realistic ICC network geometries in WT and 5-HT2B knockout models. The slow wave in the tissue models was simulated using the updated C&B ICC model. Both tissue models contained an identical gradient of intrinsic frequencies. In the WT model, slow waves were activated from near the top left corner of the simulation domain as shown in Fig 8.12, in accordance with the assignment of the highest-frequency ICCs to this region. The activation timings in this region reached a peak voltage of -25 mV approximately 140 ms after time t = 0 ms. The direction of propagation followed the orientation of the ICC network in the WT model. The subsequent activation of ICCs after the initial 140 ms in the WT model was noticeably slower, taking approximately 280 ms to activate the whole of the WT model.

The simulated slow wave activity in the 5-HT2B knockout model (Fig 8.13) demonstrated a different propagation behaviour compared to the WT model. The slow wave activity still originated from a pacemaker region near the top left corner in the 5-HT2B knockout model, but the subsequent propagation predominately progressed horizontally through the ICC network. It required an additional 40 ms approximately for the entrained slow wave activity to reach the bottom left corner of the 5-HT2B knockout model (260 ms) when compared to the normal model (220 ms). The simulation time for each tissue models was two hours for an activation time of 400 ms using a single processor of an IBM p595 HPC.
Two-dimensional Models

Figure 8.12: Simulated slow propagation in the WT model containing a normal ICC network structure. The blue colour represents the resting membrane potential and the red colour represents depolarised activity. The first image in the top row shows the $V_m$ at time $t=0$ $ms$ and each subsequent image, from left to right, then down to the next row, is 20 $ms$ apart. The propagation velocity was $\sim 1.6$ $mm/s$−1, while the main direction of depolarisation occurred from the top left corner towards to the bottom right corner of the tissue domain. Reproduced from [45].
Figure 8.13: Simulated slow propagation in the 5-HT$_{2B}$ knockout model containing a degraded ICC network structure. The blue colour represents the resting membrane potential and the red colour represents depolarised activity. The first image in the top row shows the $V_m$ at time $t=0\ ms$ and each subsequent image, from left to right, then down to the next row, is $20\ ms$ apart. The main direction of depolarisation occurred from the left to right of the tissue domain. Reproduced from [45].
Upon closer inspection of the activation wave fronts, it can be seen that the WT model demonstrates a relatively uniform propagating slow wave front, due to the relatively uniform distribution of the intact ICC network (Fig 8.14). In the 5-HT$_{2B}$ knockout model, the propagation of slow waves was impeded by the degraded ICC network structure as demonstrated by the longer time taken for the wave front to arrive at the bottom left corner of tissue domain in the 5-HT$_{2B}$ knockout model. The physiological significance of the longer activation time in the 5-HT$_{2B}$ knockout model could be a slower transit time of the intestinal contents, and recent experiment evidence supports this prediction by showing a small trend to prolonged transit time in 5-HT$_{2B}$ knockout mice [169]. It is predicted that there is a degree of surplus capacity in ICC networks, such that ICC loss up to a threshold level may be inconsequential to transit, with impairments only manifesting if ICC loss is greater than a certain threshold [169]. Future modelling studies within the framework established here could focus on predicting the level of ICC loss that will lead to functional consequences for motility and transit in the gut.

The peak $J$ in the WT model was 0.49 $\mu A mm^{-2}$ higher than in the 5-HT$_{2B}$ knockout model, and furthermore, the peak $J$ was reached 15 ms faster in the WT model compared to the 5-HT$_{2B}$ knockout model (Fig 8.14(a)). The average simulated current density in the normal model was 0.98 $\mu A mm^{-2}$, which was 0.04 $\mu A mm^{-2}$ higher than the level achieved in the 5-HT$_{2B}$ knockout model. However, the 5-HT$_{2B}$ knockout sample displayed a more gradual reduction in $J$. The recovery time taken for the $J$ to return to $\frac{2}{3}$ of its peak value was 331 ms in the 5-HT$_{2B}$ knockout model and 257 ms in the WT model. The higher peak current density in the WT model indicates that the initial component of slow wave is important for normal GI functions. Furthermore, the small difference between the peak current densities suggests that the normal GI function is also sensitive to the peak current density value. The longer recovery time in the 5-HT$_{2B}$ knockout model also indicates that the ICC degradation and the subsequent remodelling possibly redistributed the slow wave propagation to maintain some level of activation of the SMC tissue at the cost of slower propagation and less current generation. A previous study in mice stomach has also demonstrated that the amplitude of slow wave was significantly reduced due to the loss of ICC-MY, particularly in the antrum [140]. It is also possible that
breakdown in the contact between ICC and enteric neurons may also play a major role in the pathogenesis of ICC loss in the GI tissue [140].

Figure 8.14: Comparison of current density and calcium concentration in WT and 5-\(HT_{2B}\) knockout models. (a) Current density of normal model (solid line) and 5-\(HT_{2B}\) knockout model (dashed line). The normal model produced a higher peak current density than the 5-\(HT_{2B}\) knockout sample. (b) \([Ca^{2+}]_i\) density of normal and 5-\(HT_{2B}\) knockout models (same notations same as (a)). The normal model achieved consistently higher \([Ca^{2+}]_i\) density compared to the 5-\(HT_{2B}\) knockout model. Reproduced from [45].
The resting levels of simulated $[Ca^{2+}]_i$ density in both tissue models were similar at approximately 0.01 mM mm$^{-2}$ (Fig 8.14). On average, the simulated $[Ca^{2+}]_i$ density was greater in the WT model compared to the 5-HT$_{2B}$ knockout model ($0.67$ mM mm$^{-2}$ vs $0.41$ mM mm$^{-2}$). The level of $[Ca^{2+}]_i$ density over time displayed a regular sigmoidal increase for both models, with the WT model achieving a consistently higher $[Ca^{2+}]_i$ density than the 5-HT$_{2B}$ knockout model. Even though the 5-HT$_{2B}$ knockout model produced a longer recovery time (Fig 8.14(a)), the calcium density in the 5-HT$_{2B}$ knockout model was consistently lower than in the WT model. A recent experimental study has used $Ca^{2+}$-sensitive dye (Fluo-4) to image the spread of $Ca^{2+}$ from the ICC-MY to SMC in WT murine small intestine [71]. The study demonstrated that $Ca^{2+}$ matched the propagation of slow waves, which were recorded using glass pipette electrodes, in the networks of ICC-MY [71]. The study also demonstrated that loss of ICC also changed the motility patterns in the smooth muscle layer significantly [71].

8.5 Whole-organ Model

Simulated gastric slow waves in the whole organ model are illustrated in Fig 8.15. The frequency of simulated slow waves at the organ level was the same as at the cellular level (i.e., 3.0 cpm). It took approximately 60 s (i.e., one complete propagation cycle) for the activity to reach a steady state. The initial 60 s of simulated slow waves was therefore ignored. The origin of the gastric slow wave was consistently situated at the appropriate pacemaker region on the greater curvature of the proximal corpus (e.g., depolarised events (coloured red) visible at $t = 4$ and 24 s). The initial propagation from the pacemaker region formed a wide wavefront in a band by approximately $t = 4$ s, which then propagated in the antegrade direction toward the pylorus. By 24 s, the onset of the second slow wave cycle was visible in the pacemaker region of the virtual stomach model, with the first cycle of activity having reached the mid corpus. By 44 s, the onset of the third slow wave cycle was visible in the pacemaker region, while the active edge of the first cycle of slow wave activity had entered the antral region. A maximum of four simultaneous wavefronts were visible (e.g., at 60 s), while the minimum number of simultaneous wavefronts was three.
The global average of the propagation velocity was $\sim 6.4 \text{ mm s}^{-1}$, with substantial regional variations: ($\sim 7.7 \text{ mm s}^{-1}$ in the pacemaker region; $\sim 2.8 \text{ mm s}^{-1}$ in the corpus; $\sim 5.9 \text{ mm s}^{-1}$ in the antrum). The width of the active edges of the slow waves depolarised region (coloured yellow in Fig 8.15) was approximately 16 mm in the corpus and approximately 37 mm in the distal antrum. The slower local corpus velocity resulted in accrual of multiple narrow wavefront bands, with a wavefront separation displacement of approximately 50 mm. All of these modelled slow wave propagation dynamics were in accordance with experimental data [132].
Figure 8.15: Simulated slow waves in a virtual stomach model based on generic activation times of slow waves from human experiments [132]. A total of 60 s of propagation is shown. The red colour represents depolarised activity and the blue colour represents the resting membrane potentials. Reproduced from [43].
8.6 Virtual Analysis of the EGG

The simulated torso surface potentials, calculated from the organ model are presented in Fig 8.16. The majority of the upper torso was consistently at around 0 mV (Fig 8.16 in green). A distinct region of consistently positive activity occurred in the simulated torso surface potential field across the epigastrium (Fig 8.16 in yellow/red), while the recovery ‘tail’ of the torso surface potential field was located over the left hand side the lateral thorax, beneath the nipple line (Fig 8.16 in blue).

The simulated torso surface potential was also sampled over 16 virtual electrodes in a $4 \times 4$ array (Fig 8.17). The locations of the electrodes are shown in Fig 7.9 and in Fig 8.16. The mean amplitude of the EGG was $0.38 \pm 0.17$ mV. The EGG signals all followed the same periodic occurrence of 3 cpm. However, there was a large variation in the EGG amplitude between the channels, e.g., between channels 1 and 4 in the top row of Fig 8.17. Furthermore, the EGG signals did not appear to have distinctive morphological features that could be used to relate the EGG signals to the slow waves in whole-organ model.
Figure 8.16: Simulated resultant body surface potential field corresponding to the underlying slow wave activity shown in Fig 8.15. The red colour represents positive potentials and the blue colour represents negative potentials in the torso potential field. The contour lines (black) represent 0.1 mV increments. Reproduced from [43].
Figure 8.17: Simulated electrogastrography (EGG) signals over a $4 \times 4$ array of electrodes placed on the torso surface, directly over the stomach, as shown in Fig 7.9.
In order to analyse the relationship between a single trace EGG and gastric slow waves, a whole-organ model with a single cycle of slow waves was simulated. The simulation was without the presence of another simultaneous slow wave activity, and the resultant single trace EGG ($V_{EGG}$) (Fig 7.9b) was calculated (Fig 8.18). Using one a single wave freed analysis of EGG from the ‘interferences’ of other simultaneous slow waves in a normal whole-organ model, and a good initial understanding could be gained from a more straightforward relationship between the $V_{EGG}$ and a single cycle of slow waves. A realistic representation of $V_{EGG}$ could be gained next by allowing simultaneous cycles of slow waves to develop in the whole-organ model (Fig 8.15).
8. Modelling Results

Figure 8.18: Simulated single trace electrogastrography ($V_{EGG}$) signal of the electrodes placed on the torso surface and directly over the stomach, as shown in Fig 7.8 and Fig 7.9. A number of intervals were defined: $T_1$: activation of the pacemaker region; $T_2$: the end of repolarisation of the pacemaker region; $T_3$: corpus activation; $T_4$: full activation of the antrum; $T_5$ repolarisation of the antrum.
A number of characteristics in the simulated $V_{EGG}$ were related to the simulated slow waves in $G_{1-9}$ (Fig 7.8). The virtual electrode in the fundus ($G_1$) showed no active slow wave activity. The resting potential of the simulated $V_{EGG}$ was $-0.72 \text{ mV}$. The initial activation in the pacemaker region ($G_2$) was related to the beginning of a negative deflection in the $V_{EGG}$ signal, and the most negative point of the deflection ($-0.8 \text{ mV}$) in the $V_{EGG}$ signal correlated to the time when the pacemaker region was mostly activated ($T_1: \sim 3.5 \text{ s}$). The subsequent recovery of the $V_{EGG}$ to the baseline was correlated to the end of the repolarisation of slow wave activity in the pacemaker region ($T_2: \sim 8 \text{ s}$). For the next $50.5 \text{ s}$, the $V_{EGG}$ stayed close to the baseline while the slow waves propagated in the corpus ($T_3$). The beginning of a positive deflection in the simulated $V_{EGG}$ could be correlated to the time when slow wave entered $G_7$, i.e., the antrum. The peak of this deflection ($-0.63 \text{ mV}$) in the $V_{EGG}$ was reached approximately $7.5 \text{ s}$ after slow wave had entered the antrum ($T_4$), when the entire antrum was active (slow waves in $G_{7-9}$). The end of the simulated $V_{EGG}$ ($66 \text{ s}$) matched the end of repolarisation of the slow waves in the antrum.

Next, the effects of the transition from a single cycle of slow waves to simultaneous slow waves on $V_{EGG}$ was analysed (Fig 8.19).
Figure 8.19: Simulated single trace electrogastrography ($V_{EGG}$) signal of the electrodes placed on the torso surface and directly over the stomach ($G_{1-9}$), as shown in Fig 7.8 and Fig 7.9. A number of intervals were defined: $T_1$: activation of the pacemaker region; $T_2$: the end of repolarisation of the pacemaker region; $T_3$: corpus activation; $T_4$: full activation of the antrum.
The beginning of a normal whole-organ slow wave activation simulation, \textit{i.e.}, during the first cycle of slow wave activity when there were no other developing and the subsequent period when the first wave was propagating distally during which synchronous slow wave began to develop, was used as the underlying whole-organ activation model. The first cycle initially occurred without another cycle of slow waves, and 20 s into the first cycle, the second cycle had initiated; 40 s into the first cycle, the third cycle initiated (Fig 8.19); by this time the first cycle of slow waves was near the antrum. This is also the time in which the whole-organ model first contained three simultaneous cycles of waves (Fig 8.19). The initial negative deflection in the $V_{EGG}$ was identical to the case of the single cycle of slow wave (Fig 8.18). At the onset of the first simultaneous event (t = 20 s), another negative deflection in the $V_{EGG}$ signal occurred, and again at 40 s and 60 s. The most negative deflections in the $V_{EGG}$ consistently coincided with the full activation of the pacemaker region as was in the case of the single wave analysis (Fig 8.18). The peak in the $V_{EGG}$ prior to 60 s also coincided with the timings of the end of repolarisation in the pacemaker region. A ‘secondary’ peak in $V_{EGG}$ was developed as result of the the increase in $V_{EGG}$ as the antral activation ($T_{4-5}$ in Fig 8.18) was superimposed on the baseline $V_{EGG}$ activity (end of $T_4$ in Fig 8.19).

The final analysis of $V_{EGG}$ was based on a state of whole-organ activation in which three simultaneous cycles of waves were present at all times (Fig 8.20).
Figure 8.20: Simulated single trace electrogastrography (V\textsubscript{EGG}) signal of the electrodes placed on the torso surface and directly over the stomach (G\textsubscript{1-9}), as shown in Fig 7.8 and Fig 7.9. A number of intervals were defined: $T_1$: activation of the pacemaker region; $T_2$: the end of repolarisation of the pacemaker region; $T_3$: corpus activation; $T_4$: full activation of the antrum.
The three simultaneous cycles of slow waves represented the normal state of gastric slow waves [132]. The repeated activities in the single trace EGG corresponded to the dominant frequency component of the gastric slow waves in $G_{1-9}$, as demonstrated in Fig 8.21. Essentially, the results presented in Fig 8.20 are the steady-state of the results presented in Fig 8.19. Based on the results in Fig 8.18 and Fig 8.19, the end of $T_1$ in Fig 7.9 could be correlated to the time of slow wave activation in the whole pacemaker region. Similarly, the end of $T_2$ could be correlated to the end of repolarisation of slow waves in the pacemaker region; $T_3$ could be correlated to the onset of slow waves in the corpus; and the peak of the single trace EGG could be correlated to the time ($T_4$) of slow wave activation in the whole antrum.

In previous explanations of the single trace EGG, it has also sometimes been assumed that the one-to-one ratio of the EGG to slow waves was due to the fact that there is only one slow wave propagating at a time in the stomach [13, 95]. The assumption of the one-to-one ratio of the EGG to slow waves was a result of spatial-aliasing in the sparse serosal recordings [132]. As previously pointed out by Verhagen et al. [176], multiple gastric sources are simultaneously present, and the EGG is a summation of the underlying slow wave activity. The results in Fig 8.20, and based on human HR mapping data, demonstrate that the one-to-one ratio of single trace EGG signal to gastric slow waves can be better attributed to the fact that the oscillates in the single trace EGG occurred in synchrony with the repeating sequence of the underlying multiple gastric slow wave sources. More specifically, the duration of a single trace EGG representing a complete cycle of slow waves in the stomach ($\sim 65$ s) extended beyond the onset of the next activity in the single trace EGG due to the onset of the next cycle of slow waves (compare Fig 8.18 to Fig 8.20). Therefore, a proper analysis of the EGG would more likely be gained by analysing the EGG recording over a much longer duration than the currently used beat-to-beat analysis method, which is useful mainly for detection of the frequency of slow waves [95].
Figure 8.21: Spectrum of the frequency components in: (a) simulated slow wave in $G_8$; and (b) simulated EGG. In both cases, the dominant frequency component is 2.81 cpm, though the amplitude of the dominant frequency component (by taking the absolute of the transformed signal) in the slow wave signal (10.56 mV) is higher than the amplitude of the dominant frequency component in the EGG signal (0.05 mV).
Chapter 9

Conclusions and Future Directions

As Malcolm Gladwell proposed in his book ‘The Blink’ [66], it is the over-saturation of information that sometimes prevents the correct decision being made even after meticulous deliberations. Gladwell used the ‘Goldman rule’ as an example of how a set of simplified diagnostic criteria was able to achieve a better diagnostic efficiency than a set of more complicated diagnostic criteria for patients presented with cardiac related symptoms in a Chicago hospital. It was a surprise because the more complicated diagnostic procedures involved more medical tests and generated more information, which were generally perceived by clinicians for being able to present a more comprehensive view of the patient’s health, and should in theory lead to a more accurate diagnosis. However, in reality the clinicians were often distracted by all the peripheral information those tests generated and opted to ‘play it safe’ by placing the patients in the intensive care ward, putting a significant strain on the hospital’s resources; the other consequence of this conservative approach due to over-saturation of information was that patients who actually needed the intensive care sometimes could not receive adequate care and missed the optimal window of treatment.

Here, I would like to refine Gladwell’s argument. My argument is that while it is true that we are generating more information than ever before, what we need now are tools to organise, extract, and interpret those information in an integrative manner. I believe mathematical modelling is such a tool for an integrated understanding of gastrointestinal (GI) slow wave activity. The biophysical and multiscale models already contain parameters that can be directly related to physiological measurements;
with more powerful computers and efficient numerical solvers, the ability to model slow waves and more GI functions will only increase, and hopefully lead to a tighter integration between experimental data and models that will ultimately be used to explain the pathophysiology of different GI functional abnormalities.

At the conclusion of this thesis, I would like to once again highlight the key findings and discuss their relevance to research, and outline any future directions. The order of the discussions follows the order of the aims listed in Section 2.6.

9.1 Recording Platform

The first aim of this thesis was to devise and validate recording platform to reliably record GI slow waves in both animal and human subjects (Aim 1 in Section 2.6). To this end, a flexible printed-circuit-board (PCB) electrode array was developed to conduct high-resolution (HR) mapping of GI slow wave activity. The flexible PCB arrays contained gold or silver electrode contacts, with copper conducting track material. Performance of the PCBs was found to be adequate compared to a traditional epoxy-embedded electrode platform (silver contacts and wiring) in a porcine animal model (Table 5.1). Detailed slow wave characterisation was essential for accurately describing normal slow wave origin, amplitudes, and velocities in the stomachs of pigs (Fig 5.2) and human subjects (Fig 5.3) [50, 132]. Accurately quantified slow wave parameters were also invaluable for aiding development and validation of mathematical models, particularly in the case of the whole-organ model (Fig 8.15).

Flexible PCBs offered a number of advantages over traditional epoxy-embedded platforms. Once the generation 1 and 2 PCB electrode designs (G1-PCB and G2-PBC) had been finalised, machine fabrication was readily contracted to industrial suppliers, with only minimal further assembly required, i.e., soldering the SCSI plug to the PCB. Production was therefore rapid and economical in comparison to the many hours of specialised labour required to construct the epoxy-embedded platforms [103]. Machine fabrication of the PCB electrode arrays conveys precise knowledge of the spatial distribution of repeatable recordings, whereas the precision of epoxy-embedded electrodes is highly dependent on the skill of the individual undertaking their manufacture. The advantage of highly reproducible recording arrays was
particularly important when multiple electrode platforms were used simultaneously to map a large area. As shown in the simultaneous mapping of both anterior and posterior surfaces of the porcine stomach (Fig 5.4), this could be readily achieved using PCBs by adjacent tessellation via Tegaderm of multiple recording heads while maintaining the same inter-electrode distance. A potential improvement to the current PCB designs would be designing a larger PCB array with up to 128 electrodes in a 16×8 configuration either in a single layer, possibly with protruding electrodes, or adopting a multi-layered PCB design. The substrate material on which the electrodes were printed on could also be changed to a more stretchable material such as silicone [82].

The diameter of the PCB electrodes (0.3 mm) was based on the empirical evidence by Lammers et al [106]. In general, there exists a relationship where the diameter of the electrode should be inversely proportional the speed of the waves, i.e., the slower the wave, the smaller the diameter. Mintchev et al. have demonstrated that both the distance of the bipolar electrodes (if employed), and the diameter of the electrode could influence the morphology of slow wave recording [123]. The contact of the electrode could be checked by calculating the offset potential between the electrode and the ground (CMS), and/or by post-recording signal processing means.

HR mapping provided a highly detailed description of the initiation and propagation of slow wave activity [132, 106]. Further developments in software setup and signal processing were made to enhance the efficiency of data collection and analysis. The PCBs provided the opportunity to obtain the HR mapping data from pigs, from previously unrecorded locations, such as from the anterior and posterior surfaces of the porcine stomach (Fig 5.4). Another important application of the PCBs would be to record slow wave dysrhythmias in pigs and human subjects, just as Lammers et al. had conducted in canine subjects [105]. The clinical implication of being able to accurately describe slow wave dysrhythmias in human is significant, and is critical to an improved understanding of the role of slow waves in gastric diseases. Furthermore, it would also be important to correlate the slow wave recording to the motility of the stomach via a manometric catheter [192], or strain gauges [51], or other imaging modalities, e.g., MRI [36], or ex vivo studies [108].

The invasive nature of HR mapping warrants more consideration of its usage be-
9. Conclusions and Future Directions

beyond a research tool. Research is still ongoing into alternative methods that can either record slow waves with minimal incision, e.g., the laprascopic recording platform (Section 3.1.3) or eliminate incision all together. The current implementation of non-invasive slow wave recording techniques, such as electrograstrography (EGG) and magnetogastrography (MGG) have the potential for greater clinical utility (Section 2.4.2 and 3.1.4). Presently, both EGG and MGG presently lack the sensitivity required to adequately describe slow wave dysrhythmia, and the correlation between serosal slow wave activity and EGG and MGG recordings remains incompletely defined [25]. HR mapping, using the methods described here, could help bridge the gap in understanding between non-invasive recordings and slow waves.

In conclusion, the aim of developing a slow wave recording platform that could be applied in animal and human subjects was met. A validated methodology for recording, processing, and characterisation of GI slow wave via PCB arrays was developed. We anticipated that in the future PCB arrays could be exploited to map steeply curved anatomical areas of interest, such as across the greater curvature of the stomach, which were not easily accessible to epoxy-embedded electrode platforms.

9.2 Cell Models

The second aim of this thesis was to identify appropriate biophysical cell models that could be used to simulate gastric slow waves in both gastric SMCs and ICCs (Aim 2 in Section 2.6). The Corrias and Buist (C&B) SMC and ICC models provided a quantitative description of gastric slow waves [32, 33]. Other biophysical cell models, such as the ICC models by Faville et al. and Youm et al., were also considered [61, 60, 188], but the C&B models were chosen mainly because in addition to their biophysical basis, they could be applied readily to simulate gastric slow waves in tissue and whole-organ models.

The third aim of this thesis was to apply the C&B cell models to characterise response of slow waves to gastric electrical stimulation (GES) and gastric pacing at the cellular level (Aim 3 in Section 2.6). The C&B SMC was modified and applied to simulate the effects of stimulating SMCs (Section 8.1). A joint computational simulation and experimental approach was used to identify a range of minimum-energy
stimulation protocols capable of effectively invoking a supra-mechanical threshold response (-30 mV) from isolated SMCs [41, 141]. Discrepancies were noted with respect to the peak amplitude and plateau potential of the SMC membrane potential ($V_m$) in some instances (Table 8.1), however such discrepancies were partly expected due to the variations in the experimental data; and more importantly, only one set of parameters (Table 6.1) were used to match the simulated values to the recorded values. This is problematic because the particular set of parameters might not be the best set to use for all combinations of protocols tested. This is an on-going issue in many areas of science, e.g., material identification, and one way to improve this is by employing a multi-parameter optimisation method, in which the optimal set of parameters are identified by minimised the error combined through multiple initial protocol testing. In addition, the maximum conductance values were optimised to fit the initial set of $V_m$ measurements, rather than determined individually from experiments. In the future, with the accumulation of more experimental data to inform model refinement, it may be possible to further improve the agreement between the simulation and experimental results.

Relating the activities of individual SMCs to a coordinated activity at higher scales requires consideration of inputs of other systems, such as the myogenic, neuronal (the enteric nerves system) and hormonal factors [97]. An electrical stimulus will potentially impact simultaneously on more than one of these elements apart from SMCs, including the enteric nervous system and the ICCs. For example, gastric pacing may entrain ICC slow wave activity, with important implications for the coordinated activation of gastric SMCs [10]. Therefore, a number of further studies will therefore be necessary to translate the results and modelling methods described in this study to clinical utility. For example, future work will need to confirm that minimum-energy effective stimulation protocols identified through this work are also effective in vivo, and to evaluate whether motility, particularly in a chronic study, can be achieved. It will also be necessary to determine how the identified GES/-pacing protocols modulate the slow waves across and between the gastric smooth muscle layers, and to determine the effects of the stimuli on ICC entrainment and ICC-SMC coupling functions. In the future, more sophisticated multiscale models of this type may be applied to predict the physiological effects of stimulation protocols.
from cell to organ levels, and thereafter employed to identify effective protocols for incorporation into new implantable stimulation devices.

In conclusion, the C&B SMC model was adapted in a simulation-assisted study of the response of rat gastric SMCs to a range of GES and pacing protocols. Defining minimum-energy effective stimulation protocols and the use of pulse-trains, were identified as important potential strategies for improving the power efficiency of implantable long-pulse GES devices that seek to modulate SMC electrophysiology and motility. The gastric pacing/GES simulation study suggested that effective and efficient stimulation protocols could be adapted to include trains (40 Hz) of short pulse width (3-6 ms), which are longer than the pulse-widths (<0.5 ms) administrated by commercially available GES pacemakers on the market. Customised and validated computational simulations were shown to be a worthwhile tool in improving the efficiency of GES research.

9.3 

9.3.1 Automata Model

The automata model was used to simulate normal slow wave propagation (Fig 8.9), and the effects of a typical pacing protocol (Fig 8.10). Simulated normal slow waves were successfully shown to originate from the location of pacing needles and the results showed propagation in the antegrade and retrograde directions at the designated velocities. The automata model was a preliminary step in understanding the effects of slow wave propagation and interactions between two entrained waves.

In the automata model, the location of the origin of entrained slow waves, the number of origins (normal and ectopic), and the frequency of each entrained slow wave could be readily adjusted. The zones of entrainment (ZOE) achieved by each
individual channel could therefore be predicted under different pacing frequencies (Fig 8.11), presenting a useful initial tool for the computational screening of effective pacing protocols at the organ scale. In particular, multichannel gastric pacing has been proposed as means of reducing the energy demand required of the effective pacing protocols [113]. However, protocol development for pacing is a complex task, because a vast range of possible parameter combinations (principally pulse-width, amplitude, frequency and on/off times) must be considered and evaluated at each channel. To date, trial-and-error has been the dominant strategy for evaluating the efficacy of the various pacing protocols, requiring laborious animal testing [57]. With further refinement, the automata model could be used to investigate the interactions of the timings of the stimuli with respect to the entrained slow waves in multichannel pacing studies.

Future research should also address two important limitations of the automata model, in order to allow a more sophisticated platform for pacing simulations with improved predictive capabilities for all pacing parameters. First, the ICC algorithm was deterministic, and a biophysical ICC model, e.g., C&B ICC model, should ideally be incorporated. Second, the 2D regular grid of ICCs and SMCs was relatively simplistic, and incorporating a multi-layered structure based on a realistic gastric micro-structure would provide an improved basis for predictive simulations, as has been achieved in previous cardiac work [172]. Additional sub-classes of ICCs, such as ICC-IM, ICC-MY, and ICC-DMP would need to be incorporated to create a more realistic model of gastric slow wave propagation.

Ultimately, the clinical translation of gastric pacing and/or GES for dysmotility syndromes will require demonstrated improvements in motility and symptoms. To date, only modest improvements in clinical symptom scores have been demonstrated following pacing in gastroparetic patients [120], partly because motility also involves a complex interaction of neural, hormonal, and paracrine factors, besides gastric slow waves [25]. In the future, computational simulations and HR entrainment mapping may assist in addressing the challenge of improving motility via pacing. For example, the SMC stimulation study (Section 6.1.1); HR mapping of entrainment slow wave activity has also been used to study the effects of pacing on slow waves in the porcine stomach [134].
Another possible use of the automata model is to incorporate it into an electromechanical model, in which the slow wave activation is simulated using the automata model first and related to a set of constitutive laws and mechanical governing equations. In such a modelling framework, additional inputs from the neural and endocrine systems may also be required.

In conclusion, the automata model offered an initial platform to simulate propagation of slow waves and test the effects of competing entrained slow waves. The automata model also demonstrated good initial predictive value for pacing simulations, and with improvements, we anticipated it would prove a useful platform for clinically relevant simulations, including evaluating and optimising protocols for multichannel gastric pacing.

9.3.2 Biophysical ICC Network Model

The tissue-specific models presented in Section 7.5.2 enabled correlation of the structure of ICC networks with physiological functions in GI tissues, and this was utilised to quantify some of the physiological differences in entrainment outcomes between the WT and 5-HT$_{2B}$ knockout models (Fig 8.12 and 8.13). The simulation demonstrated quantifiable differences with regard to the current (J) and [Ca$^{2+}$]$_i$ density (Fig 8.14), which could not have been demonstrated under previous modelling frameworks that employed simplistic phenomenological or automata representations for ICC networks [42].

Two assumptions employed in the biophysical tissue model warranted further comment. First, the simulations were conducted on ICC network geometries that were compressed into 2D images. The thinness of the ICC-MY layer was principally used to justify the 2D modelling approach. However, human ICC and smooth muscle layers demonstrate substantially different morphologies than murine layers and therefore future modelling studies employing human-derived geometries may need to reconsider the validity of this assumption [87]. Second, the C&B ICC model was formulated to reproduce the characteristics of gastric slow waves, whereas the tissue geometries in this study were sourced from murine jejunum. The choice of adopting a gastric ICC model was justified because only the activation phase of one slow wave cycle was simulated in the tissue blocks, meaning that differences in gastric and
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intestinal plateau durations and frequencies were inconsequential in this case. For future studies, organ-specific slow wave models may be used to simulate the recovery phase of slow waves in the stomach and intestines.

One important limitation of this study was the lack of information regarding the orientation of the ICC-MY networks in relation to the muscle fibre orientation in the intestine. While in the wild-type the ICC-MY structure appeared to be relative uniform, the $5-HT_{2B}$ knockout model appeared to loss ICC predominantly in one direction (Fig 7.7). This preferential loss of ICC may have significant impact on the coordination of motility in a specific direction of organ, but first the orientation of the ICC network in relation to the organ must be known. Another potential limitation is the assumption of using a 2D model to represent the thin ICC-MY network. This was shown to be adequate in the intestine model, but the ICC-MY in the human intestine has been shown to be much thicker [107]. Future studies may require more sophisticated 3D models of ICC networks to be implemented.

The finding of the relationship between structural loss of ICCs and slow waves were of relevance to clinical conditions such as diabetic gastroparesis, in which ICC network depletion and degradation is a central pathogenic feature [59, 140]. ICC loss in diabetic gastroparesis is a consequence of factors that promote ICC death, such as via inhibition of the protective enzyme heme oxygenase-1, as well as factors that reduce ICC survival or regeneration, such as via inhibiting the ICC-promoting hormones insulin and IGF-1 [140]. To investigate how the physiological consequences of ICC network degradation quantified by the tissue model translate to functional outcomes in GI smooth muscles behaviour, it would be useful in the future to couple anatomical and biophysical representations of the SMC models into the ICC tissue models. For the tissue model, the non-ICC tissue was assumed to possess no active voltage response to the simulated slow waves, whereas in reality this space will be partly occupied by smooth muscle cells. The threshold activation C&B SMC model (Eqn 6.9) could be also applied within the tissue models in the future. Furthermore, the same simulation technique is also applicable to tissue models that represent larger physical dimensions.

While both diabetic gastroparesis and the $5-HT_{2B}$ knockout phenotypes have been characterised by a widespread general depletion of ICC populations, the ICC loss in
diabetes has been recognised as having a more patchy distribution [140]. Slow wave dysrhythmias have been commonly recognised to occur in gastroparesis patients [21], and a recent study has demonstrated that gastric dysrhythmia can feature complex focal events and re-entrant behaviours that are similar to the mechanisms underlying atrial fibrillation [105, 147]. Detailed tissue models have been effectively applied in cardiology to define the role of complex tissue architectures in the generation and maintenance of fibrillation-type behaviours [191], and the tissue models provide a platform for similar studies to investigate the structural basis of slow wave dysrhythmia. For example, future work may investigate whether uncoordinated propagation circuits arise within the heterogeneously-depleted ICC networks of patients with diabetic gastroparesis, and whether re-entrant slow wave propagation behaviours could arise as a result. Another point worth noting is the accuracy of the conductivity values used to calculate the current density in Eqn 7.7, where the bulk conductivity values were used. A more accurate current density could be calculated by using conductivity values that are more specific to the cell membrane properties.

Existing GI mechanical models have typically modelled the movements of the GI luminal contents by imposing the mechanical deformation of the GI wall from imaging evidence [142]. A next step is to link slow wave activity to motility, offering the opportunity to translate the established multi-scale continuum modelling techniques to the GI field - in order to red obtain a better understanding of the interplay of slow wave and GI motility across multiple spatiotemporal scales. However, as outlined in Section 2.2, the unique and complex physiology of the GI tract also necessitates novel adaptations and approaches. In a recent study, a preliminary model of GI electromechanical coupling has been proposed [47]. The study presents a proof-of-concept framework and perspectives for cellular electromechanical coupling of ICC-SMC-contraction, and electromechanical activity in a 2D model. However, significant limitations still exist: (i) the first limitation is the absence of an mechanoelectrical feedback mechanism at the cellular level; (ii) the second limitation is the appropriate and specific constitutive law for GI smooth muscles; and (iii) the third limitation is the incomplete knowledge of the co-regulators of GI motility, particularly those concerning the effects of hormones, ENS, and the effects of neuromuscular-affecting agents on motility [47].

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Future GI electromechanical models should also consider the $Ca^{2+}$ modulation mechanism (through myosin light chain kinase (MLCK) activation). It is understood that the MLCK activation occurs when the $[Ca^{2+}]_i$ exceeds a certain threshold concentration [141]; once $[Ca^{2+}]_i$ is triggered in the SMC, many $Ca^{2+}$ sensitisation mechanisms have been identified to contribute to contraction and also relaxation [154, 16]. Another anatomical study has demonstrated that the ICCs and the neighbouring SMCs form interlocking invginations and protrusions in the cell membranes, which may act to enhance the mechanosensitivity in the GI tissue [170].

Calcium induced calcium release (CICR) may also play a role in the mechanosen-sitivity of the ICC membrane. A recent study has demonstrated that amplitude of slow wave is increased when the intestinal tissue undergoes stretch [179], and this response could be eliminated by ryanodine $Ca^{2+}$ current, which is mediated by CICR. Together with the dynamic interlocking junctions between ICCs and SMCs during contraction and $Ca^{2+}$ sensitisation, these mechanisms form a mechanoelectrical feedback pathway in both ICCs and SMCs. The combined modelling framework can be used to test the effects of pharmaceutical agents, e.g., anaesthesia, on slow wave activation and motility.

In conclusion, the tissue-specific model has presented an integrated biophysical and multiscale modelling framework for simulating slow wave entrainment within realistic tissue structures. The framework was successfully applied to quantify differences in the peak current density and density of $[Ca^{2+}]_i$ between a normal and a degraded ICC network geometry, which could help to explain functional impairments that arise when ICC populations are depleted. The framework developed here can now be applied to better understand the pathological consequences of ICC network defects in GI motility disorders such as diabetic gastroparesis.

### 9.4 3D Models

The fifth aim of this thesis was to incorporate the experimental recordings to simu-late slow wave activity in a whole-organ model (Aim 5 in Section 2.6). The whole-organ model presented an improved multiscale model of human gastric slow wave activity, integrating biophysical GI cell models and a large volume of recent ex-
9. Conclusions and Future Directions

Experimental evidence, including the results from human gastric HR mapping studies (Section 5.3) [132]. The final aim of thesis was to use quantify the relationship between EGG and gastric slow waves (Aim 6 in Section 2.6). The EGG was simulated using established forward simulation techniques [147], and the whole-organ model was applied to simulate virtual EGG over an anatomical torso model, to compare slow waves and EGG simultaneously.

9.4.1 Whole-organ Model

The whole-organ model presented a realistic slow wave activation pattern in an anatomically realistic model of the human stomach (Fig 8.15). The whole-organ model adopted a uniform distribution of ICC-SMC at each continuum unit, whereas in reality several different types of ICC network arrangements interact with SMC in different ways throughout the GI tract [155]. While the whole-organ presented in this thesis model was sufficient for forward-modelling applications, tissue-specific models incorporating detailed and information of ICC network geometries should be preferred when representing and evaluating slow wave propagation at finer biological scales [45]. A recent ‘tridomain’ framework for multiple cell types proposed by Buist et al. would be a potential platform for incorporating both ICC and SMC slow waves simultaneously in a whole organ and/or tissue model [14].

There was also likely to be some error in the anatomical relationships assigned in establishing this model. The gastric CT images were taken prior to surgery, when the stomach was distended by contrast agents, whereas the HR mapping data was obtained during surgery and after fasting. The HR mapping data was obtained based on best-estimates relative to the gastric anatomical landmarks and was matched manually to the virtual stomach model [132]. The HR mapping data was also prescribed using several patches of recordings from the anterior serosa, with extrapolation of data to the posterior serosa based on assumptions from porcine HR mapping data (Fig 5.4) [50]. Despite these limitations, the approach employed in the whole-organ model did provide a significantly improved foundation for EGG interpretation over previous mathematical models. Nevertheless, these sources of error might be further reduced in the future by improved anatomical registration of the HR mapping arrays (via concurrent mapping and imaging), and by simultaneously mapping a larger
serosal surface area.

In the future, the whole-organ model may be improved by incorporating experimental evidence regarding the effects of carbon monoxide, which is known to be responsible for regulating the gastric resting membrane potential gradient in SMCs and possibly ICCs [58], as has recently been applied in another modelling study [14]. An accurate description of the resting membrane potentials would be particularly important if the $V_m$ of ICCs were also taken into account. A more accurate description of the variations in the upstroke of slow wave membrane potentials ($\frac{\partial V_m}{\partial t}$), e.g., $\sim 10$ mV s$^{-1}$ in the corpus and $\sim 20$ mV s$^{-1}$ in the antrum [78], should also be modelled because of their potential contributions to the dipole calculation (Eqn 7.8).

Studies have also demonstrated a spatial variation in ICC-MY and ICC-IM in the stomach [75, 78], which may contribute to the heterogeneity in the slow waves in different regions of the stomach.

In conclusion, the whole-organ model effectively integrated biophysical cell models and a large volume of recent experimental evidence, including the results from human gastric HR mapping studies. The model also provided the means to simulate the resultant EGG over an anatomical torso model, to establish the correlation of the slow wave dynamics in the whole-organ model with the resultant EGG on the torso surface.

### 9.4.2 EGG Analysis

The presence of multiple slow waves in the whole-organ model is likely to have significant implications on the interpretations of far-field recording techniques as such EGG and MGG. It may mean that the far-field recordings cannot be directly related back to the separate single slow wave events (in an inverse problem), and that the recording corresponds to a summation of all these events (in a forward problem) [26].

The analysis of the simulated EGG in relation to the gastric slow waves presented an improved understanding of the composition of EGG signals (Section 8.6). A single EGG trace ($V_{EGG}$) was calculated by adapting Einthoven’s Triangle over the torso region (Fig 7.9). Previously, it has sometimes been simply assumed that the distal antral slow waves dominate the EGG signal, because of the higher slow wave amplitude and velocity seen on serosal recordings from the distal stomach, and because
of the close distance between the abdominal wall and the gastric antrum [163, 187].

The simulated V_{EGG} of a single cycle of slow wave (Fig 8.18) suggested that a far-field representing a single cycle of slow waves was comprised of two components, a negative deflection that correlated to slow wave activations in the pacemaker region, and a positive deflection that correlated to slow wave activations in the antrum. The amplitudes of the two deflections were similar. With the occurrence of simultaneous cycles of gastric slow waves, the simulated V_{EGG} showed the more typical 3 cpm oscillations (Fig 8.20). The beginning of each new cycle in the simulated V_{EGG} correlated to the onset of slow waves in the pacemaker region, and the peak of the simulated EGG correlated to activation of the antrum from the cycle two before the present cycle (Fig 8.19). This observation suggested that a single cycle of slow waves should be analysed over a much longer duration of EGG recording, i.e., at least up to 60 s, than the typical beat-to-beat analysis [95].

The amplitude of the simulate EGG is inversely related to the conductance of the torso, which was assumed to be homogeneous. A potential improvement would be to incorporate more structural details, e.g., muscle and fat layers, into the torso model, and study their effects on the amplitude and morphology of EGG. The combined effects of the slow wave activation sequences, torso material anisotropy, and stomach orientation will need to be experimentally validated in animal and/or human subjects by recording EGG concurrently with HR mapping.

The results of the EGG study suggest that the sources of the EGG are significantly more complex, partly because of the following three observations (Fig 8.15): (i) besides the antral slow waves, the pacemaker region also contains high-amplitude events; (ii) the corpus contains the most number of simultaneous wavefronts; (iii) a physiological resting membrane potential gradient that modulates regional activities may cause a further distortion of the dipole directions. In combination, these three factors contributed to a ‘distortion’ in the equivalent dipole calculated by Eqn 7.9, such that the contribution of the antral slow waves to the EGG was moderated by the combined effects of the summated slow wave activity occurring in the mid and proximal stomach regions. The ‘view’ by the V_{EGG} in Fig 8.20 of gastric slow wave propagation thus reflected a combined contribution from all of the electrically active regions in the stomach.
The torso model now provides a valuable virtual platform and hypothesis testing tool to predict the effects of various types of gastric dysrhythmias on the EGG, which offers means to define the electrophysiological basis of the abnormal EGG. While the simulated normal EGG could be interpreted as a superposition of three overlapping waves (Fig 8.20), a recent work which employed HR mapping has defined the spatiotemporal dynamics of gastric dysrhythmias in canine subjects, finding complex focal events and fibrillation-type behaviours [105]. Before simulations of dysrhythmic EGG are performed using this model, it would therefore be highly desirable to first develop a clearer understanding of dysrhythmic slow wave activity in the human stomach, via human HR mapping in disease states such as gastroparesis. The contributions of other signal sources, e.g., intestine, colon, and the heart may also be combined in future studies to simulate a more realistic ensemble of signals.

In conclusion, the virtual EGG analysis offered new insights into the electrophysiological basis of the EGG, and it should be noted that major technical hurdles also remain before the EGG would likely become a widely used valuable diagnostic adjunct. Most significantly, cutaneous recordings of gastric slow waves are of very low amplitude; movement artefacts and noise (such as the ECG) routinely make visual the EGG analysis unreliable, and can also mislead the interpretation of automated analyses [176]. Furthermore, orientation of the stomach and abdominal thickness are likely to influence the interpretation of EGG as well, as previously demonstrated [125]. Until more sophisticated recording and analysis techniques overcome these technical challenges, EGG interpretation will remain challenging. Nevertheless, the significantly improved understanding of the electrophysiological basis of the EGG, offered in the torso model, does potentially eliminate one barrier limiting the improved clinical application of the EGG.

9.5 Concluding Remarks

In this thesis the development of the flexible PBC electrodes, and its use for HR mapping studies in pigs and human patients were discussed. Current work is ongoing into the analyses of dysrhythmic slow waves in patients with gastroparesis at the University of Mississippi Medical Center, Jackson, MS, USA. The prospect of
relating dysrhythmic slow waves to functional disorders will be essential for a clinical translation of the slow wave activity.

The applications of mathematical models to simulate gastric slow waves were also discussed. Attention was paid to the modifications of cell models because of the fundamental information they conveyed in the continuum modelling framework. The incorporation of a modified ICC model in anatomical ICC networks was used to quantitatively demonstrate the effects of ICC degradation on tissue physiology. The whole-organ model was used to integrate HR mapping data and slow wave propagation in an anatomically realistic human stomach model. A direct comparison was made between the activation timings of slow waves in the whole-organ model and the resultant EGG trace to identify a number of characteristics in the EGG trace. The models now await incorporation and integration of the dysrhythmic slow wave data. Further work in electromechanical coupling between slow waves and GI motility should also be considered. Our understanding of GI electrophysiology will be improved with the continuing development of experimental and modelling techniques; and the clinical utility of these modelling techniques is looking promising in the near future.
Appendices
Appendix A

Awards and Publications

A.1 Scholarship and Awards

1. The University of Auckland Doctoral Scholarship 2008-2011

2. Runner-up, Three Minute Thesis Competition, The University of Auckland, New Zealand, 2011

3. The John Carman prize for the best oral presentation at Medical Sciences Congress 2010, Queenstown, New Zealand

4. Selected as the University of Auckland delegate to the sixth International Student Forum, Beijing, China, 2010

5. SPARK 100K Challenge, third place, 2010

6. SPARK 100K Challenge Qualifier Challenge, Team leader of ‘The G.I. Joes’, 2010

7. SPARK Ideas Challenge, i-Volve Biotechnology winner, Team leader of ‘The G.I. Joes’, 2010

8. Top student paper award, Engineering Medicine and Biology Conference, Minneapolis, MN, USA, 2009

10. Abstract of Distinction, Digestive Disease Week, San Diego, CA, USA, 2008

11. Sir Logan Campbell Travel Grant, 2008

A.2 Patent


A.3 Chapters


A.4 Peer-reviewed Journal Articles


A.5 Peer-reviewed Journal Letters


A.6 Conference Proceedings


A.7 Conference Abstracts


Appendix B

Scripts

- Section B.1: The Matlab file for plotting activation maps and calculating velocity fields (.m file).

- Section B.2: Modified SMC model, with a threshold-term that enables the SMC model to respond to a stimulus term, i.e. $I_{ICC}$ (.m file).

- Section B.3: Modified and combined C&B SMC and interstitial cell of Cajal (ICC) models. The ICC component of the combined ICC-SMC model included an IP$_3$-dependent mechanism for simulation of entrainment (.m file).

- Section B.4: Automata model implemented in a 2D finite-difference grid (.m file).

- Section B.5: Simulation of slow wave entrainment in a linear 1D model (.com file).

- Section B.6: Simulation of slow wave entrainment in a linear 2D model using realistic ICC images (.com file).

- Section B.7: Simulation of gastric slow wave activation in a cubic Hermite 3D model of the human stomach (.com file).

- Section B.8: Simulation of EGG in a cubic Hermite 3D model of the human torso (.com file).
B. Scripts

B.1 Activation Maps and Velocity Field

% Peng Du
% 14 July 2009
% Auckland Bioengineering Institute, New Zealand
%
% This is a Matlab script that reads in marked slow wave data and display
% them in a combined set of graphs.
%
% v1. (14 July, 09): removed the amplitude plotting function, added fitting
% error output
% v2. (15 December, 09): proper implement of the of higher order LS fitting method
%
% added graph plotting functions; rearranged folders
% v2.1 (16 December, 10): better interface with GEPS output (activation
% times and amplitudes

clear all;
close all;

% Add working directories
addpath('Configuration') % configuration
addpath('Data/Thesis') % processed data
addpath('Fitting') % fitting files
addpath('Plotting') % plot functions
addpath('Support') % support functions

% Read in file names
DIR_FILE = 'human7exp2-Deci_20140';

DIR_FILE_CONFIG = [DIR_FILE,'_ElecConf.txt'];
DIR_FILE_TIME = [DIR_FILE,'_00X.txt'];
DIR_FILE_TRACE = [DIR_FILE,'electrogram.txt'];

Fid(1) = fopen(DIR_FILE_CONFIG);
Fid(2) = fopen(DIR_FILE_TIME);
isFile = Fid(1)*Fid(2);

%Fs = 1000;
Fs = 1;  % Hz (samples/second), from SmoothMap305 onwards
% Checking for files
if isFile < 0
    disp('One or more files not found')
    return;
else
    % Read configuration
    [nx ny inter_elec config] = config_read(DIR_FILE_CONFIG);
% Modify configuration if required
nx ny config = config_mod(config, 1:nx, 1:ny);

% Read times
[elec time amp] = marked_time_read_GEMS(DIR_FILE_TIME, Fs, 1);

% Allocate time and electrode coordinates
[TIME AMP] = allocate_selected_time_GEMS(elec, config, time, nx, ny, inter_elec, amp);

ELECTRODE.nx = nx;
ELECTRODE.ny = ny;
ELECTRODE.inter = inter_elec;
ELECTRODE.config = config;

% Allocate times as configuration
NX = nx+1;
NY = ny+1;
[TIME_nonINTERP, TIME_INTERP, AMP_nonINTERP, AMP_INTERP, MIN, MAX, XI, YI] = allocate_time_GEMS(ELECTRODE, TIME, AMP, NX, NY, 'linear');

% TIME_nonINTERP = TIME_nonINTERP + MIN;
% TIME_INTERP = TIME_INTERP + MIN;

% Calculate velocity field
GRAD_FLAG = 0;
{dF vel_mean vel_std err TIME_polyINTERP} = velocity_field(ELECTRODE, XI, YI, TIME_INTERP, TIME_nonINTERP, 3, GRAD_FLAG);

TIME_GridINTERP = TIME_INTERP;
TIME.PolyINTERP = TIME_polyINTERP;
TIME.nonINTERP = TIME_nonINTERP;

% Plot activation maps, velocity fields and trace
plot_dt = 2;
plot_all_GEMS(XI, YI, nx, ny, inter_elec, TIME_INTERP, AMP_INTERP, plot_dt, MAX, dF, [10 5]);
plot_trace_GEMS(DIR_FILE_TRACE, [6 3], [0 70]);

disp(sprintf('rms error of the fitted activation field is %g s',err));
disp(sprintf('mean velocity is %g mm/s', vel_mean));
disp(sprintf('std of velocity is %g mm/s', vel_std));
end

% Print
set(gcf, 'PaperPositionMode', 'auto')
output_dir = [pwd, '\output', DIR_FILE];
saveas(gcf, output_dir, 'epsc');
B. Scripts

B.2 Modified SMC Model

% CellML model: SMC_model
% Conversion from CellML 1.0 to MATLAB (init) was done using COR (0.9.31.1279)

function dY = SMC_v4(time, Y)

% Initial conditions

% Y = [0.0, 0.00008, -68.0, 0.02, 0.0, 0.99, 0.95, 1.0, 0.05787, 0.0, 0.005, ←
% 0.00414, 0.72, 0.0, 0.82];

% YNames = {'local_t (time_units) (in I_stim)', 'Ca_i (millimolar) (in SM_Membrane)',
% 'VmSM (voltage_units) (in SM_Membrane)', 'd_LVA_SM (dimensionless) (in d_LVA_SM)',
% 'd_Ltype_SM (dimensionless) (in d_Ltype_SM)', 'f_LVA_SM (dimensionless) (in f_LVA_SM)',
% 'f_Ltype_SM (dimensionless) (in f_Ltype_SM)', 'f_CA_Ltype_SM (dimensionless) (in f_CA_Ltype_SM)',
% 'h_Na_SM (dimensionless) (in h_Na_SM)', 'm_NSCC_SM (dimensionless) (in m_NSCC_SM)',
% 'm_Na_SM (dimensionless) (in m_Na_SM)', 'xa1_SM (dimensionless) (in xa1_SM)',
% 'xa2_SM (dimensionless) (in xa2_SM)', 'xr1_SM (dimensionless) (in xr1_SM)',
% 'xr2_SM (dimensionless) (in xr2_SM)'};

% YUnits = {'time_units', 'millimolar', 'voltage_units', 'dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', 'dimensionless'};

% YComponents = {'I_stim', 'SM_Membrane', 'SM_Membrane', 'd_LVA_SM', 'd_Ltype_SM',
% 'f_LVA_SM', 'f_Ltype_SM', 'f_CA_Ltype_SM', 'h_Na_SM', 'm_NSCC_SM', 'm_Na_SM',
% 'xa1_SM', 'xa2_SM', 'xr1_SM', 'xr2_SM'};

% State variables

% 1: local_t (time_units) (in I_stim)
% 2: Ca_i (millimolar) (in SM_Membrane)
% 3: VmSM (voltage_units) (in SM_Membrane)
% 4: d_LVA_SM (dimensionless) (in d_LVA_SM)
% 5: d_Ltype_SM (dimensionless) (in d_Ltype_SM)
% 6: f_LVA_SM (dimensionless) (in f_LVA_SM)
% 7: f_Ltype_SM (dimensionless) (in f_Ltype_SM)
% 8: f_CA_Ltype_SM (dimensionless) (in f_CA_Ltype_SM)
% 9: h_Na_SM (dimensionless) (in h_Na_SM)
% 10: m_NSCC_SM (dimensionless) (in m_NSCC_SM)
% 11: m_Na_SM (dimensionless) (in m_Na_SM)
% 12: xa1_SM (dimensionless) (in xa1_SM)
% 13: xa2_SM (dimensionless) (in xa2_SM)
% 14: xr1_SM (dimensionless) (in xr1_SM)
% 15: xr2_SM (dimensionless) (in xr2_SM)

% Constants

Ca_o = 2.5; % millimolar (in Environment)
F = 96486.0; % F_units (in Environment)
\[ K_o = 5.9; \quad \text{\% millimolar (in Environment)} \]
\[ \text{RToF} = 26.7133; \quad \text{\% voltage_units (in Environment)} \]
\[ T_{\text{correction, Ca}} = 2.6235; \quad \text{\% dimensionless (in Environment)} \]
\[ T_{\text{correction, K}} = 1.4986; \quad \text{\% dimensionless (in Environment)} \]
\[ T_{\text{correction, Na}} = 3.2056; \quad \text{\% dimensionless (in Environment)} \]
\[ E_{K_1} = -88.0; \quad \text{\% voltage_units (in E_{BK, SM})} \]
\[ G_{\text{max, BK}} = 60.0; \quad \text{\% conductance_units (in I_{BK, SM})} \]
\[ G_{\text{max, LVA}} = 0.018; \quad \text{\% conductance_units (in I_{LVA, SM})} \]
\[ G_{\text{max, Ltype}} = 65.0; \quad \text{\% conductance_units (in I_{Ltype, SM})} \]
\[ E_{\text{NSCC}} = -28.0; \quad \text{\% voltage_units (in I_{NSCC, SM})} \]
\[ G_{\text{max, NSCC_SM}} = 50.0; \quad \text{\% conductance_units (in I_{NSCC, SM})} \]
\[ E_{Na} = 69.0; \quad \text{\% voltage_units (in I_{Na, SM})} \]
\[ G_{\text{max, Na_SM}} = 3.0; \quad \text{\% conductance_units (in I_{Na, SM})} \]
\[ E_{K_2} = -88.0; \quad \text{\% voltage_units (in E_{BK, SM})} \]
\[ G_{\text{max, bk}} = 0.0144; \quad \text{\% conductance_units (in I_{bk, SM})} \]
\[ G_{\text{max, ka_SM}} = 9.0; \quad \text{\% conductance_units (in I_{ka, SM})} \]
\[ E_{K_3} = -88.0; \quad \text{\% voltage_units (in E_{BK, SM})} \]
\[ G_{\text{max, kr_SM}} = 35.0; \quad \text{\% conductance_units (in I_{kr, SM})} \]
\[ \text{G_{couple}} = 1.3; \quad \text{\% conductance_units (in I_{stim})} \]
\[ V_{\text{decay}} = 37.25; \quad \text{\% voltage_units (in I_{stim})} \]
\[ V_{\text{threshold}} = -65.0; \quad \text{\% voltage_units (in I_{stim})} \]
\[ \text{delta_VICC} = 59.0; \quad \text{\% voltage_units (in I_{stim})} \]
\[ t_{ICC_{\text{stimulus}}} = 10.0; \quad \text{\% time_units (in I_{stim})} \]
\[ t_{ICC_{\text{peak}}} = 0.098; \quad \text{\% time_units (in I_{stim})} \]
\[ t_{ICC_{\text{plateau}}} = 7.582; \quad \text{\% time_units (in I_{stim})} \]
\[ J_{\text{max, CaSR}} = 0.31705; \quad \text{\% millimolar_per_second (in J_{CaSR, SM})} \]
\[ \text{Ach} = 0.00001; \quad \text{\% millimolar (in Neural_input)} \]
\[ C_{\text{SM}} = 77.0; \quad \text{\% capacitance_units (in SM_Membrane)} \]
\[ \text{I_{stim}} = 0.0; \quad \text{\% current_units (in SM_Membrane)} \]
\[ K_{-i} = 164.0; \quad \text{\% millimolar (in SM_Membrane)} \]
\[ \text{Vol_{SM}} = 3500.0; \quad \text{\% volume_units (in SM_Membrane)} \]
\[ t_{\text{d_LVA_SM}} = 7.8706; \quad \text{\% time_units (in d_LVA_SM)} \]
\[ t_{\text{d_Ltype_SM}} = 1.2331; \quad \text{\% time_units (in d_Ltype_SM)} \]
\[ t_{\text{f_Ltype_SM}} = 225.6231; \quad \text{\% time_units (in f_Ltype_SM)} \]
\[ t_{\text{f_ca_Ltype_SM}} = 5.247; \quad \text{\% time_units (in f_ca_Ltype_SM)} \]
\[ t_{\text{f_xr1_SM}} = 119.8843; \quad \text{\% time_units (in xr1_SM)} \]

\% Computation

\% time (time_units)

\[ \text{d_{BK_SM}} = 1.0/(1.0+\exp(Y(3)/-17.0-2.0*\log(Y(2)/0.001))); \]
\[ \text{I_{BK_SM}} = G_{\text{max, BK}}*\text{d_{BK_SM}}*(Y(3)-E_{K_1}); \]
\[ \text{E_{Ca_1}} = 0.5*RToF*\log(Ca_o/Y(2)); \]
\[ \text{I_{LVA_SM}} = G_{\text{max, LVA}}*Y(6)*Y(4)*(Y(3)-E_{Ca_1}); \]
\[ \text{E_{Ca_2}} = 0.5*RToF*\log(Ca_o/Y(2)); \]
\[ \text{I_{Ltype_SM}} = G_{\text{max, Ltype}}*Y(7)*Y(5)*Y(8)*(Y(3)-E_{Ca_2}); \]
\[ f_{ca_{NSCC_SM}} = 1.0/(1.0+(Y(2)/0.0002)^{-4.0}); \]
rach\_NSCC\_SM = 1.0/(1.0+0.01/Ach);
I\_NSCC\_SM = G\_max\_NSCC\_SM\*Y(10)*f\_ca\_NSCC\_SM*rach\_NSCC\_SM*(Y(3)-E\_NSCC);
I\_Na\_SM = G\_max\_Na\_SM\*Y(9)*Y(11)*(Y(3)-E\_Na);
I\_bk\_SM = G\_max\_bk\*(Y(3)-E\_K_2);
E\_K_4 = RToF*log(K_o/K_i);
I\_ka\_SM = G\_max\_ka\_SM\*Y(12)/Y(13)/Y(3)-E\_K_4);
I\_kr\_SM = G\_max\_kr\_SM\*Y(14)/Y(15)*(Y(3)-E\_K_3);

if (Y(3) > V\_threshold)
    dY(1, 1) = 1.0;
elseif (((Y(3) < V\_threshold) & (Y(1) > 0.0)) & (Y(1) > 0.0))
    dY(1, 1) = -1.0*Y(1);
else
    dY(1, 1) = 0.0;
end;

if (Y(3) > V\_threshold)
    local_time = Y(1);
else
    local_time = 100.0;
end;

if (local_time < t\_ICCpeak)
    I\_stim = G\_couple*delta_VICC;
elseif (((local_time >= t\_ICCpeak) & (local_time <= t\_ICCplateau)) & (local_time <= t\_ICCstimulus))
    I\_stim = G\_couple*V\_decay*1.0/(1.0+exp((local_time-8.0)/1.0));
else
    I\_stim = 0.0;
end;

J\_CaSR\_SM = J\_max\_CaSR*(Y(2)*1.0)*1.34;
dY(3, 1) = -1000.0/Cm\_SM*(I\_Na\_SM+I\_Ltype\_SM+I\_LVA\_SM+I\_kr\_SM+I\_ka\_SM+I\_BK\_SM+I\_NSCC\_SM+I\_bk\_SM+I\_stim+-1.0*I\_stim)+1.0*Stim);
dY(2, 1) = (-(1000.0*I\_Ltype\_SM+-1.0*I\_LVA\_SM)/2.0+0.001*F*Vol\_SM)+1000.0*←
J\_CaSR\_SM;
d\_inf\_LVA\_SM = 1.0/(1.0+exp((Y(3)+27.5)/-10.9));
dY(4, 1) = 1000.0*(d\_inf\_LVA\_SM-Y(4))/tau\_d\_LVA\_SM;
d\_inf\_Ltype\_SM = 1.0/(1.0+exp((Y(3)+17.0)/-4.3));
dY(5, 1) = 1000.0*(d\_inf\_Ltype\_SM-Y(5))/tau\_d\_Ltype\_SM;
f\_inf\_LVA\_SM = 1.0/(1.0+exp((Y(3)+15.8)/7.0));
tau\_f\_LVA\_SM = T\_correction_Ca*7.58*exp(Y(3)*0.00817);
dY(6, 1) = 1000.0*(f\_inf\_LVA\_SM-Y(6))/tau\_f\_LVA\_SM;
f\_inf\_Ltype\_SM = 1.0/(1.0+exp((Y(3)+43.0)/8.9));
dY(7, 1) = 1000.0*(f\_inf\_Ltype\_SM-Y(7))/tau\_f\_Ltype\_SM;
f\_ca\_inf\_Ltype\_SM = 1.0-1.0/(1.0+exp((Y(2)-0.00012401)/-0.0000131));
dY(8, 1) = 1000.0*(f\_ca\_inf\_Ltype\_SM-Y(8))/tau\_f\_ca\_Ltype\_SM;
\[ h_{\text{inf}}_{\text{Na}} = \frac{1}{1.0 + \exp((Y(3) + 78.0) / 3.0)}; \]
\[ \tau_{h_{\text{Na}}} = \frac{T_{\text{correction}_{\text{Na}}}(Y(3) + 0.25 \times 1.0 + 5.5)}{\text{B6}}; \]
\[ dY(9, 1) = 1000.0 \times (h_{\text{inf}}_{\text{Na}} - Y(9)) / \tau_{h_{\text{Na}}}; \]
\[ m_{\text{inf}}_{\text{NSCC}_{\text{SM}}} = \frac{1}{1.0 + \exp((Y(3) + 25.0) / -20.0)}; \]
\[ \tau_{m_{\text{NSCC}_{\text{SM}}} = \frac{T_{\text{correction}_{\text{Na}}}(Y(3) + 66.0) / -26.0)}{\text{B6}} \times 150.0; \]
\[ dY(10, 1) = 1000.0 \times (m_{\text{inf}}_{\text{NSCC}_{\text{SM}}} - Y(10)) / \tau_{m_{\text{NSCC}_{\text{SM}}}}; \]
\[ m_{\text{inf}}_{\text{Na}} = \frac{1}{1.0 + \exp((Y(3) + 47.0) / -4.8)}; \]
\[ \tau_{m_{\text{Na}}} = \frac{T_{\text{correction}_{\text{Na}}}(Y(3) - 0.017 \times 1.0 + 0.44)}{\text{B6}}; \]
\[ dY(11, 1) = 1000.0 \times (m_{\text{inf}}_{\text{Na}} - Y(11)) / \tau_{m_{\text{Na}}}; \]
\[ xa_{1_{\text{inf}}}_{\text{SM}} = \frac{1}{1.0 + \exp((Y(3) + 26.5) / -7.9)}; \]
\[ \tau_{xa_{1_{\text{SM}}} = \frac{T_{\text{correction}_{\text{K}}}(31.8 + 175.0 \times \exp(-0.5 \times ((Y(3) + 44.4) / 22.3)^2.0))}{\text{B6}}; \]
\[ dY(12, 1) = 1000.0 \times (xa_{1_{\text{inf}}}_{\text{SM}} - Y(12)) / \tau_{xa_{1_{\text{SM}}}}; \]
\[ xa_{2_{\text{inf}}}_{\text{SM}} = 0.1 \times 0.9 / (1.0 + \exp((Y(3) + 65.0) / 6.2)); \]
\[ \tau_{xa_{1_{\text{SM}}} = \frac{T_{\text{correction}_{\text{K}}}(90.0)}{\text{B6}}; \]
\[ dY(13, 1) = 1000.0 \times (xa_{2_{\text{inf}}}_{\text{SM}} - Y(13)) / \tau_{xa_{2_{\text{SM}}}}; \]
\[ xr_{1_{\text{inf}}}_{\text{SM}} = \frac{1}{1.0 + \exp((Y(3) + 27.0) / -5.0)}; \]
\[ dY(14, 1) = 1000.0 \times (xr_{1_{\text{inf}}}_{\text{SM}} - Y(14)) / \tau_{xa_{1_{\text{SM}}}}; \]
\[ xr_{2_{\text{inf}}}_{\text{SM}} = 0.6 \times 0.8 / (1.0 + \exp((Y(3) + 58.0) / 10.0)); \]
\[ \tau_{xa_{2_{\text{SM}}} = \frac{T_{\text{correction}_{\text{K}}}(707.0 + 1481.0 \times \exp((Y(3) + 36.0) / 95.0))}{\text{B6}}; \]
\[ dY(15, 1) = 1000.0 \times (xr_{2_{\text{inf}}}_{\text{SM}} - Y(15)) / \tau_{xa_{2_{\text{SM}}}}; \]

% End of file
B.3 Modified ICC-SMC Model

% CellML model: ICC_SMC_combined_model
% Conversion from CellML 1.0 to MATLAB (init) was done using ODR (0.9.31.1279)

function dY = ICC_SMC_v5c(time, Y)

% Initial conditions

% Y = [0.00000993087, 0.00008, -67.0, -68.0, 0.007299, 0.0000902, 0.000136, \cdots
% 0.000637203283598439, 0.0, 0.0, 0.02, 0.0, 0.0, 0.0, 0.0, \cdots
% 0.99, 0.95, 1.0, 1.0, 1.0, 1.0, 1.0, 0.05787, 0.0, 0.005, 0.00414, 0.72, 0.0, \cdots
% 0.82];

% YNames = {'Ca_i', 'Ca_i_SM', 'Vm', 'Vm_SM', 'Ca_ER', 'Ca_PU', 'Ca_m', 'IP3', '\cdots
% d_CaCl', 'd_ERG', 'd_LVA_SM', 'd_Ltype_SM', 'd_NSCC', 'd_Na', '\cdots
% d_VDDR', 'd_kv11', 'f_LVA_SM', 'f_Ltype_SM', 'f_Na', 'f_VDDR', '\cdots
% f_ca_Ltype_SM', 'f_ca_Ltype', 'f_kv11', 'h_Na_SM', 'm_NSCC_SM', 'm_Na_SM', '\cdots
% x1_SM', 'xa2_SM', 'xa1_SM', 'xa2_SM'};

% YUnits = {'millimolar', 'millimolar', 'voltage_units', 'voltage_units', '\cdots
% millimolar', 'millimolar', 'millimolar', 'millimolar', 'dimensionless', '\cdots
% dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', '\cdots
% dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', '\cdots
% dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', '\cdots
% dimensionless', 'dimensionless', 'dimensionless', 'dimensionless', '\cdots
% dimensionless', 'dimensionless', 'dimensionless'};

% YComponents = {'Membrane', 'Membrane', 'Membrane', 'Membrane', 'PU_unit', '\cdots
% PU_unit', 'PU_unit', 'PU_unit', 'd_CaCl', 'd_ERG', 'd_LVA_SM', 'd_Ltype_SM', '\cdots
% d_VDDR', 'd_kv11', 'f_LVA_SM', 'f_Ltype_SM', '\cdots
% f_Ltype', 'f_Na', 'f_VDDR', 'f_ca_Ltype_SM', 'f_ca_Ltype', 'f_kv11', 'h_Na_SM', '\cdots
% m_NSCC_SM', 'm_Na_SM', 'x1_SM', 'xa2_SM', 'xa1_SM', 'xa2_SM'};

% State variables

% 1: Ca_i (millimolar) (in Membrane)
% 2: Ca_i_SM (millimolar) (in Membrane)
% 3: Vm (voltage_units) (in Membrane)
% 4: Vm_SM (voltage_units) (in Membrane)
% 5: Ca_ER (millimolar) (in PU_unit)
% 6: Ca_PU (millimolar) (in PU_unit)
% 7: Ca_m (millimolar) (in PU_unit)
% 8: IP3 (millimolar) (in PU_unit)
% 9: d_CaCl (dimensionless) (in d_CaCl)
% 10: d_ERG (dimensionless) (in d_ERG)
% 11: d_LVA_SM (dimensionless) (in d_LVA_SM)
% Modified ICC-SMC Model

% 12: \( d_{Ltype_{SM}} \) (dimensionless) (in \( d_{Ltype_{SM}} \))
% 13: \( d_{Ltype} \) (dimensionless) (in \( d_{Ltype} \))
% 14: \( d_{NSCC} \) (dimensionless) (in \( d_{NSCC} \))
% 15: \( d_{Na} \) (dimensionless) (in \( d_{Na} \))
% 16: \( d_{VDDR} \) (dimensionless) (in \( d_{VDDR} \))
% 17: \( f_{kv11} \) (dimensionless) (in \( f_{kv11} \))
% 18: \( f_{LVA_{SM}} \) (dimensionless) (in \( f_{LVA_{SM}} \))
% 19: \( f_{Ltype_{SM}} \) (dimensionless) (in \( f_{Ltype_{SM}} \))
% 20: \( f_{Ltype} \) (dimensionless) (in \( f_{Ltype} \))
% 21: \( f_{Na} \) (dimensionless) (in \( f_{Na} \))
% 22: \( f_{VDDR} \) (dimensionless) (in \( f_{VDDR} \))
% 23: \( f_{ca_{Ltype_{SM}}} \) (dimensionless) (in \( f_{ca_{Ltype_{SM}}} \))
% 24: \( f_{ca_{Ltype}} \) (dimensionless) (in \( f_{ca_{Ltype}} \))
% 25: \( f_{kv11} \) (dimensionless) (in \( f_{kv11} \))
% 26: \( h_{Na_{SM}} \) (dimensionless) (in \( h_{Na_{SM}} \))
% 27: \( m_{NSCC_{SM}} \) (dimensionless) (in \( m_{NSCC_{SM}} \))
% 28: \( m_{Na_{SM}} \) (dimensionless) (in \( m_{Na_{SM}} \))
% 29: \( xa1_{SM} \) (dimensionless) (in \( xa1_{SM} \))
% 30: \( xa2_{SM} \) (dimensionless) (in \( xa2_{SM} \))
% 31: \( xr1_{SM} \) (dimensionless) (in \( xr1_{SM} \))
% 32: \( xr2_{SM} \) (dimensionless) (in \( xr2_{SM} \))

% Constants

\( Ca_o = 2.5 \); \% millimolar (in Environment)
\( Ca_o_{SM} = 2.5 \); \% millimolar (in Environment)
\( F = 96.4846 \); \% F units (in Environment)
\( FoRT = 0.0374 \); \% Inverse Voltage units (in Environment)
\( RToF = 26.7137 \); \% voltage units (in Environment)
\( T_{correction_{Ca}} = 2.6235 \); \% dimensionless (in Environment)
\( T_{correction_{K}} = 1.4986 \); \% dimensionless (in Environment)
\( T_{correction_{Na}} = 3.2056 \); \% dimensionless (in Environment)
\( G_{max_{BK_{1}}} = 37.3 \); \% conductance units (\( G_{max_{BK}} \) in \( I_{BK} \))
\( G_{max_{BK_{2}}} = 60.0 \); \% conductance units (\( G_{max_{BK}} \) in \( I_{BK_{SM}} \))
\( G_{max_{CaCl}} = 10.1 \); \% conductance units (in \( I_{CaCl} \))
\( G_{max_{ERG}} = 2.5 \); \% conductance units (in \( I_{ERG} \))
\( G_{couple} = 60.0 \); \% conductance units (in \( I_{Junc} \))
\( G_{max_{LVA}} = 0.18 \); \% conductance units (in \( I_{LVA_{SM}} \))
\( G_{max_{Ltype_{1}}} = 2.0 \); \% conductance units (\( G_{max_{Ltype}} \) in \( I_{Ltype} \))
\( G_{max_{Ltype_{2}}} = 65.0 \); \% conductance units (\( G_{max_{Ltype}} \) in \( I_{Ltype_{SM}} \))
\( G_{max_{NSCC}} = 12.15 \); \% conductance units (in \( I_{NSCC} \))
\( G_{max_{NSCC_{SM}}} = 50.0 \); \% conductance units (in \( I_{NSCC_{SM}} \))
\( G_{max_{Na}} = 20.0 \); \% conductance units (in \( I_{Na} \))
\( G_{max_{Na_{SM}}} = 3.0 \); \% conductance units (in \( I_{Na_{SM}} \))
\( G_{max_{VDDR}} = 3.0 \); \% conductance units (in \( I_{VDDR} \))
\( G_{max_{bk_{1}}} = 0.15 \); \% conductance units (\( G_{max_{bk}} \) in \( I_{bk} \))
\( G_{max_{bk_{2}}} = 0.0144 \); \% conductance units (\( G_{max_{bk}} \) in \( I_{bk_{SM}} \))
\( G_{max_{ka_{SM}}} = 9.0 \); \% conductance units (in \( I_{ka_{SM}} \))
B. Scripts

\[ G_{\text{max}}_{kr\_SM} = 35.0; \quad \% \text{conductance\_units (in I}_{kr\_SM} \]  
\[ G_{\text{max}}_{kv11} = 6.3; \quad \% \text{conductance\_units (in I}_{kv11} \]  
\[ J_{\text{max}}_{CaSR} = 317.05; \quad \% \text{millimolar\_per\_second (in J}_{CaSR\_SM} \]  
\[ J_{\text{max}}_{PMCA} = 0.09; \quad \% \text{millimolar\_per\_second (in J}_{PMCA} \]  
\[ C_n = 0.025; \quad \% \text{capacitance\_units (in Membrane)} \]  
\[ C_{n\_SM} = 77.0e^{-3}; \quad \% \text{capacitance\_units (in Membrane)} \]  
\[ E_{Cl} = -11.23; \quad \% \text{voltage\_units (in Membrane)} \]  
\[ E_{K} = -76.0; \quad \% \text{voltage\_units (in Membrane)} \]  
\[ E_{K\_SM} = -67.5; \quad \% \text{voltage\_units (in Membrane)} \]  
\[ E_{Na} = 40.57; \quad \% \text{voltage\_units (in Membrane)} \]  
\[ E_{Na\_SM} = 69.0; \quad \% \text{voltage\_units (in Membrane)} \]  
\[ I_{\text{stim}}_{ICC} = 0.0; \quad \% \text{current\_units (in Membrane)} \]  
\[ Na_i = 30.0; \quad \% \text{millimolar (in Membrane)} \]  
\[ T_{start} = 0.0; \quad \% \text{time\_units (in Membrane)} \]  
\[ V_{cyto} = 7.0e^{-13}; \quad \% \text{volume\_units (in Membrane)} \]  
\[ Vol_{SM} = 3.5e^{-3}; \quad \% \text{volume\_units (in Membrane)} \]  
\[ f_c = 0.01; \quad \% \text{dimensionless (in Membrane)} \]  
\[ A_{ch} = 0.00001; \quad \% \text{millimolar (in Neural\_input)} \]  
\[ J_{\text{ERleak}} = 1.67; \quad \% \text{rate\_constants\_units (in PU\_unit)} \]  
\[ J_{\text{max}}_{leak} = 0.01; \quad \% \text{rate\_constants\_units (in PU\_unit)} \]  
\[ J_{\text{max}}_{IP3} = 50000.0; \quad \% \text{rate\_constants\_units (in PU\_unit)} \]  
\[ J_{\text{max}}_{NaCa} = 0.05; \quad \% \text{millimolar\_per\_second (in PU\_unit)} \]  
\[ J_{\text{max}}_{serca} = 1.8333; \quad \% \text{millimolar\_per\_second (in PU\_unit)} \]  
\[ J_{\text{max}}_{uni} = 5000.0; \quad \% \text{rate\_constants\_units (in PU\_unit)} \]  
\[ K_{Ca} = 0.003; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ K_{Na} = 9.4; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ K_{act} = 0.00038; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ K_{trans} = 0.006; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ L = 50.0; \quad \% \text{dimensionless (in PU\_unit)} \]  
\[ P_{mV} = 1.33e^{-5}; \quad \% \text{millimolar\_per\_second (in PU\_unit)} \]  
\[ V_m = 3.33e^{-5}; \quad \% \text{millimolar\_per\_second (in PU\_unit)} \]  
\[ b = 0.5; \quad \% \text{dimensionless (in PU\_unit)} \]  
\[ beta = 2.6e^{-5}; \quad \% \text{millimolar\_per\_second (in PU\_unit)} \]  
\[ conc = 0.001; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ d_{ACT} = 0.001; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ d_{IP3} = 0.00025; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ delta_{Psi} = 164.0; \quad \% \text{voltage\_units (in PU\_unit)} \]  
\[ delta_{Psi\_star} = 91.0; \quad \% \text{voltage\_units (in PU\_unit)} \]  
\[ eta = 0.015; \quad \% \text{rate\_constants\_units (in PU\_unit)} \]  
\[ fe = 0.01; \quad \% \text{dimensionless (in PU\_unit)} \]  
\[ fn = 0.0003; \quad \% \text{dimensionless (in PU\_unit)} \]  
\[ h = 0.9396; \quad \% \text{dimensionless (in PU\_unit)} \]  
\[ k_4 = 0.0005; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ k_{serca} = 0.00042; \quad \% \text{millimolar (in PU\_unit)} \]  
\[ kr = -58.0; \quad \% \text{voltage\_units (in PU\_unit)} \]  
\[ n = 2.0; \quad \% \text{dimensionless (in PU\_unit)} \]
\( na = 2.8; \) % dimensionless (in PU
unit)
\( r = 8.0; \) % dimensionless (in PU
unit)
\( u = 4.0; \) % dimensionless (in PU
unit)
\( \tau_{d, CaCl} = 0.03; \) % time
units (in d
CaCl)
\( \tau_{d, ERG} = 0.0051; \) % time
units (in d
ERG)
\( \tau_{d, LVA_SM} = 7.8706e-3; \) % time
units (in d
LVA_SM)
\( \tau_{d, Ltype_SM} = 1.2331e-3; \) % time
units (in d
Ltype_SM)
\( \tau_{d, Ltype} = 0.0026; \) % time
units (in d
Ltype)
\( \tau_{d, Na} = 0.01; \) % time
units (in d
Na)
\( \tau_{d, VDDR} = 0.02; \) % time
units (in d
VDDR)
\( \tau_{d, kv11} = 0.0085; \) % time
units (in d
kv11)
\( \tau_{f, Ltype_SM} = 225.6231e-3; \) % time
units (in f
Ltype_SM)
\( \tau_{f, Ltype} = 0.2256; \) % time
units (in f
Ltype)
\( \tau_{f, Na} = 0.005; \) % time
units (in f
Na)
\( \tau_{f, VDDR} = 0.1; \) % time
units (in f
VDDR)
\( \tau_{f, ca} = 5.247e-3; \) % time
units (in f
ca)
\( \tau_{f, kv11} = 0.0085; \) % time
units (in f
kv11)
\( \tau_{x,r1, SM} = 119.8843e-3; \) % time
units (in x
r1_SM)

% Computed variables

% I
BK (current
units) (in I
BK)
% I
BK_SM (current
units) (in I
BK_SM)
% I
CaCl (current
units) (in I
CaCl)
% I
ERG (current
units) (in I
ERG)
% I
Junc (current
units) (in I
Junc)
% E
Ca_SM_1 (voltage
units) (E
Ca in I
LVA_SM)
% I
LVA_SM (current
units) (in I
LVA_SM)
% E
Ca_1 (voltage
units) (E
Ca in I
Ltype)
% I
Ltype (current
units) (in I
Ltype)
% E
Ca_SM_2 (voltage
units) (E
Ca in I
Ltype_SM)
% I
Ltype_SM (current
units) (in I
Ltype_SM)
% I
NSCC (current
units) (in I
NSCC)
% I
NSCC_SM (current
units) (in I
NSCC_SM)
% f
ca_SM (dimensionless) (in I
NSCC_SM)
% rach_NSCE_SM (dimensionless) (in I
NSCC_SM)
% I
Na (current
units) (in I
Na)
% I
Na_SM (current
units) (in I
Na_SM)
% E
Ca_2 (voltage
units) (E
Ca in I
VDDR)
% I
VDDR (current
units) (in I
VDDR)
% I
bk (current
units) (in I
bk)
% I
bk_SM (current
units) (in I
bk_SM)
% I
ka_SM (current
units) (in I
ka_SM)
% I
kr_SM (current
units) (in I
kr_SM)
% I
kv11 (current
units) (in I
kv11)
% J
CaSR_SM (millimolar_per_second) (in J
CaSR_SM)
% J_Pmca (millimolar_per_second) (in J_Pmca)
% V_coor (dimensionless) (in Membrane)
% J_ERout (millimolar_per_second) (in PU_unit)
% J_NaCa (millimolar_per_second) (in PU_unit)
% J_SERCA (millimolar_per_second) (in PU_unit)
% J_leak (millimolar_per_second) (in PU_unit)
% J_uni (millimolar_per_second) (in PU_unit)
% MMC (millimolar) (in PU_unit)
% d_BK_SM (dimensionless) (in d_BK_SM)
% d_BK (dimensionless) (in d_BK)
% d_inf_CaCl (dimensionless) (in d_CaCl)
% d_inf_ERG (dimensionless) (in d_ERG)
% d_inf_LVA_SM (dimensionless) (in d_LVA_SM)
% d_inf_Ltype_SM (dimensionless) (in d_Ltype_SM)
% d_inf_Ltype (dimensionless) (in d_Ltype)
% d_inf_NSCC (dimensionless) (in d_NSCC)
% d_inf_Na (dimensionless) (in d_Na)
% d_inf_VDDR (dimensionless) (in d_VDDR)
% d_inf_kv11 (dimensionless) (in d_kv11)
% f_inf_LVA_SM (dimensionless) (in f_LVA_SM)
% tau_f_LVA_SM (time_units) (in f_LVA_SM)
% f_inf_Ltype_SM (dimensionless) (in f_Ltype_SM)
% f_inf_Ltype (dimensionless) (in f_Ltype)
% f_inf_Na (dimensionless) (in f_Na)
% f_inf_VDDR (dimensionless) (in f_VDDR)
% f_ca_inf_Ltype_SM (dimensionless) (in f_ca_Ltype_SM)
% f_ca_inf_Ltype (dimensionless) (in f_ca_Ltype)
% f_inf_kv11 (dimensionless) (in f_kv11)
% h_inf_Na (dimensionless) (in h_Na_SM)
% tau_h_Na (time_units) (in h_Na_SM)
% m_inf_NSCC_SM (dimensionless) (in m_NSCC_SM)
% tau_m_NSCC_SM (time_units) (in m_NSCC_SM)
% m_inf_Na (dimensionless) (in m_Na_SM)
% tau_m_Na (time_units) (in m_Na_SM)
% tau_xa1_SM (time_units) (in xa1_SM)
% xa1_inf_SM (dimensionless) (in xa1_SM)
% tau_xa2_SM (time_units) (in xa2_SM)
% xa2_inf_SM (dimensionless) (in xa2_SM)
% xe1_inf_SM (dimensionless) (in xe1_SM)
% tau_xe2_SM (time_units) (in xe2_SM)
% xe2_inf_SM (dimensionless) (in xe2_SM)

% Computation

d_BK = 1.0/(1.0+exp(Y(3)/-17.0-2.0*log(Y(1)/0.001)));
I_BK = G_max_BK_1*d_BK*(Y(3)-E_K);
Modified ICC-SMC Model

d_{BK_SM} = 1.0/(1.0+\exp(Y(4)/-17.0-2.0*\log(Y(2)/0.001)));
I_{BK_SM} = G_{max_BK} \cdot d_{BK_SM} \cdot (Y(4)-E_K_SM);
I_{CaCl} = G_{max_CaCl} \cdot Y(9) \cdot (Y(3)-E_Cl);
I_{ERG} = G_{max_ERG} \cdot Y(10) \cdot (Y(3)-E_K);
I_{Junc} = G_{couple} \cdot (Y(3)-Y(4));
E_{Ca_SM_1} = 0.5*\exp\log(Ca_o/SM/Y(2));
I_{LVA_SM} = G_{max_LVA} \cdot Y(18) \cdot (Y(4)-E_Ca_SM_1);
E_{Ca_SM_2} = 0.5*\exp\log(Ca_o/Y(1));
I_{Ltype} = G_{max_Ltype} \cdot Y(20) \cdot Y(13) \cdot Y(24) \cdot (Y(3)-E_Ca_1);
E_{Ca_SM_2} = 0.5*\exp\log(Ca_o/SM/Y(2));
I_{Ltype_SM} = G_{max_Ltype_2} \cdot Y(19) \cdot Y(12) \cdot Y(23) \cdot (Y(4)-E_Ca_SM_2);
I_{NSCC} = G_{max_NSCC} \cdot (Y(14) \cdot (Y(3)-E_NSCC});
f_{ca_NSCC_SM} = 1.0/(1.0+(Y(2)/0.0002)^{-4.0});
rach_NSCC_SM = 1.0/(1.0+0.01/Ach);
I_{NSCC_SM} = G_{max_NSCC_SM} \cdot (Y(27) \cdot f_{ca_NSCC_SM} \cdot rach_NSCC_SM \cdot (Y(4)-E_NSCC_SM});
I_{Na} = G_{max_Na} \cdot Y(21) \cdot Y(15) \cdot (Y(3)-E_Na);
I_{Na_SM} = G_{max_Na_SM} \cdot Y(26) \cdot Y(28) \cdot (Y(4)-E_Na_SM);
E_{CA_2} = 0.5*\exp\log(Ca_o/Y(1));
I_{VDDR} = G_{max_VDDR} \cdot Y(22) \cdot Y(16) \cdot (Y(3)-E_Ca_2);
I_{bk} = G_{max_bk} \cdot Y(3)-E_K;
I_{bk_SM} = G_{max_bk} \cdot Y(4)-E_K_SM;
I_{ka_SM} = G_{max_ka} \cdot Y(29) \cdot Y(30) \cdot (Y(4)-E_K_SM);
I_{kr_SM} = G_{max_kr} \cdot Y(31) \cdot Y(32) \cdot (Y(4)-E_K_SM);
I_{kv11} = G_{max_kv11} \cdot Y(25) \cdot Y(17) \cdot (Y(3)-E_K);
J_{CaSR_SM} = I_{max_CaSR} \cdot (Y(2) \cdot 1.0) \cdot 1.34;
J_{PMCA} = I_{max_PMCA} \cdot 1.0/(1.0+0.000298/Y(1));

if (time < T_start)
    V_coor = 0.0;
else
    V_coor = 1.0;
end;

dY(3, 1) = -1.0*V_coor/Cm*(-I_stim_ICC+I_Na+I_Ltype+I_VDDR+I_kv11+I_ERG+I_BK+---)
    I_{CaCl1+I_NSCC}+I_{bk}+J_{PMCA}+2.0*1000000.0*1000000.0*F\cdot V_cyto);
J_{leak} = J_{max_leak} \cdot (Y(6)-Y(1));
dY(1, 1) = f_C\cdot V_coors((-1.0+I_{Ltype}+I_{VDDR})/(2.0*1000000.0*1000000.0*F\cdot V_cyto)---
    )+J_{leak}+1.0*J_{PMCA};

dY(4, 1) = -1.0/Cm_sM\cdot (I_{Na_SM}+I_{Ltype_SM}+I_{LVA_SM}+I_{kr_SM}+I_{ka_SM}+I_{bk_SM}+---
    I_{NSCC_SM}+I_{bk_SM}+1.0*I_{Junc});

\text{other equations...}
\[ J_{NaCa} = J_{\text{max, NaCa}} \exp(b \cdot \text{FoRT} + (\text{deltaPsi} - \text{deltaPsi_*star}))/((1.0 + (K_{Na}/Na_i)^n) \cdot (1.0 + K_{Ca}/Y(7))) \];
\[ dY(6, 1) = V_{\text{coor}} \cdot \text{fc}((J_{NaCa} - J_{\text{uni}}) \cdot 128.7 + (J_{ERout} - J_{\text{SERCA}}) \cdot 100.0) \];
\[ dY(7, 1) = V_{\text{coor}} \cdot \text{fm}(J_{\text{uni}} - J_{\text{NaCa}}) \];
\[ dY(5, 1) = V_{\text{coor}} \cdot \text{fe}(J_{\text{SERCA}} - J_{\text{ERout}}) \];
\[ dY(8, 1) = V_{\text{coor}} \cdot ((\beta - \text{etas}(Y(8) - Vm4) + Y(8) \cdot u)/(k4 \cdot u + Y(8) \cdot u) + PmV \cdot (1.0 - Y(3) \cdot r)/(Y(3) \cdot r + kr \cdot r)) \];
\[ d_{\text{inf, CaCl}} = 1.0/(1.0 + (0.00014/Y(1)) \cdot 3.0) \];
\[ dY(9, 1) = V_{\text{coor}} \cdot (d_{\text{inf, CaCl}} - Y(9))/\text{tau}_d_{\text{CaCl}} \];
\[ d_{\text{inf, ERG}} = 0.2 + 0.8/(1.0 + \exp((Y(3) + 20.0)/-1.8)) \];
\[ dY(10, 1) = V_{\text{coor}} \cdot (d_{\text{inf, ERG}} - Y(10))/\text{tau}_d_{\text{ERG}} \];
\[ d_{\text{inf, LVA, SM}} = 1.0/(1.0 + \exp((Y(4) + 27.5)/-10.9)) \];
\[ dY(11, 1) = (d_{\text{inf, LVA, SM}} - Y(11))/\text{tau}_d_{\text{LVA, SM}} \];
\[ d_{\text{inf, Ltype}} = 1.0/(1.0 + \exp((Y(3) + 17.0)/-4.3)) \];
\[ dY(13, 1) = V_{\text{coor}} \cdot (d_{\text{inf, Ltype}} - Y(13))/\text{tau}_d_{\text{Ltype}} \];
\[ d_{\text{inf, Ltype, SM}} = 1.0/(1.0 + \exp((Y(4) + 17.0)/-4.3)) \];
\[ dY(12, 1) = (d_{\text{inf, Ltype, SM}} - Y(12))/\text{tau}_d_{\text{Ltype, SM}} \];
\[ d_{\text{inf, NSCC}} = 1.0/(1.0 + (0.0000745/Y(6))/-85.0) \];
\[ dY(14, 1) = V_{\text{coor}} \cdot (d_{\text{inf, NSCC}} - Y(14))/\text{tau}_d_{\text{NSCC}} \];
\[ d_{\text{inf, Na}} = 1.0/(1.0 + \exp((Y(3) + 47.0)/-4.8)) \];
\[ dY(15, 1) = V_{\text{coor}} \cdot (d_{\text{inf, Na}} - Y(15))/\text{tau}_d_{\text{Na}} \];
\[ d_{\text{inf, VDDR}} = 1.0/(1.0 + \exp((Y(3) + 26.0)/-6.0)) \];
\[ dY(16, 1) = V_{\text{coor}} \cdot (d_{\text{inf, VDDR}} - Y(16))/\text{tau}_d_{\text{VDDR}} \];
\[ d_{\text{inf, kV11}} = 1.0/(1.0 + \exp((Y(3) + 25.0)/-7.7)) \];
\[ dY(17, 1) = V_{\text{coor}} \cdot (d_{\text{inf, kV11}} - Y(17))/\text{tau}_d_{\text{kV11}} \];
\[ f_{\text{inf, LVA, SM}} = 1.0/(1.0 + \exp((Y(4) + 15.8)/7.0)) \];
\[ \text{tau}_f_{\text{LVA, SM}} = T_{\text{correction, Ca}} \cdot 7.58 \cdot 3 \cdot \exp(Y(4) \cdot 0.00817) \];
\[ dY(18, 1) = (f_{\text{inf, LVA, SM}} - Y(18))/\text{tau}_f_{\text{LVA, SM}} \];
\[ f_{\text{inf, Ltype}} = 1.0/(1.0 + \exp((Y(3) + 43.0)/8.9)) \];
\[ dY(20, 1) = V_{\text{coor}} \cdot (f_{\text{inf, Ltype}} - Y(20))/\text{tau}_f_{\text{Ltype}} \];
\[ f_{\text{inf, Ltype, SM}} = 1.0/(1.0 + \exp((Y(4) + 43.0)/8.9)) \];
\[ dY(19, 1) = (f_{\text{inf, Ltype, SM}} - Y(19))/\text{tau}_f_{\text{Ltype, SM}} \];
\[ f_{\text{inf, Na}} = 1.0/(1.0 + \exp((Y(3) + 78.0)/7.0)) \];
\[ dY(21, 1) = V_{\text{coor}} \cdot (f_{\text{inf, Na}} - Y(21))/\text{tau}_f_{\text{Na}} \];
\[ f_{\text{inf, VDDR}} = 1.0/(1.0 + \exp((Y(3) + 66.0)/6.5)) \];
\[ dY(22, 1) = V_{\text{coor}} \cdot (f_{\text{inf, VDDR}} - Y(22))/\text{tau}_f_{\text{VDDR}} \];
\[ f_{\text{ca, inf, Ltype}} = 1.0 - 1.0/(1.0 + \exp((Y(1) - 0.0001 - 0.000214)/-0.0000271)) \];
\[ dY(24, 1) = V_{\text{coor}} \cdot (f_{\text{ca, inf, Ltype}} - Y(24))/\text{tau}_f_{\text{ca, Ltype}} \];
\[ f_{\text{ca, inf, Ltype, SM}} = 1.0 - 1.0/(1.0 + \exp((Y(2) - 0.00012401)/-0.0000131)) \];
\[ dY(23, 1) = (f_{\text{ca, inf, Ltype, SM}} - Y(23))/\text{tau}_f_{\text{ca, Ltype, SM}} \];
\[ f_{\text{inf, kV11}} = 0.5 + 0.5/(1.0 + \exp((Y(3) + 44.8)/4.4)) \];
\[ dY(25, 1) = V_{\text{coor}} \cdot (f_{\text{inf, kV11}} - Y(25))/\text{tau}_f_{\text{kV11}} \];
\[ b_{\text{inf, Na}} = 1.0/(1.0 + \exp((Y(4) + 78.0)/3.0)) \];
\[ \text{tau}_b_{\text{Na}} = T_{\text{correction, Na}} \cdot (Y(4) \cdot -0.25 \cdot 1.0 \cdot -3 + 5.5 \cdot 3) \];
\[ dY(26, 1) = (b_{\text{inf, Na}} - Y(26))/\text{tau}_b_{\text{Na}} \];
\[ m_{\text{inf, NSCC, SM}} = 1.0/(1.0 + \exp((Y(4) + 25.0)/-20.0)) \];
\[ \text{tau}_m_{\text{NSCC, SM}} = 1.0/(1.0 + \exp((Y(4) + 66.0)/-26.0)) \cdot 0.15 \];
\[ dY(27, 1) = (m_{\text{inf, NSCC, SM}} - Y(27))/\text{tau}_m_{\text{NSCC, SM}} \];
\[ m_{\text{inf}}_{\text{Na}} = \frac{1.0}{1.0 + \exp\left(\left(Y(4) + 47.0\right)/-4.8\right)}; \]
\[ \tau_{m_{\text{Na}}} = T_{\text{correction}_{\text{Na}}} (Y(4) + -0.017 + 1.0e-3 + 0.44e-3); \]
\[ dY(28, 1) = \left( m_{\text{inf}}_{\text{Na}} - Y(28) \right)/\tau_{m_{\text{Na}}}; \]
\[ xa1_{\text{inf}}_{\text{SM}} = \frac{1.0}{1.0 + \exp\left(\left(Y(4) + 26.5\right)/-7.9\right)}; \]
\[ \tau_{xa1_{\text{SM}}} = T_{\text{correction}_K} * (31.8e-3 + 175.0e-3 * \exp(-0.5 * ((Y(4) + 44.4)/22.3)^2.0)); \]
\[ dY(28, 1) = \left( xa1_{\text{inf}}_{\text{SM}} - Y(29) \right)/\tau_{xa1_{\text{SM}}}; \]
\[ xa2_{\text{inf}}_{\text{SM}} = 0.1 + 0.9/(1.0 + \exp\left(\left(Y(4) + 65.0\right)/6.2\right)); \]
\[ \tau_{xa2_{\text{SM}}} = T_{\text{correction}_K} * 90.0e-3; \]
\[ dY(29, 1) = \left( xa2_{\text{inf}}_{\text{SM}} - Y(30) \right)/\tau_{xa2_{\text{SM}}}; \]
\[ xr1_{\text{inf}}_{\text{SM}} = 1.0/(1.0 + \exp\left(\left(Y(4) + 27.0\right)/-5.0\right)); \]
\[ dY(30, 1) = \left( xr1_{\text{inf}}_{\text{SM}} - Y(31) \right)/\tau_{xr1_{\text{SM}}}; \]
\[ xr2_{\text{inf}}_{\text{SM}} = 0.6 + 0.8/(1.0 + \exp\left(\left(Y(4) + 58.0\right)/10.0\right)); \]
\[ \tau_{xr2_{\text{SM}}} = T_{\text{correction}_K} * (-707.0e-3 + 1481.0e-3 * \exp((Y(4) + 36.0)/95.0)); \]
\[ dY(31, 1) = \left( xr2_{\text{inf}}_{\text{SM}} - Y(32) \right)/\tau_{xr2_{\text{SM}}}; \]

\% End of file
B.4 Automata 2D Model

close all;
clear all;

% Finite-difference parameters
% This section defines the model parameters
nx = 16;
ny = 16;
dx = 4;  
% in mm
dy = 4;  
% in mm
dt = 0.1;  
% in seconds
printfrequency = 0.1;  
% output frequency

% ICC-MY parameters
% IICC setup, all parameters in seconds
peakstimuluslenght = 0.098;
plateaustimuluslenght = 7.582;
totalstimuluslenght = 10;
localt(1:nx+ny) = 0;

v_icc_vertical = 4;  
% mm/s
v_icc_horizontal = 6;  
% mm/s

node_tag = zeros((nx+2),(ny+2));
for i = 2:(nx+1)
    for j = 2:(ny+1)
        node_tag(i,j) = (i-2)*nx+j-1;
    end
end

pace_maker_node = [[4;3],[13;15]];
pace_maker_node_number = (pace_maker_node(1,:)-1).*nx + pace_maker_node(2,:);
dead_icc = [];

stimulusfrequency(1:nx*ny) = 20000;  
% time before the first ICC excites
stimulusfrequency(pace_maker_node_number) = 60/3;

stimulusinstant(1:nx*ny) = 20.001;
stimulusinstant(pace_maker_node_number) = -2.000;

ICC_magnitude = 60;
Gcouple = 1.3;
Iexternal(1:nx*ny) = 0.0;
IexternalDecay(1:nx*ny) = 0.0;

finalt = 600;
tspan = [0 dt];

% model parameters
% the new parameters added are the diffusion coefficient (D), and cell
% surface area (Am). Both parameters were obtained from the Aliev paper
% D = 0.005; % diffusion coefficient (input)
Cm = 77; % cell capacitance in pF
Am = 0.0041; % estimated cell surface in mm^2

% Initial conditions of SMC
y0 = [0.00008, -69.75, 0.02, 0.0, 0.99, 0.95, 1.0, 0.05787, 0.0, 0.005, ...
     0.00414, 0.72, 0.0, 0.82];
% 1: SM_Membrane__Ca_i (millimolar)
% 2: SM_Membrane__Vm_SM (voltage units)
% 3: d_LVA_SM__d_LVA_SM (dimensionless)
% 4: d_Ltype_SM__d_Ltype_SM (dimensionless)
% 5: f_LVA_SM__f_LVA_SM (dimensionless)
% 6: f_Ltype_SM__f_Ltype_SM (dimensionless)
% 7: f_ca_Ltype_SM__f_ca_Ltype_SM (dimensionless)
% 8: h_Na_SM__h_Na_SM (dimensionless)
% 9: m_NSCC_SM__m_NSCC_SM (dimensionless)
% 10: m_Na_SM__m_Na_SM (dimensionless)
% 11: xa1_SM__xa1_SM (dimensionless)
% 12: xa2_SM__xa2_SM (dimensionless)
% 13: xr1_SM__xr1_SM (dimensionless)
% 14: xr2_SM__xr2_SM (dimensionless)

Y0 = zeros(nx*ny,14);
Vm = 1:nx*ny;
Iion = 1:nx*ny;

for i = 1:nx*ny
    Y0(i,1:14) = y0;
    Vm(i) = y0(2);
end

% Finite-difference discretisation
% A central difference scheme (1, -2, 1) is used to approximately the
% difference term. Natural BCs are applied to the boundary cells.

% Setup the A matrix
\[ A = \text{eye}(nx*ny); \]

\textbf{for} \ i = 2:(nx-1) \ \textbf{for} \ j = 2:(ny-1) \\
\quad \text{node} = (i-1)*nx + j;
\quad A(\text{node}, \text{node}-nx) = dt*D/(Cm*Am*dy*dy); \\
\quad A(\text{node}, \text{node}-1) = dt*D/(Cm*Am*dx*dx); \\
\quad A(\text{node}, \text{node}) = 1 - 2*dt*D/(Cm*Am*dx*dx) - 2*dt*D/(Cm*Am*dy*dy); \\
\quad A(\text{node}, \text{node}+1) = dt*D/(Cm*Am*dy*dy); \\
\quad A(\text{node}, \text{node}+nx) = dt*D/(Cm*Am*dy*dy); \\
\textbf{end} \\
\textbf{end} \\

LHS = \text{eye}(nx*ny,nx*ny); \\
k = 1; \\
bc(1:(2*nx+(ny-2)*2)) = 0; \\
\textbf{for} \ i = 1:ny \\
\textbf{\% -x direction} \\
\quad \text{node} = 1 + (i-1)*nx; \\
\quad \text{LHS}(\text{node}, \text{node}) = -3/(2*dx); \\
\quad \text{LHS}(\text{node}, \text{node}-1) = 2/dx; \\
\quad \text{LHS}(\text{node}, \text{node}+2) = -1/(2*dx); \\
\quad bc(k) = \text{node}; \\
\quad k = k + 1; \\
\textbf{\% +x direction} \\
\quad \text{node} = nx*i; \\
\quad \text{LHS}(\text{node}, \text{node}) = 3/(2*dx); \\
\quad \text{LHS}(\text{node}, \text{node}-1) = -2/dx; \\
\quad \text{LHS}(\text{node}, \text{node}-2) = 1/(2*dx); \\
\quad bc(k) = \text{node}; \\
\quad k = k + 1; \\
\textbf{end} \\

\textbf{\% the y-direction} \\
\textbf{for} \ j = 2:(nx-1) \\
\textbf{\% - y direction} \\
\quad \text{node} = j; \\
\quad \text{LHS}(\text{node}, \text{node}) = -3/(2*dy); \\
\quad \text{LHS}(\text{node}, \text{node} + nx) = 2/dy; \\
\quad \text{LHS}(\text{node}, \text{node} + 2*nx) = -1/(2*dy); \\
\quad bc(k) = \text{node}; \\
\quad k = k + 1; \\
\textbf{\% + y direction} \\
\quad \text{node} = nx*(ny-1)+j; \\
\quad \text{LHS}(\text{node}, \text{node}) = 3/(2*dy); \\
\quad \text{LHS}(\text{node}, \text{node} - nx) = -2/dy; \\
\quad \text{LHS}(\text{node}, \text{node} - 2*nx) = 1/(2*dy);
bc(k) = node;
k = k + 1;
end
LHS = inv(LHS);

% Output file headers
% SMC Vm to sm_write.txt; ICC to signal_write.txt
sm_write = fopen('sm_hist.txt', 'w');
signal_write = fopen('signal_hist.txt', 'w');
zoi_write = fopen('zoi_hist.txt', 'w');

% Integrate in time using a (RD) first-order operator splitting
% there're two possible explicit schemes here,
% 1) update Vm then individual gating channels (implemented)
% 2) update individual gating channels then Vm (pseudo-explicit)

for t = 0:dt:final

fprintf('%4.1f %s\n', t, 'second');

[stimulusinstant, node_tag, pace_1, pace_2, localt, Iexternal] = input_signal_conditioner_circular(totalstimuluslenght, ...
stimulusfrequency, stimulusinstant, nx, dx, ny, dy, pace_make_node, node_tag, dt, localt, Iexternal, dead_icc);

[Iinternal, localt, IexternalDecay, stimulusinstant] = input_signal_train(
    totalstimuluslenght, peakstimuluslenght, plateaustimuluslenght, localt, t, ...
dt, ...
    ICC_magnitude, Gcouple, Iexternal, stimulusinstant, IexternalDecay);
RHS(bc) = 0;
Iexternal(bc) = 0;

% Solve for SMC Vm
for i = 1:nx*ny
    Y0(i, 2) = Vm(i);
    [T, Y] = ode15s(@t, y, corrias_buist_2007_compute(t, y, Iexternal(i)), tspan, Y0(i, :));
    l = size(Y);
    Y0(i, :) = Y(l(1), :);
    Ion(i) = -Cm*(Y(2) - Vm(i))/dt;
end

RHS = -dt*(Ion - Iexternal)/Cm + (A*(Vm'))';
RHS(bc) = 0;

Vm = LHS*RHS';
Vm = Vm';
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```c
% Write data to file
fprintf(sm_write,'%.6f\n',Vn);
fprintf(signal_write,'%.6f\n',Iexternal);
fprintf(zoi_write,'%.6f\n',pace_1,pace_2);
end
fclose(sm_write);
fclose(signal_write);
fclose(zoi_write);
```
B.5 1D Model

# 1D linear model of slow wave entrainment
# Peng Du
# 10 April 2009
# Auckland Bioengineering Institute, New Zealand

# Define output directory
$OUT="output"; unless (-d $OUT) { mkdir $OUT};

# Define output conditions
$EXPORT=1;
$HISTFILE="$OUT/stomach";

# Specify simulation parameters, nodes, elements (linear)
fem define para:r:min;
fem define coor:r:1d_coor;
fem define node:r:1d_line;
fem define base:r:1d_line;
fem define elem:r:1d_line;
fem group element 1 as ELEM_1D;

# Define grid points
fem define grid:r:1d_line;
fem update grid geometry;
fem update grid metric;
fem group grid external as BOUNDARY;
fem group grid elem ELEM_1D as ICC;

# Define equations and cell model (ICC-SMC:v5b)
fem define equa:r:1d_line class 1,2;
fem define cell:r:1d_line class 1,2;

# Define fibre orientation
fem define fibr:r:1d_line;
fem define elem:r:1d_line fibre;

if ($EXPORT)
{
    fem export node;"$OUT/stomach" as stomach;
fem export elem;"$OUT/stomach" as stomach;
fem export elem;"$OUT/grid" as stomach grid_numbers;
fem export elem;"$OUT/field" as stomach field;
}

# Setup cell and fibre material properties
# 'beta' is varied linearly across ELEM_ID
fem define mate;r;1d_line class 1,2 cell;
fem define mate;r;1d_line class 1,2;
fem update grid material class 1,2;

# Setup initial conditions
fem define init;r;1d_line class 1,2

# Setup solvers
fem define solv;r;CGJacIt_LSGDA class 1;
fem define solv;r;LU_Euler class 2;

if ($HISTORY){
    # Open a history file for ICCs.
    fem open history;$HISTFILE write variables yqs niqslist 1,8;
}

# Solution loop
$Tend = 180;
$Tstart = 0.0;
$dt = 0.1;

for ($time=0;$time<=$Tend;$time+=$dt) {
    if ($time==0) {
        fem solve to 0 class 1,2;
    }
    else {
        fem solve restart to $time class 1,2;
    }
    if ($HISTORY) {
        # Write history information.
        fem write history time $time variables yqs niqslist 1,8 class 1 hist;
    }
    print "*** Time $time\n***";
}
fem quit;
B.6 2D Model

# 2D linear model of slow wave propagation with realistic ICC network structures
# Peng Du
# 1 May 2009
# Auckland Bioengineering Institute, New Zealand

# Define input (for ICC network) and output directory
$OUT="output"; unless (-d $OUT) {mkdir $OUT};
$INPUT = "ipgrgp"; unless (-d $INPUT) {mkdir $INPUT};

# Define output conditions
$EXPORT=1;
$HISTFILE="$OUT/stomach";

# Specify simulation parameters, node, 4x elements (linear)
fem define para: r:min;
fem define coor: r:2d_coor;
fem define node: r:2d_slice;
fem define base: r:2d_slice;
fem define elem: r:2d_slice;
fem group element 1..4 as ELEM_all;

# Define fibre
fem define fibr: r:2d_slice;
fem define elem: r:2d_slice fibre;

# Allocate grid points
fem define grid: r:2d_slice;
fem update grid geometry

# Read in ICC groups (one group per element)
fem group grid: r:$INPUT/ICCelem_0001;
fem group grid: r:$INPUT/ICCelem_0002;
fem group grid: r:$INPUT/ICCelem_0003;
fem group grid: r:$INPUT/ICCelem_0004;

# Read in background/SM (one group per element)
fem group grid: r:$INPUT/Otherselem_0001;
fem group grid: r:$INPUT/Otherselem_0002;
fem group grid: r:$INPUT/Otherselem_0003;
fem group grid: r:$INPUT/Otherselem_0004;

# Group ipgroups
fem group grid grid ICCelem_0001,ICCelem_0002,ICCelem_0003,ICCelem_0004 as ICC;
fem group grid grid Otherselem_0001,Otherselem_0002,Otherselem_0003,
B. Scripts

Otherselem_0004 as SM;
fem group grid external as BOUNDARY;

# Define equations and cell model (dud.xml; ICC_v4.xml)
fem define equa;r;2d_slice class 1,2;
fem define cell;r;2d_slice class 1,2;

# Define material and cell properties
fem define mate;r;2d_slice class 1,2;
fem define mate;r;2d_slice class 1,2 cell;

# Define initial conditions
fem define init;r;2d_slice class 1,2;

# Define solvers
fem define solv;r;CGJacIt_LSODA class 1;
fem define solv;r;LU.Euler class 2;

if($EXPORT)
{
    fem export node;"$OUT/stomach" as stomach;
fem export elem;"$OUT/stomach" as stomach;
fem export elem;"$OUT/grid" as stomach grid_numbers;
fem export elem;"$OUT/field" as stomach field;
}

if ($EXPORT)
{
    # Identify the index of the export cell variable; open a history file
    fem inquire cell_variable Ca_i return_variables VM_ARRAY,VM_IDX;
fem open history;$HISTFILE write variables $VM_ARRAY niqslist $VM_IDX;
}

# Solve (in s; Tstart = start recording time; Tend = end time; dt = dt)
$Tstart = 0.0;
$Tend = 0.5;
$dt = 0.001;
$STEP_TOTAL = ($Tend - $Tstart)/$dt;
$STEP = 0;

for ($time=0;$time<=$Tend;$time+=$dt) {
    if ($time==0) {
        fem solve to 0 class 1,2;
    }
    else {
        fem solve restart to $time class 1,2;
    }
    if ($time > $Tstart) {

if ($EXPORT) {
    # Export Vm
    $FILENAME=sprintf("field\%05d",$STEP);
    fem export elem:"OUT/$FILENAME" field as stomach class 1;
    system( "gzip -f "OUT/$FILENAME.exelem" &" );

    # Export current dipole (mA/mm^2)
    fem def source:; grid one_dipole fixed_position grregion 1 grclass←
        1 time $STEP;
    print "*** Exporting at Time $time and Step $STEP of $(STEP_TOTAL←
        )\n";
    $STEP = $STEP + 1;
    if ($EXPORT) {
        # Write history information.
        fem write history time $time variables $VM_ARRAY;
    }
}
}

fem define sour:;u:;dipole grid grregion 1 grclass 1;
fem export sour:;OUT/dipole_all as dipole;

fem quit;

# Additional file of interest
# perl script:
# AverageingValues_iphist.pl (Convert .iphist into averaged measure (in grid point←
# ) for each dt)
# output: AverageCa.txt
# to run: perl ./AverageingValues_iphist.pl stomach.iphist
# note: need to multiply by 1/(dxdy) to obtain mM/mm^2

# opgrid2EXDATA_3D_nopotential.pl (Convert .opgrid into .exdata, need to fem list ←
# grid points first)
# output: user specified
# to run: perl opgrid2EXDATA_3D_nopotential.pl $input.opgrid $output.exdata
# note: only works if one element is used
B. Scripts

B.7 3D Model

# 3D Full slow wave activation model of stomach (subject ID: H009).
# Peng Du
# 18 February 2010
# Auckland Bioengineering Institute, New Zealand

# Define input and output directory
$INPUT="input";
$OUT="output"; unless (-d $OUT) { mkdir $OUT};

# Define output conditions
$EXPORT=1;
$HISTORY=1; #to export to unemap
$HISTFILE="$OUT/stomach";

# Define class variables
$Vm=1;
$PHI_e=2;

# Specify simulation parameters, node, 88 elements (cubic hermite)
fem define para:r:min;
fem define coor:r:3d_coor;
fem define node;r:$INPUT/H009_full;
fem define base;r:3d_full;
fem define elem;r:$INPUT/H009_full;

# Scale the geometric model to match measurements made in surgery
fem change node scale by 2,2,2;
fem update nodes derivative;

# Group elements into anatomical regions/boundary
fem group element 1..88 as ELEM_all;
fem group element 1..24,68,67,66,65,72,71,65..68,73..75,80 as FUNDUS;
fem group element 69,70,77,78 as PM;
fem group element 76,79,27,26,25,28..32,81..88,33..40 as CORPUS;
fem group element 41..48,49..56,57..64 as ANTRUM;
fem group grid external as BOUNDARY;

# Allocate grid points (10x10x2 per element, can scale-up)
fem define grid;r:3d_full;
fem update grid geometry;

# Define fibre
fem define fibr;r:3d_full;
fem define elem;r:3d_full fibre;
# Define equations and cell model (ICC-SMC:v5c)
fem define equa:3d_full class $V_m,$PHI_e;
fem define cell:3d_full class $V_m,$PHI_e;

if($EXPORT)
{
    #fem export node:"$OUT/stomach" as stomach;
fem export elem:"$OUT/stomach" as stomach;
fem export elem:"$OUT/grid" as stomach grid_numbers;
fem export elem:"$OUT/field" as stomach field;
}

# Define material and cell properties
# RMP and activation times assigned to nodes and linearly interpolated across each element
fem define mate:3d_full class $V_m,$PHI_e;
fem define mate:3d_full class $V_m,$PHI_e cell;

# Define initial conditions
fem define init:3d_full class $V_m,$PHI_e;

# Define solvers
fem define solv:CGJacIt_LSODA class $V_m$
fem define solv:LU_Euler class $PHI_e$

if ($HISTORY$
{
    # Open a history file, niqlist 1 is the location of $V_m$SM (see: fem list cell)
    fem open history;$HISTFILE write variables yqs niqlist 1 binary;
}

# Solve (in s; Tstart = start recording time; Tend = end time; dt = dt)
$Tstart = 59.0$;
$Tend = 150$;
$dt = 0.1$;
$STEP_TOTAL = ($Tend − $Tstart)/$dt$;
$STEP = 0$;

for ($time=0;$time<=$Tend;$time+=dt) {

    if ($time==0$) {
        fem solve to 0 class 1,2;
    }
    else {
        fem solve restart to $time$ class $V_m,$PHI_e;
    }
}
if ($time >= $start) {
    if ($export) {
        # Export Vm
        $filename = sprintf("field%05d","step");
        fem export elem;"$OUT/$filename" field as stomach class 1;
        system( "gzip -f "$OUT/$filename.exelm" &" );
        # Export Phi_e
        $filename_PHI_E = sprintf("PHI_E_field%05d","step");
        #fem export elem;"$OUT/$filename_PHI_E" field as stomach class 2;
        #system( "gzip -f "$OUT/$filename_PHI_E.exelm" &" );
        # Export dipole
        fem def source;c grid one_dipole fixed_position grregion 1 grclass←
        1 time $step;
        fem def source;c grid one_dipole grregion 1 grclass 1 time $step;
        fem def source;c grid grregion 1 grclass 1 time $step;
        $step = $step + 1;
        if ($history) {
            # Write history information.
            fem write history time $step variables yqs niqlist 1 class←
            1,2 hist all binary;
        }
        }
    }
    print "*** Time $time***";
}

# Export current dipole exdata
fem define source:v dipole grid grregion 1 grclass 1;
fem export source:$/OUT/dipole_all as dipole;
if ($history) {
    fem close history binary;
    # Export electrode for 1D line to text signal.
    $signalfile1="$OUT/SEL_SW"
    $comppts1="452,2408,1386,1640"
    fem eval elect:$signalfile1 history $histfile from grid yqs niqlist 1 class 1,2←
    elect $comppts1 binary;
    fem def export;r;unemap_16;
    fem export signal:$signalfile1 electrode signal $signalfile1;
    fem evaluate electrode:$signalfile1 history $histfile from grid yqs niqlist 1←
class 1,2 elect $COMPPTS1 binary;

# Tidy up temporary files
system(" rm $HISTFILE.binhis ");

fem quit;
B.8 EGG Model

# EGG simulation of a whole-organ model embedded in a torso model
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# 1 May 2010
# Auckland Bioengineering Institute, New Zealand

# Define output directory
$OUT = "output"; unless (~d $OUT) {mkdir $OUT};

# Define output conditions
$EXPORT_TORSO = 1;  # torso geometry
$EXPORT = 1;  # dipoles

$TORSO = 1;
$LAPLACE = 1;

use File::Temp;  # Create temporary file for binhist file
($FILEHANDLE, $HISTFILE)=File::Temp::tempfile();

$OFFSET = 40000;
$OFFSET = 0;

# Specify simulation parameters; torso geometry: node, elements
fem define parameters: r:torso;
fem define coordinates 3,1;
fem define nodes: r:torso;
fem define bases: r:torso;
fem define elements: r:torso;

# Transform the torso model
fem change node translate by -4.130,-85;
fem change node rotate by -32 by 0,0,0 axis 1,0,0;
fem change node translate by 0,38,65;
fem change node scale by 1.33,1.33,1.33;
fem update node derivative;

if ($EXPORT_TORSO)
{
  fem export nodes:torso as torso region $TORSO offset $OFFSET;
  fem export elements:torso as torso region $TORSO offset_elem $OFFSET;
}

# Group nodes to be applied for the .ipinit.
fem group nodes 1 as BOTTOM region $TORSO;
fem group nodes 254 as TOP region $TORSO;
fem group nodes 2..253 as MIDDLE region $TORSO;

# Define the laplace problem in the torso.
fem define equation: r; skin region $TORSO class $LAPLACE;
fem define material: r; skin region $TORSO class $LAPLACE;
fem define initial: r; skin region $TORSO class $LAPLACE;
fem define solve: r; skin region $TORSO class $LAPLACE;

# Open a history file for signals
fem open history; $HISTFILE write variables yp niylist 1 binary reg $TORSO;

# Solve (in s; Tstart = start recording time; Tend = end time; dt = dt)
$Tend = 600;
$dt = 1;
$STEP = 0;

for ($time=0; $time<=$Tend; $time+= $dt) {
  if ($time==0) {
    fem solve region $TORSO class $LAPLACE;
    fem define source: r; dipole region $TORSO scale -1;
  }
  else {
    fem update source region $TORSO class $LAPLACE time $time;
    fem solve region $TORSO class $LAPLACE;
  }
}

if ($EXPORT) {
  # Export torso potential
  $FILENAME = sprintf("torso_pot%05d", $time);
  fem export nodes; "$OUT/$FILENAME" region $TORSO class $LAPLACE field as ←
  torso offset $OFFSET;
  fem write history time $time variables yp niylist 1 class 1 hist 1 binary;

  # Export dipole
  fem define source: w; $OUT/dipole_changed as dipole;
  fem export source; "$OUT/dipole_changed as dipole;

  $STEP = $STEP + 1;
}
print "*** Time $time
***";
}

fem close history binary;

# Export to unemap
$SIGNALFILE="EGG_all";
$COMPPTS="182,183,184,185,158,159,160,161,134,135,136,137,110,111,112,113";
fem eval elect; $SIGNALFILE hist $HISTFILE from nodes elect $COMPPTS bin reg $TORSO←
B. Scripts

; fem def export;r:unemap;
fem export sign:$SIGNALFILE signal $SIGNALFILE electrodes;

fem quit;

# Notes
# To convert .signal file to .txt file, use the following command
# /hpc/cmiss/unemap/utilities/i686-linux/unemap/sig2text EGG_all.signal EGG_all.tgt

txt
References


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