High-Resolution Mapping Techniques for Slow Wave Recovery Analysis

Henry Han
bhan957@aucklanduni.ac.nz

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

Doctor of Philosophy in Bioengineering

The University of Auckland

2022
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapters 3 and 6: H. Han, L. K. Cheng, and N. Paskaranandavadivel, "High-Resolution In Vivo Monophasic Gastric Slow Waves to Quantify Activation and Recovery Profiles," in Neurogastroenterology & Motility. 2022; 00:e14422. doi: 10.1111/nmo.14422

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Leo K. Cheng</td>
<td>Experiment design, data collection, and review of manuscript</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadivel</td>
<td>Experiment design, data collection, analysis, and review of manuscript</td>
</tr>
</tbody>
</table>

CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:
- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry Han</td>
<td></td>
<td>21/12/2021</td>
</tr>
<tr>
<td>Prof. Leo K. Cheng</td>
<td></td>
<td>21/12/2021</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadivel</td>
<td></td>
<td>21/12/2021</td>
</tr>
</tbody>
</table>
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 4: H. Han, L. K. Cheng, R. Avci and N. Paskaranandavadiivel, "Quantification of Gastric Slow Wave Velocity Using Bipolar High-Resolution Recordings," in IEEE Transactions on Biomedical Engineering, vol. 69, no. 3, pp. 1063-1071, March 2022, doi: 10.1109/TBME.2021.3112955.

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Experiment design, data collection, analysis and writing of manuscript</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>80</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Leo K. Cheng</td>
<td>Experiment design, data collection, and review of manuscript</td>
</tr>
<tr>
<td>Dr. Recep Avci</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadiivel</td>
<td>Experiment design, data collection, analysis and review of manuscript</td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:

❖ the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and

❖ that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry Han</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Prof. Leo K. Cheng</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Dr. Recep Avci</td>
<td></td>
<td>11/11/2021</td>
</tr>
</tbody>
</table>
This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. Please include one copy of this form for each co-authored work. Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.


<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Extent of contribution by PhD candidate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment design, data collection, analysis and writing of manuscript</td>
<td>80</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Leo K. Cheng</td>
<td>Experiment design, data collection, and review of manuscript</td>
</tr>
<tr>
<td>Dr. Recep Avci</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadivel</td>
<td>Experiment design, data collection, analysis and review of manuscript</td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry Han</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Prof. Leo K. Cheng</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Dr. Recep Avci</td>
<td></td>
<td>11/11/2021</td>
</tr>
</tbody>
</table>
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. Please include one copy of this form for each co-authored work. Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.


<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Leo K. Cheng</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Tim R. Angeli-Gordon</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadivel</td>
<td>Experiment design, data collection, analysis and writing of manuscript</td>
</tr>
</tbody>
</table>

CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Leo K. Cheng</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Tim R. Angeli-Gordon</td>
<td>Data collection and review of manuscript</td>
</tr>
<tr>
<td>Dr. Nira Paskaranandavadivel</td>
<td>Experiment design, data collection, analysis and review of manuscript</td>
</tr>
</tbody>
</table>

Certification by Co-Authors

The undersigned hereby certify that:

❖ the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
❖ that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry Han</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Prof. Leo K. Cheng</td>
<td></td>
<td>11/11/2021</td>
</tr>
<tr>
<td>Dr. Tim R. Angeli-Gordon</td>
<td></td>
<td>11/11/2021</td>
</tr>
</tbody>
</table>
Abstract

Gastrointestinal (GI) bio-electrical slow waves are, in part, responsible for coordinating motility. Abnormal slow wave propagation has been associated with functional disorders such as gastroparesis and chronic unexplained nausea and vomiting. Experimental slow wave recordings provide an improved understanding of the mechanisms that initiate and perpetuate these disorders. Two types of slow wave morphologies, biphasic and monophasic, are commonly recorded extracellularly. The activation phase of biphasic slow waves has been quantified through high-resolution (HR) mapping of normal and abnormal propagation patterns. Monophasic recordings, in comparison to biphasic recordings, enables more reliable capture of the recovery phase of slow waves which can aid in improving our understanding of normal and abnormal propagation patterns. For example, in the cardiac field, monophasic recordings have enabled investigations into restitution curve to improve our understanding of normal and abnormal rhythm. Current methods in the GI field have employed only a limited number of electrodes for monophasic slow wave recordings. The additional information that can be provided by monophasic slow waves deserves more attention. In addition, HR monophasic slow wave recording methods are required to provide information about the spatiotemporal characteristics of monophasic potentials. This thesis aimed to improve the understanding of the mechanisms that underpin GI dysrhythmia, by analysing monophasic slow waves recorded from pig stomachs using novel electrodes and computational analysis methods.

Three types of electrodes were designed and fabricated to record monophasic slow waves: (i) bipolar contact electrode, (ii) dry suction electrode, (iii) wet suction electrode. These designs were validated against conventional HR flexible printed circuit (FPC) surface contact electrode arrays. The performance of the designs was compared in terms of the ability to record gastric monophasic slow waves. The results showed that from 86% pig studies, the wet suction electrode design recorded monophasic slow waves. This rate of success was higher than the other 2 designs (67% for bipolar contact electrode design and 60% for dry suction electrode design). The HR wet suction electrode array was therefore used for all the subsequent studies in this thesis.

To quantify slow wave propagation velocity, the typical approach is to employ a standard “marking-and-grouping” approach along with manual review. This method is time-consuming, especially when analysing HR slow wave recordings of a long duration. A bipolar velocity estimation (BVE) method was developed, where unipolar in vivo HR recordings were used to construct bipolar recordings in different directions. Then, the local directionality of the slow wave was extracted by calculating time delay information. The accuracy of the method was verified using synthetic data and then validated with in vivo HR pig experimental recordings. The BVE algorithm resulted in 19.2 ± 1.7° of direction error and 2.0 ± 0.2 mm/s of speed error, when compared to the standard “marking-and-grouping” method. As a result, gastric slow wave wavefront velocities were estimated using the BVE algorithm efficiently.
In addition to the BVE algorithm, a variable threshold wavelet (VTW) algorithm was developed, featuring wavelet decomposition, to compute the derivative to detect the slow wave activation times (ATs) of monophasic signals. The performance of the VTW algorithm was compared against an existing falling-edge, variable threshold (FEVT) algorithm. Varying levels of synthetic noise were added to the \textit{in vivo} slow wave recordings. Sensitivity, positive-predictive value, area under the curve (A_{roc}) metric and percentage improvement metric of AT identification accuracy were calculated. Compared to the existing FEVT algorithm, the VTW algorithm improved the A_{roc} by 11.1\% in the presence of high-frequency noise, thereby reducing the time needed for manual review and correction for the slow wave analysis workflow.

HR wet suction and conventional FPC electrode arrays were applied on pig stomachs to record monophasic and biphasic slow waves for the following analysis, including validation of monophasic slow wave recordings and characterisation of recovery phases. The coverages of activation and recovery maps for both electrode types (the ratio of the areas of detectable AT and recovery time in the whole array) were calculated and compared. Monophasic slow waves had a more pronounced recovery phase with a higher absolute gradient than biphasic slow waves (0.5 ± 0.1 mV·s\(^{-1}\) vs. 0.3 ± 0.1 mV·s\(^{-1}\)). For suction and FPC electrode arrays, the recovery map coverage in comparison to the activation map decreased by 4 ± 6\% and 43 ± 11\% respectively. Slow wave recovery phase analysis could be performed more efficiently on monophasic signals compared to biphasic signals, due to their more identifiable recovery phases.

HR monophasic slow wave recordings were acquired from different regions of the stomach (upper corpus, mid-corpus, and antrum) using multi-channel wet suction electrode arrays, and were used to define the gastric activation to recovery interval (ARI) distribution, where the ARIs of the antrum region were significantly shorter than the other regions. The slope and the p-value of the linear fit between the ARIs of adjacent wavefronts were related to the slow wave propagation pattern change. The slopes and the p-values between normal pacemaker propagation patterns were around 0.8 and less than 0.05 individually. Before and during a propagation pattern change, the slope decreased or increased to a lower or higher level. The p-value of the regression increased beyond 0.05 during a propagation pattern change. In addition, an investigation on the relation of ARI and slow wave propagation speed was conducted across the stomach. The linear fits had positive mean slopes in the upper corpus region, whereas negative mean slopes in the mid-corpus region.

The main findings from the thesis are that monophasic slow wave recordings can be reliably recorded by the novel HR wet suction electrode array. Furthermore, the propagation pattern of the recovery phase and the morphology of the monophasic slow waves were analysed to understand the genesis and mechanisms of GI dysrhythmia. In addition, the linearity of ARIs was proposed as an indicator of slow wave propagation pattern change. The analysis of recovery dynamics are predictive of pattern change and can be used as an indicator to assess transition to normal pattern for new and existing interventions. Based on this hypothesis, new therapies will be developed. All of these findings aid in the understanding and the treatment of GI disorders.
Acknowledgements

First of all, I would like to thank my supervisors Dr. Nira Paskaranandavadivel and Prof. Leo K. Cheng. From the remote interview before admission to the thesis writing, I can feel their passion to science, which influenced and motivated me all the way along the doctoral journey. Nira has always been curious and creative. I am grateful for his inspirational ideas. Leo is always calm and insightful. I cannot thank him enough for his attention to detail when performing experiments and reviewing manuscripts. I am privileged to be under their supervision.

Secondly, I would like to thank the Health Research Council of New Zealand for supporting this research with funding. I would like to thank the University of Auckland and the Auckland Bioengineering Institute for providing additional financial support due to delays associated with COVID-19.

Thirdly, I would like to thank all the members of the Auckland Bioengineering Institute. The whole institute has provided supportive and productive environment for this research. Especially, I would like to thank Dr. Timothy R. Angeli-Gordon and Ms. Linley Nisbet for their professional skills, and Dr. Recep Avci for his efforts in continuously improving the software for signal processing and in reviewing manuscripts. Also, I would like to thank Mr. Stephen Oding and Dr. Paul Roberts for their help and support with rapid prototyping of parts, Dr. June-Chiew Han for his valuable advice in purchasing consumables and Ms. Naomi Kelly for her helpful administrative support. Moreover, I would like to thank Assoc. Prof. Peng Du for his friendship and Dr. Saeed Alighale for his help in designing and building the pressure control system.

Last but not least, I would like to thank my family. I cannot accomplish this research without the encouragement from my wife Mia and my daughter Ophelia, who was born during this doctoral journey. I would like to thank my mother Xiuqi and my parents-in-law Jing and Hui, for their help in taking care of the family in Ophelia’s early days. Also, I would like to thank my other family members Cai and Luo, thank you for being with me and giving me warm smiles whenever I am blue.
Contents

List of Figures xix

List of Tables xxiii

Nomenclature xxv

1 Motivations and Objectives 1
  1.1 Background ................................................................. 1
  1.2 Motivations ................................................................. 1
  1.3 Objectives ................................................................. 2

2 Introduction 3
  2.1 Gastric Electrophysiology ............................................. 3
    2.1.1 Slow Wave Generation and Propagation ......................... 3
    2.1.2 Slow Wave Origin .................................................... 6
    2.1.3 Slow Wave Frequency ............................................... 6
    2.1.4 Slow Wave Amplitude ............................................. 8
    2.1.5 Slow Wave Propagation Direction ................................. 9
  2.2 Slow Wave Morphologies .............................................. 12
    2.2.1 Biphasic Slow Waves ............................................... 12
    2.2.2 Monophasic Slow Waves ......................................... 14
    2.2.3 Relation Between the Morphologies ............................... 16
  2.3 Methods to Record Monophasic Slow Waves ......................... 19
    2.3.1 Intracellular Methods ............................................ 19
    2.3.2 Pseudo-Intracellular Methods ................................... 21
    2.3.3 Contactless Methods ............................................. 26
  2.4 Slow Wave Analysis Algorithms in the Gastrointestinal Field ... 27
    2.4.1 Activation Time Detection Algorithms ......................... 27
    2.4.2 Other Algorithms ................................................. 28
  2.5 Summary ................................................................. 31

3 Monophasic Slow Wave Recording Electrode Designs 33
  3.1 Methods ................................................................. 33
3.1.1 Bipolar Contact Electrode Design and Manufacture 33
3.1.2 Dry Suction Electrode Design and Manufacture 34
3.1.3 Wet Suction Electrode Design and Manufacture 39
3.1.4 Experimental Methods 41
3.1.5 Signal Processing and Slow Wave Analysis 42
3.2 Results 42
3.2.1 Bipolar Contact Electrode 42
3.2.2 Dry Suction Electrode 44
3.2.3 Wet Suction Electrode 44
3.3 Discussion 45
3.4 Summary 46

4 Quantification of Gastric Slow Wave Velocity Using Bipolar High-Resolution Recordings 47
4.1 Methods 47
4.1.1 Synthetic Signal Generation 48
4.1.2 The Bipolar Velocity Estimation Algorithm 48
4.1.3 Visualisation and Performance Measurements 53
4.2 Results 54
4.2.1 The Bipolar Velocity Estimation Algorithm 54
4.2.2 Visualisation 56
4.2.3 Performance Measurements 56
4.3 Discussion 58
4.4 Summary 60

5 Detection of Monophasic Slow Wave Activation Time Using Wavelet Decomposition 61
5.1 Methods 61
5.1.1 The Variable Threshold Wavelet Algorithm 61
5.1.2 Performance Measurements 62
5.2 Results 64
5.3 Discussion 65
5.4 Summary 66

6 Monophasic Gastric Slow Waves to Quantify Activation and Recovery Profiles 67
6.1 Methods 67
6.1.1 Categorisation of Monophasic Slow Wave 68
6.1.2 Average Morphology, Amplitude, Activation to Recovery Interval, and Gradient 69
6.1.3 Coverage Loss Percentage Calculation 69
6.1.4 Activation and Recovery Map Generation 70
6.2 Results 70
6.2.1 Categorisation of Monophasic Slow Wave and Average Morphology 70
List of Figures

2.1 Anatomy of the human stomach .................................................. 4
2.2 ICC network in the canine gastric antrum ..................................... 4
2.3 Mouse gastric antrum slow waves of various morphologies ............... 5
2.4 Slow wave recordings 1 week and 3 weeks after the longitudinal gastric bisection and reunion of the stomach ................................................................. 7
2.5 Recordings of multiple slow waves simultaneously propagating .......... 8
2.6 Slow wave amplitude distribution in the canine and human stomach ...... 9
2.7 Slow wave recordings from the human antrum .................................. 10
2.8 Activation maps from different regions of the stomach ..................... 11
2.9 Abnormal slow wave propagation patterns .................................... 12
2.10 Biphasic action potential and slow wave ...................................... 13
2.11 Design of the differential electrode used to record gastric biphasic slow waves ................................................................. 13
2.12 Design of the HR FPC electrode array ......................................... 14
2.13 Extracellular monophasic cardiac action potential and GI slow wave .... 15
2.14 Comparison of intracellular and extracellular potentials ................. 17
2.15 Comparison of cardiac ARIs between biphasic and monophasic action potentials ................................................................. 17
2.16 Comparison of slow waves recorded by an agar-Tyrode filled glass capillary tube from different distances above the serosa of cat jejunum .... 18
2.17 Hypothetical circuit to explain recording morphologies ................... 18
2.18 Comparison of intestinal slow wave activity before and after administration of nifedipine ................................................................. 19
2.19 Gastric slow wave recordings ...................................................... 20
2.20 Contact electrode designed for cardiac monophasic action potential recording ................................................................. 21
2.21 A contact electrode design ......................................................... 22
2.22 A simplified and a clinical contact electrode designs ....................... 23
2.23 Wet suction electrode designs .................................................... 24
2.24 Dry suction electrode designs used to record slow wave activity ......... 25
2.25 X-ray photo of the application of a multi-channel NG electrode .......... 26
2.26 An example of optical slow wave recording ................................... 27
2.27 Flowchart and intermediate results of a GI spike removal algorithm ....... 29
2.28 Flowchart and intermediate results of a GI AT detection algorithm ....... 30
2.29 An example of an automated RT detection .................................... 31
List of Figures

3.1 Single-channel bipolar contact electrode design drawing and photos .......................... 35
3.2 Multi-channel bipolar contact electrode array design drawing and photos ...................... 36
3.3 Single-channel dry suction electrode design drawing and photos ............................... 37
3.4 Multi-channel dry suction electrode array design drawing and photos ......................... 38
3.5 The flowchart and a photo of the pressure control system ...................................... 39
3.6 Single-channel wet suction electrode ........................................................................ 40
3.7 Multi-channel wet suction electrode array .................................................................. 41
3.8 Examples of bipolar contact electrode recordings ....................................................... 43
3.9 Examples of dry suction electrode recordings ............................................................. 44
3.10 Examples of wet suction electrode recordings ............................................................ 45

4.1 Flowchart of the BVE algorithm .................................................................................... 49
4.2 Kurtosis of signal gradient calculation for experimental recordings ............................. 50
4.3 Patch size configurations for the BVE algorithm ......................................................... 51
4.4 Electrode pair configurations ...................................................................................... 52
4.5 Activation maps of the 3 synthetically generated slow wave profiles ............................ 54
4.6 Intermediate results of the BVE algorithm ................................................................. 55
4.7 Absolute direction and speed error results in comparison to the gold standard ............ 57
4.8 Two example tracings of synthetic high-frequency noise added signals ...................... 58

5.1 Wavelet decomposition using algorithme à trous ......................................................... 62
5.2 Flowchart of the VTW algorithm .................................................................................. 63
5.3 Examples of ventilator noise added and high-frequency noise added data ................... 63
5.4 A case where the VTW algorithm outperformed the FEVT algorithm when processing high-frequency noise added signal with an SNR of 52 dB .................................................. 65
5.5 Performance measurement results of both the algorithms .......................................... 66

6.1 Measurement of the slow wave amplitude and the early repolarisation amplitude ........ 68
6.2 Average morphology of biphasic slow wave, monophasic slow wave, and monophasic slow wave morphology 1 and 2 ................................................................. 71
6.3 Box plots quantifying the amplitude, ARI, AT gradient, and RT gradient, for biphasic slow waves, monophasic slow waves, and monophasic slow wave morphology 1 and 2 .................................................. 73
6.4 Comparison of coverage loss percentages between the FPC electrode arrays and the wet suction electrode arrays .................................................................................. 74
6.5 Activation and recovery maps with the positions of electrode arrays ............................ 75

7.1 Representative monophasic slow waves and regional variations in ARI across the stomach 79
7.2 The activation maps and the location of the electrodes of case 1 .................................. 80
7.3 Linear fits between the ARIs from adjacent wavefronts and the slope and the p-value of all the linear fits for case 1 ..................................................................................... 81
7.4 The activation maps and the location of the electrodes of case 2 .................................. 83
7.5 Linear fits between the ARIs from adjacent wavefronts and the slope and the p-value of all
the linear fits for case 2 ................................................................. 84
7.6 The activation maps and the location of the electrodes of case 3 ............................ 85
7.7 Linear fits between the ARIs from adjacent wavefronts and the slope and the p-value of all
the linear fits for case 3 ................................................................. 86
7.8 The activation, recovery, ARI, and speed maps, the linear fit between ARI and speed, and
the location of the electrodes of an example wavefront from the upper corpus region .... 87
7.9 The activation, recovery, ARI, and speed maps, the linear fit between ARI and speed, and
the location of the electrodes of an example wavefront from the mid-corpus region .... 88
7.10 The linear fit slope comparison between slow wave ARI and propagation speed from
different regions of the stomach ................................................... 88
List of Tables

3.1 Summary of experiments conducted with 3 types of electrodes . . . . . . . . . . . . . . 43
4.1 Mean absolute direction and speed errors for synthetic signals for 4 noise levels . . . . 56
Nomenclature

3D  Three-Dimensional  
AdTh  Adaptive Threshold  
APD  Action Potential Duration  
ARI  Activation to Recovery Interval  
AT  Activation Time  
BNC connector  Bayonet Neill-Concelman Connector  
BVE  Bipolar Velocity Estimation  
cpm  Cycles per Minute  
FEVT  Falling-Edge, Variable Threshold  
FPC  Flexible Printed Circuit  
GI  Gastrointestinal  
HR  High-Resolution  
ICC  Interstitial Cell of Cajal  
ICC – IM  ICC in the Intramuscular Region  
ICC – MY  ICC in the Myenteric Region  
KuGr  Kurtosis of Signal Gradient  
NEO  Non-Linear Energy Operator  
NG  Nasogastric  
PID  Proportional-Integral-Derivative  
PIM  Percentage Improvement Metric  
PPV  Positive Predictive Value
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAI</td>
<td>Recovery to Activation Interval</td>
</tr>
<tr>
<td>RT</td>
<td>Refractory Time</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to Noise Ratio</td>
</tr>
<tr>
<td>VTW</td>
<td>Variable Threshold Wavelet</td>
</tr>
</tbody>
</table>
Chapter 1
Motivations and Objectives

In this chapter, a brief background of the gastrointestinal (GI) system is presented, followed by the motivations and the objectives of this thesis. The overall aim of this thesis is to help understand the GI electrophysiology, especially to help understand the pathways that initiate and maintain GI disorders.

1.1 Background

GI diseases affect 60 to 70 million people annually in the United States [98]. The stomach is a key organ of the GI system and is responsible for digestion. The motility of the GI system is coordinated, in part, by bio-electrical events known as slow waves. These slow waves contain vital information that can help improve the understanding of the mechanisms underlying normal and abnormal GI motility. For example, the activation and the recovery phases of the slow wave recordings denote the depolarisation and the repolarisation time courses of the cells around the recording site. High-resolution (HR) flexible printed circuit (FPC) electrode arrays enabled recordings of spatiotemporal slow waves and characterisation of propagation patterns. The activation times (ATs) of slow wave events have been identified and visualised by generating activation maps. Furthermore, abnormal slow wave propagation patterns have been observed and associated with a number of functional GI motility disorders.

1.2 Motivations

Further GI research is important to understand the mechanisms that underlie disorders and to develop targeted therapies. Biphasic potentials have been recorded and analysed in HR for over a decade. To date, the electrodes used to record monophasic potentials are sparse and of a low channel count. The corresponding recordings were not able to provide propagation information of the slow wave. To analyse the spatiotemporal information from monophasic potentials, as has been conducted in HR biphasic potential recordings, new recording device designs, experimental techniques, and analysis methods are required.

The morphology of monophasic slow waves differs from biphasic slow waves and provides additional information of the recovery phase of the slow wave. However, this additional information has not been
Motivations and Objectives

fully investigated. Compared to biphasic slow waves, monophasic slow waves are less commonly studied in the GI field. High-amplitude and high-velocity slow wave propagation has been associated with gastric dysrhythmia [84], but the genesis and maintenance mechanisms of these abnormalities are poorly understood. In comparison, in the neuronal and cardiac fields, additional information of cell repolarisation has been obtained by analysing monophasic potentials. Vital findings such as cardiac electrical restitution have been established and used extensively to understand the cardiac electrophysiology and to explain the genesis of arrhythmias. In the GI field, the additional information that can be provided by monophasic slow waves deserves more attention. The additional information of cell repolarisation provided by monophasic slow wave recordings can help improve our understanding of the genesis and maintenance mechanisms of gastric disorders. It can also be used to help define the electrical restitution across the stomach with the use of pacing or drugs. These mechanisms will be essential in developing novel therapeutics for functional motility disorders.

1.3 Objectives

The objectives of this thesis were to record and analyse HR monophasic slow waves using in vivo extracellular mapping techniques. The goal was to develop a framework capable of recording (hardware) and analysing (software) HR monophasic slow waves. For the hardware component, novel electrode designs were developed and applied in animal studies to record HR monophasic slow waves from gastric serosa. For the software component, automated algorithms were proposed to analyse the spatiotemporal information from HR slow wave recordings. Finally, investigations of HR monophasic slow wave recordings were conducted to help understand the progression of gastric disorders.
Chapter 2

Introduction

In this chapter the electrophysiological mechanisms that underpin gastric activity are reviewed. Electrode designs previously used to record GI bio-electrical slow wave activity are described, in particular the evolution of suction electrodes, which were used to record the recovery phase of the slow wave. Existing algorithms for analysing slow waves are outlined, with a focus on HR recording analysis.

2.1 Gastric Electrophysiology

The stomach is a key organ of the GI system and is responsible for digestion. A schematic diagram of human stomach anatomy is shown in Figure 2.1. The stomach can be anatomically divided into 4 regions: fundus, corpus, antrum, and pylorus. The fundus usually contains the gas produced from digestion and relaxes to allow more food to be stored. The corpus is where most of the food is stored. In the antrum, chyme is produced by mixing the food. The rate at which chyme enters the intestine is regulated by the pylorus.

The motility is, in part, governed by 2 types of bioelectrical events termed slow waves and spikes. Spikes have been related to the amplitude and the duration of contractions in human small intestine [86]. Slow waves are generated and propagated by interstitial cells of Cajal (ICC)s in the musculature [105], and play a key role in coordinating gastric motility. ICCs act as intermediates between the smooth muscle cells and the enteric nervous system [50]. The enteric nervous system, which comprises of neurons that lie along the gut, that play key role in regulating motility, along with affecting other gut function such as local blood flow, mucosal transport and modulation of immune and endocrine function. The loss of ICCs has been associated with abnormal slow wave activity and gastric disorders such as gastroparesis [44, 82], chronic unexplained nausea and vomiting [5], and functional dyspepsia [110]. In this section, slow wave generation, propagation, origin, frequency, amplitude, and direction were described by reviewing previous studies.

2.1.1 Slow Wave Generation and Propagation

ICCs have been found throughout the GI tract and have been classified into sub-populations based on their location within the musculature. An ICC network was formed by these sub-populations [123, 28].
Different regions of the GI tract have variations in the density and the function of the ICCs. For example, the structure of the ICC network in the canine gastric antrum is shown in Figure 2.2. ICCs in the myenteric region (ICC-MY) generate the slow waves, whereas ICCs in the intramuscular region (ICC-IM) transmit these slow waves to smooth muscle cells [28, 122]. In another study, ICC-IM was also found to generate the slow waves [51]. In addition, ICCs in the septa region between the circular muscle bundles further transmit these slow waves to distant bundles of circular muscle [56]. In another study, slow waves of various configurations have been recorded from different layers of the gastric serosa [55]. Figure 2.3 shows 3 types of signal morphology intracellularly recorded from the cells of the first 3 layers underneath the serosa, termed “follower cells”, “pacemaker cells”, and “slow wave cells”. The variance of the morphology was because of the inconsistent density of ICCs in different regions of the stomach. This finding suggested that the generation of slow waves was related to at least ICC-MY and ICC-IM.

Ca\(^{2+}\) is involved in slow wave generation and propagation. The Ca\(^{2+}\) released by intracellular IP\(_3\) receptor-operated Ca\(^{2+}\) stores activated Ca\(^{2+}\)-activated chloride channels in ICCs [106, 43, 57]. The efflux of Cl\(^-\) generated a spontaneous inward current, which gave rise to the slow waves. For slow wave
Figure 2.3: Mouse gastric antrum slow waves of various morphologies. A and B were intracellularly recorded from the first layer of cells underneath the serosa termed the “follower cells”. C and D show the signal morphology intracellularly recorded from the layer of cells underneath the “follower cell” layer termed the “pacemaker cells”. The third signal morphology are shown in E and F and was intracellularly recorded from the third layer of cells underneath the serosa termed the “slow wave cells”. Adapted from [55].
propagation, two mechanisms have been proposed. The first mechanism is that slow waves propagate serially from ICC to ICC because of the voltage dependence of ICCs [18]. The alternative proposed mechanism is that slow waves propagate through coupled oscillator-based entrainment [59]. The coupled oscillator-based entrainment involve the $\text{Ca}^{2+}$ release-refill cycle, where the $\text{Ca}^{2+}$-dependent membrane is current modulated and $\text{Ca}^{2+}$ is stored and released through cell-coupling. This mechanism couples the frequencies of adjacent cells by voltage-dependent modulated release of $\text{Ca}^{2+}$ store and underpins the entrainment of slow wave propagation. Gastric ICCs have a proximal-to-distal gradient in intrinsic pacemaker frequency, which was unified by this mechanism during slow wave propagation [105]. The loss of slow wave entrainment has been associated with the decrease of ICC counts and gastric dysrhythmias [82, 73].

2.1.2 Slow Wave Origin

The origin of slow waves within the stomach has been studied since the 20th century [64], and was termed the gastric pacemaker. Kelly et al. conducted a gastric bisection to isolate the greater curvature from the lesser curvature, followed by a reunio using sutures. Slow waves were then recorded for 3 months [64]. The slow wave recordings 1 and 3 weeks after the reunion are shown in Figure 2.4. The slow waves recorded 1 week after the reunion showed that the lesser curvature was uncoupled from the greater curvature. The greater curvature slow wave recordings had regular slow wave rhythm unlike the lesser curvature. For the recordings from 3 weeks after the reunion, the slow wave coupling between the greater and lesser curvature was re-established. This re-establishment is also termed the re-entrainment of the pacemaker. Thus, the location of the dominant pacemaker in canine was in the greater curvature half of the proximal corpus. Furthermore, Hinder et al. located the gastric pacemaker of human in the mid-corpus along the greater curvature 5 - 7 cm distal to the cardia [53]. This location has recently been verified by the HR recording techniques [72, 83]. In comparison, for dysrhythmic subjects, the pacemaker location can be varying and unstable [84].

2.1.3 Slow Wave Frequency

Slow wave frequency varies between species. The frequency of gastric slow waves is approximately 5 cycles per minute (cpm) in dogs [64]. This was confirmed by Lammers et al. using HR recording techniques [72]. In humans, the frequency of slow waves has been measured at around 3 cpm [53]. Miedema et al. confirmed that the mean frequency of slow wave activities in human stomachs was 3.2 ± 0.1 cpm [78]. In addition, O’Grady et al. utilised HR recording techniques to show the frequency at different regions of the stomach (pacemaker, corpus, and antrum) was consistent (2.83 ± 0.35 cpm) [83].

There are multiple slow waves propagating in the stomach at the same time. The number of slow waves simultaneously propagating in the canine stomach is 3 to 5 [72]. In the human stomach, this number is 3 to 4. HR slow waves were recorded from human stomachs with an average period of about 20 s, as shown in Figure 2.5(D) [83]. Isochronal maps were generated using manually marked ATs to visualise the pathway of the slow wave propagation. In Figure 2.5(E), electrodes are denoted as black dots and the area
Figure 2.4: Slow wave recordings (A) 1 week and (B) 3 weeks after the longitudinal gastric bisection and reunion of the stomach. (A) The greater curvature recordings showed regular slow wave rhythm unlike the lesser curvature. (B) Coupling between the greater and lesser curvature was re-established after 3 weeks. Adapted from [64].
Figure 2.5: Recordings of multiple slow waves simultaneously propagating. (A) The position of the HR FPC electrode array (192 channels). (B) The activation map. Velocity map is shown in (C). (D) The recordings of 18 representative channels indicated in (A) and (B). (E) Within 10 seconds, three slow waves (a, b, and c) were recorded simultaneously propagating. Adapted from [83].

of slow wave propagation per 2 s is shown as colour bands. It can be observed from Figure 2.5(D - E) that 3 slow waves (a, b, and c) were simultaneously propagating across the mapped area.

2.1.4 Slow Wave Amplitude

Slow wave amplitude varies across different regions of the stomach. Slow waves of different amplitudes were recorded by Bozler in 1945 [17]. Lammers et al. conducted a study to measure the slow wave amplitude variation in the canine stomach [72]. As shown in Figure 2.6(A), slow waves propagating in the pacemaker region were of high amplitude (1.8 ± 1.0 mV). In the rest of the corpus and fundus, the slow waves were of low amplitude (0.8 ± 0.5 mV). In the antrum, the slow waves had the highest amplitude (2.0 ± 1.3 mV). However, no slow waves were recorded from the proximal fundus, cardia, and the region between antrum and pylorus [53, 72]. The fundus was described as electrically “silent” [23]. By using HR recording techniques, slow waves were identified to propagate towards fundus and cardia for approximately 38 mm before terminating in human stomachs [83].

The distribution of slow wave amplitudes in human and canine stomachs are similar. A comparison between the slow wave amplitude in canine and human stomachs is shown in Figure 2.6. For both
2.1 Gastric Electrophysiology

Figure 2.6: Extracellular slow wave amplitude distribution in the (A) canine and (B) human stomach. The slow wave amplitude was highest in the pacemaker (proximal fundus) and the antrum regions of the stomach. The central mark of each box represents the median. The bottom and top edges of the boxes represent the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles. The whiskers extend to the most extreme data points that are within 1.5 times the interquartile range away from the bottom or top of the box. Adapted from [72, 83].

Canine and human stomachs, the amplitudes are in general higher in the pacemaker and the antrum regions, compared to the corpus region [78]. The introduction of HR recording techniques enabled spatiotemporal analysis for slow wave recordings, including amplitude measurements. By utilising HR recording techniques, this distribution was verified [83].

A study focusing on HR terminal antrum slow wave recording was carried out in 2016 [12]. The slow wave recordings from the antrum are shown in Figure 2.7. In the antrum, slow wave amplitude started to increase until peak amplitude was reached and sustained, in the area 36 to 14 mm proximal to the pyloric ring, as indicated by the black arrows beside the amplitude maps in Figure 2.7(C - D). The slow waves were then terminated at 14 mm proximal to the pyloric ring. In this study, HR mapping helped in understanding the structure function relation of the anatomical regions in the stomach.

2.1.5 Slow Wave Propagation Direction

Normal slow waves originate at the pacemaker region in the upper corpus and propagate towards the pylorus [64, 72]. In the pacemaker region, slow waves propagate isotropically [83]. Proximal to the pacemaker region, slow waves propagated for a limited distance towards the fundus and cardia. Distal to the pacemaker region, slow waves propagated toward the pylorus in a circumferential band. In both the corpus and the antrum, slow waves propagate in an antegrade direction, along the longitudinal axis of the stomach, and from the greater curvature to the lesser curvature along the transverse axis in the mid-corpus [53, 78]. HR recording techniques were applied on different regions of the stomach to identify the slow wave direction across the stomach. The activation maps of the recordings were generated (Figure 2.8),
Figure 2.7: Slow wave recordings from the human antrum. Two examples from the human antrum slow wave recordings are shown in (A) and (B). The corresponding activation, amplitude, and velocity maps are shown in (C) and (D). The first 3 channels in each example (A1 - A3 and C1 - C3) were located in the proximal antrum, while the last 3 channels (B1 - B3 and D1 - D3) were located in the prepyloric antrum. Higher amplitude slow waves were recorded in the prepyloric antrum than the proximal antrum. The black arrows beside the amplitude maps show the regions where slow wave amplitude started to increase until peak amplitude was reached and sustained. Adapted from [12].
Figure 2.8: Activation maps from different regions of the stomach. (A - C) The locations of HR electrode arrays: (A) the pacemaker region, (B) the lower fundus region, and (C) the antrum region. (D - F) The corresponding activation maps. Slow waves origin from the pacemaker region, propagate towards distal along the longitudinal axis of the stomach. Adapted from [83].

which verified the slow wave direction results from early studies. In addition, slow waves propagated rapidly circumferentially, and slowly propagated inferiorly in a longitudinal manner [83].

Abnormal slow wave propagation patterns were also recorded, which include retrograde propagation, ectopic pacemaker propagation, conduction block propagation, and re-entry propagation, as shown in Figure 2.9. An example of retrograde propagation pattern and the corresponding electrode array location are shown in Figure 2.9(A - B), where slow waves travel from distal to proximal in the mid-corpus. An example of ectopic pacemaker propagation pattern is shown in Figure 2.9(C), where slow waves generated from the ectopic pacemaker (lower) collided with the slow waves from the normal pacemaker above the electrode array. An example of conduction block propagation pattern is shown in Figure 2.9(D). In this case, slow waves changed their normal propagation direction to activate around the blocked area. An example of re-entry propagation pattern is shown in Figure 2.9(E). Slow waves travelled around the
2.2 Slow Wave Morphologies

Slow waves of various morphologies have been recorded in previous studies. Slow waves have been categorised into 2 major groups based on their morphology: biphasic and monophasic.

2.2.1 Biphasic Slow Waves

The morphology of biphasic slow waves (Figure 2.10) was described as a positive deflection followed by a negative deflection (activation phase) and a gradual return to the baseline (recovery phase) [14]. The activation phase is when the cells at the recording site get depolarised, whereas the recovery phase is when these cells get repolarised as shown in Figure 2.10(A). The AT and the recovery time (RT) are defined at the steepest points during the activation phase and the recovery phase.

In the cardiac field, the bio-electrical activities, similar to slow waves in the GI system, are termed action potentials. Silver wire unipolar electrodes were placed softly on the epicardium to record in vivo biphasic action potentials in 1990 [52]. An example of a cardiac biphasic action potential is shown in Figure 2.10(B). The morphology of biphasic action potentials generally contains an upstroke away from the baseline, a downstroke returning and passing down the baseline, and an upstroke back to baseline.

GI biphasic slow wave recorded by a unipolar contact electrode is shown in Figure 2.10(C). Bozler used a differential (bipolar) contact electrode to record in vivo gastric biphasic slow waves in 1945 [17]. The diagram of the electrode and the recording from this study are shown in Figure 2.11. The morphology of the recording is similar to Figure 2.10(C). Later in 1967, biphasic slow waves were recorded in vitro by placing unipolar electrodes 1 mm above the serosa in Tyrode solution [15].
2.2 Slow Wave Morphologies

Figure 2.10: Biphasic action potential and slow wave. (A) Genesis of biphasic recordings. A wave of cell depolarisation passed underneath the recording electrode. The corresponding recordings are shown on the left at 3 different times. (B) A cardiac biphasic action potential recording. The vertical line indicates the AT. (C) A GI biphasic slow wave recording. The morphology of both recordings is similar. Adapted from [118, 52, 6]

Figure 2.11: Design of the differential electrode used to record gastric biphasic slow waves. (A) The surface in contact with the muscle. (B) The side view. The capillary electrode E was enlarged and shown in (C). S is the shell of the electrode, which was made of glass or enamelled brass. Each of the 2 capillary electrodes contained a platinum wire. (D) Gastric biphasic slow wave recorded by the electrode. Reproduced from [17].
HR recording techniques were introduced to record GI biphasic slow waves in spatiotemporal detail [70]. The design of an HR FPC electrode array is shown in Figure 2.12 [29]. This design incorporated 32 contact electrodes with 4 mm inter-electrode distance on a piece of FPC. HR FPC electrode arrays have been used in vivo to record HR biphasic slow waves in pig and human studies [6, 29, 12, 83, 82]. The additional spatial information has been used to map slow wave propagation in an HR manner, which was not possible with traditional sparse electrodes. The pathway of the slow wave activation propagation was identified and visualised using isochronal activation maps (Section 2.4).

In addition to the activation phase, studies on the recovery phase have been conducted. In the cardiac field, under variable cycle lengths, the relationship between action potential duration (APD) and the preceding diastolic interval is termed the cardiac electrical restitution and was defined with the use of pacing or drugs [116, 101]. Non-uniform shortening of the APD in different myocardial regions upon heart rate acceleration was used to explain arrhythmia [89, 124]. In the GI field, the term activation to recovery interval (ARI) was more commonly used to refer APD, and was defined as the time difference between the AT and the corresponding RT. In addition, the recovery to activation interval (RAI) was defined as the time difference between the RT and the next AT. These metrics have been related to dysrhythmic slow wave propagations. Biphasic slow waves recorded by HR FPC electrode arrays were analysed. Normal propagations have longer ARI and shorter RAI than dysrhythmic propagations [93].

### 2.2.2 Monophasic Slow Waves

The morphology of extracellular monophasic slow waves (Figure 2.13) was described as an upstroke (activation phase) followed by a plateau and a downstroke (recovery phase) [32]. In some cases, early repolarisation exists as the rapid but transient downstroke between the upstroke and the plateau of the
2.2 Slow Wave Morphologies

Figure 2.13: Extracellular monophasic (A) cardiac action potentials (paced), (B) GI slow wave and their underlying ionic currents. Similar morphologies are observed for the slower components in (A) and (B). The arrows in (A) indicate early repolarisation. More information about the ionic currents underlying the monophasic slow wave morphology is described in Section 2.1.1. Adapted from [66, 6].

monophasic slow wave, as shown in Figure 2.13(A) [77, 109]. Similar to the biphasic slow wave, the AT and RT of monophasic slow waves are defined at the steepest points during the activation phase and the recovery phase.

In the cardiac field, monophasic action potentials were recorded in vitro in a study conducted by Knollmann et al. [66]. These monophasic action potentials were recorded from intact mouse heart using a bipolar contact electrode design described in Section 2.3.2 (Figure 2.22), and then validated against intracellular potentials. The recording from this study is shown as an example of cardiac monophasic action potential in Figure 2.13(A). Later in 1990, floating intracellular microelectrodes were used to record in vivo monophasic action potentials inside cells of dog epicardium [52]. The morphology of cardiac monophasic action potentials begins with an upstroke away from the baseline (activation phase), a plateau, an early repolarisation, and a downstroke back to the baseline (recovery phase). The phase of early repolarisation has been used as a characteristic to diagnose J wave syndrome, which is a cause of idiopathic ventricular fibrillation and leads to sudden cardiac death [77, 109].

An example of GI monophasic slow wave is shown in Figure 2.13(B). The morphology is similar to Figure 2.13(A). In 1942, monophasic slow waves were recorded in vitro by Bozler from rabbit and cat small intestine stripes [16]. In a study conducted by Bortoff in 1967, in vitro monophasic slow waves...
from cat jejunum were recorded by both needle electrodes (intracellular) and glass pipette electrodes [15]. The intracellular recordings were transmembrane slow wave recordings [38]. In addition, slow waves of 3 morphology types were recorded in this study, with different distances or pressures between the electrodes and the recording sites. Later in 1978, *in vitro* transmembrane monophasic slow waves from canine and human gastric smooth muscles were recorded [32]. The morphologies of transmembrane slow waves from dogs and humans were similar to those from rabbits and cats. Recently, *in vivo* extracellular monophasic slow waves were recorded from pigs by a single point suction electrode [6].

The morphology of the monophasic action potentials was previously explained by 2 hypotheses [38]. The first hypothesis stated that the suction electrodes injured the cells underneath and as a result monophasic action potentials were recorded between the injured and uninjured cells. It was unclear whether the source of the monophasic action potentials was the injured cells (depolarising electrode) or the uninjured cells (reference electrode). The second hypothesis is based on the “volume conductor” theory, where in the tissue around the capillary glass tube wall, a localised depolarising effect was caused by the negative pressure. A “sink” or “source” current was created between the affected tissue (depolarising electrode) and the adjacent unaffected tissue (reference electrode) and gave rise to the monophasic morphology. This hypothesis suggested that the source of the monophasic action potentials was the reference electrode. The latter hypothesis was supported through simulation studies in the cardiac and the GI field, in which the potential voltage of the affected tissue was fixed, resulting in monophasic morphology [6, 119]. In addition to the modelling studies, experimental studies have also confirmed that the reference electrode as the source of monophasic action potentials [27]. The underlying ionic currents that contribute to the monophasic morphology are summarised in Figure 2.13(C - D). More details about the ionic currents underlying the monophasic slow wave morphology are described in Section 2.1.1. Compared to the cardiac action potential, the GI monophasic slow wave is less investigated and therefore not well understood.

### 2.2.3 Relation Between the Morphologies

The intracellular slow waves typically have a monophasic morphology, whereas extracellular slow waves typically have a biphasic morphology. In the neuronal field, the relation between these 2 morphologies was studied by recording potentials from hippocampal slices of pigs, using intracellular and extracellular electrodes simultaneously [25]. The recordings from this study are shown in Figure 2.14. The intracellular and extracellular potentials were of monophasic and biphasic morphologies. In addition, the extracellular potentials approximated the double differential of the corresponding intracellular potentials. However, the morphology of extracellular potentials was not always proportional to the double differential of the intracellular potential morphology. In a modelling study, it was found that the morphology of extracellular potentials was directly related to the spatial distribution of the intracellular potentials [114].

In the cardiac field, biphasic action potentials have been shown to be proportional to the second temporal derivative of the monophasic action potentials [52]. This relation was confirmed by comparing ARIIs from the action potentials of both morphologies. A detailed comparison is shown in Figure 2.15. In this comparison, the cardiac ARI differences were negligible between biphasic and monophasic action
2.2 Slow Wave Morphologies

Figure 2.14: Comparison of intracellular (Intra) and extracellular (Extra) potentials. These potentials were recorded from hippocampal slices of pigs. The intracellular potential had a monophasic morphology, whereas the extracellular potential had a biphasic morphology. The extracellular potential approximated the double differential of the intracellular potential. Adapted from [25].

Figure 2.15: Comparison of cardiac ARIs between biphasic and monophasic action potentials. Electrogram (Eg) had a biphasic morphology, whereas the transmembrane action potential (TMAP) had a monophasic morphology. The ARIs are displayed in ms and are comparable between TMAP and Eg. Adapted from [52].

In the GI field, extracellular monophasic slow waves have similar configurations with intracellular monophasic slow waves [32, 6]. In 1942, monophasic slow waves were recorded from tissues within 1 mm from the injury regions (created by pinching) of small intestine preparations from rabbits and cats. Biphasic slow waves were recorded otherwise [16]. Similar to Bozler’s study, three different morphologies of slow waves were recorded *in vitro* by an agar-Tyrode filled glass capillary tube, with different distances to the serosa of cat jejunum [15]. Slow waves of the three morphologies are shown in Figure 2.16. When the electrode was placed about 1 mm above the serosa, slow waves of a biphasic morphology were recorded as shown in Figure 2.16(A). When the electrode was lightly pressed onto the serosa, slow waves of a monophasic morphology with early repolarisation were recorded as shown in Figure 2.16(B). When the electrode was firmly pressed onto the serosa, slow waves of a monophasic morphology without early repolarisation were recorded as shown in Figure 2.16(C). A hypothetical circuit was proposed (Figure 2.17) to explain the cause of the morphologies. In the circuit, $P_I$ and $P_{II}$ represent the indifferent and recording electrodes. $R_v$, $R_s$, $R_m$, $R_m'$ and, $R_i$ represent the resistance of the medium between the electrodes, the
Figure 2.16: Comparison of slow waves recorded by an agar-Tyrode filled glass capillary tube from different distances above the serosa of cat jejunum. (A) The potential recorded from about 1 mm above serosa in Tyrode solution. (B) The potential recorded with the electrode lightly pressed on serosa. This morphology was termed intermediate type slow wave. (C) The potential recorded with the electrode firmly pressed on serosa. This morphology was termed monophasic type slow wave. Adapted from [15].

Figure 2.17: Hypothetical circuit to explain recording morphologies. $P_I$ and $P_{II}$ represent the indifferent electrode and the recording electrode. $R_v$, $R_s$, $R_m$, $R_m'$, and $R_i$ represent the resistance of the medium between the electrodes, the resistance around the tip of the recording electrode due to the indentation or penetration of the issue beneath it, the resistance of adjacent segments of the membrane, the resistance of the recording segment of the membrane, and the internal resistance of the membrane. $E_m$ represents the battery of adjacent segments of the membrane. Adapted from [15].

Resistance around the tip of the recording electrode due to indentation or penetration of the issue beneath it, the resistance of adjacent segments of the membrane, the resistance of the recording segment of the membrane, and the internal resistance of the membrane. $E_m$ represents the battery of adjacent segments of the membrane. The voltage across $R_v$ represents the field potential, which had a biphasic morphology. The voltage across $R_s$ represents the transmembrane slow wave, which was proportional to $E_m$, with a monophasic morphology. The voltage summation across $R_v$ and $R_s$ represents the signal recorded. Under light pressure condition, due to the indentation or penetration of the issue beneath the recording electrode, $R_s$ was greater than the no pressure condition. The voltage cross $R_s$ was comparable with that cross $R_v$. Thus, a superposition of field potentials and transmembrane slow waves was recorded. Under the firm pressure condition, $R_s$ further increased. The domination of transmembrane slow waves was recorded. In addition, biphasic slow waves approximated the double differential of monophasic slow waves in this study [15].
2.3 Methods to Record Monophasic Slow Waves

Recently, slow waves were recorded from pig jejunum in the presence and absence of contractions as shown in Figure 2.18, where nifedipine was administered to suppress motion [6]. This observation confirmed that recorded slow wave potentials were not caused by movement artifacts. In addition, the monophasic slow waves were validated to approximate the double differential of biphasic slow waves (Figure 2.19). This was consistent with the finding in the neuronal field [25].

In summary, it was confirmed in previous studies that biphasic slow waves can be recorded from the surface of a muscle (extracellular methods). In comparison, monophasic slow waves can be recorded intracellularly from inside the muscle cells (intracellular methods) or through methods that record extracellularly while deforming the recording surface (pseudo-intracellular methods).

2.3 Methods to Record Monophasic Slow Waves

Monophasic slow wave recording techniques were commonly used to understand how drugs affected the slow waves. More specifically, to analyse the refractory phases of slow wave events when drugs were administered. Two types of contact methods were used: (i) intracellular and (ii) pseudo-intracellular methods. In addition, an emerging contactless method was also reviewed.

2.3.1 Intracellular Methods

In the cardiac field, intracellular monophasic action potentials have been recorded using glass microelectrode from in vitro preparations. With an impalement of an individual cell, monophasic action potentials were recorded as intracellular potentials. Monophasic action potentials were first recorded as a phenomena accompanying the excitatory state in the ventricle of frog hearts in 1880 [20]. In 1990, monophasic action potentials from the epicardium were recorded in vivo with floating microelectrode [52]. In this study, unipolar electrograms were shown synchronised to transmembrane action potentials. Furthermore, in vitro monophasic action potentials from the epicardium of mice were recorded using same techniques [66]. In this study, monophasic action potential recording extracellular contact electrodes were validated against intracellular methods. In the GI field, Bozler recorded monophasic slow waves from rabbit and cat small intestine strips in 1942 [16]. It was shown that the slow wave conduction mechanism in the smooth muscle of the intestine was the same as other excitable tissues. A large amount of intracellular recording studies were conducted afterwards [55, 32, 15, 14, 33, 65].
Figure 2.19: Gastric slow wave recordings. ATs and RTs are denoted as dot and plus signs. (A) Gastric monophasic slow waves. (B) The smoothed differential of (A). (C) The smoothed double differential of (A). (D) The biphasic slow waves recorded simultaneously. The morphology is similar to (C). Reproduced from [6].
2.3 Methods to Record Monophasic Slow Waves

2.3.2 Pseudo-Intracellular Methods

Pseudo-intracellular methods are another approach to record monophasic slow waves. These type of methods are not intracellular methods but extracellular methods, which approximate intracellular recordings by slightly injuring the recording surface. Three main types of electrodes have been designed and applied in previous studies: (i) bipolar contact electrodes, (ii) wet suction electrodes, (iii) dry suction electrodes.

Bipolar Contact Electrode

Bipolar contact electrodes were primarily used to record cardiac monophasic action potentials. In 1935, monophasic action potentials were recorded from mammalian heart using contact electrodes [61]. The design of the electrode is shown in Figure 2.20. The glass electrode (D) was filled with saline solution, and then pressed towards the epicardium with moderate pressure. Yarn wicks (C) were frequently moisturised with saline solution. Consistent monophasic action potentials were recorded if the pressure was maintained. A modified design was proposed in 1987 [102]. The cardiac electrode design is shown in Figure 2.21(A). Moreover, a electrode holder was designed for this electrode as shown in Figure 2.21(B - E). A piece of sponge was used to replace yarn wicks from the previous design. The active electrode protruded from the opening of the holder. Thus, pressure was present between the active electrode and the recording site when the holder was placed on the epicardium. The reference electrode was in contact with the saline soaked sponge around it, where the sponge was in contact with the epicardium.

In 2001, the cardiac electrode design was simplified. The diagram of this simplified design is shown in Figure 2.22(A) [66]. *In vitro* monophasic action potentials were recorded from mouse epicardium using two twisted silver chloride wires. These wires were insulated by Teflon except for the tips. The reference electrode was 1 mm shorter than the active electrode, and was in contact with the bath medium near the recording site, while the active electrode was pressing onto the epicardium. Various electrode tip diameters were tested. The tip with a diameter of 0.25 mm recorded better monophasic action potentials than 1.0 and
Figure 2.21: A contact electrode design. (A) The design drawing of the electrode. The active electrode protruded from the end of the catheter. The reference electrode was located on the side of the catheter, 5 mm from the end. (B) The drawing of the electrode holder designed for (A). (C) The electrode holder with (A) assembled in centre. (D) The piece of sponge used in this design. (E) The electrode holder with (A) and (D) assembled. Adapted from [102].
2.3 Methods to Record Monophasic Slow Waves

Figure 2.22: A (A) simplified and a (B) clinical contact electrode designs for monophasic action potential (MAP) recording. (A) The reference electrode was 1 mm shorter than the tip (active) electrode, and was in contact with the bath medium near the recording site, while the tip electrode was pressing onto the tissue. (B) A cardiac catheter design for clinical MAP recording. The MAP recording electrode at the end of the catheter (active electrode) was pressed onto the endocardium, whereas the other MAP recording electrode (reference electrode) was kept in the blood close to the recording site. Adapted from [66, 38].

Clinical cardiac monophasic action potential recordings from humans were carried out using the electrode shown in Figure 2.22(B). High grade Ag-AgCl powder was suspended in a special polymeric matrix to make the electrodes [38]. The recording electrode at the end of the catheter (active electrode) was pressed onto the endocardium, whereas the other recording electrode (reference electrode) was kept in the blood close to the recording site.

Wet Suction Electrode

The second type of pseudo-intracellular methods to record monophasic slow waves is through the use of wet suction electrodes. Isotonic solution such as KCl or saline solution was used as the medium to apply suction. Both unipolar and bipolar designs were proposed in the literature and reviewed in this section. Wet suction electrodes were introduced in the cardiac field before applied in the GI field. Monophasic action potentials were recorded in situ from ventricular endocardium by unipolar suction electrodes filled with KCl solution, which was used to depolarise the tissue [67]. The structure of the suction electrode was then simplified to be utilised with saline solution in human cardiac recording [87] (Figure 2.23). A bipolar suction electrode was used with one wire inside the catheter 1 mm proximal to the distal end and another wire on the outer surface of the catheter 3 mm from the distal end. Both of the electrodes were made of silver or stainless steel wires of 0.2 - 0.3 mm thick.

In a study conducted in 2013, unipolar glass suction electrodes filled with saline solution were used to record monophasic slow waves from gastric serosa [6]. A photo of the electrode is shown in Figure 2.23(B). The electrode was made from a capillary glass tube with a diameter of 1.2 mm and a segment of silver wire. The electrode was lowered and placed on the serosa. The capillary tube was then filled with saline solution. A gentle negative pressure was applied and held by the stopcock. A portion of the serosa was...
Figure 2.23: Wet suction electrode designs. (A) Suction electrode used in human cardiac monophasic action potential recording. A bipolar suction electrode was used with one wire inside the catheter 1 mm proximal to the distal end and another wire on the outer surface of the catheter 3 mm from the distal end. (B) Glass suction electrode used to record *in vivo* monophasic serosal slow waves. The electrode was made from a capillary glass tube with a diameter of 1.2 mm and a segment of silver wire. (C) A photo of the recording site. A portion of the serosa was invaginated into the capillary glass tube and had a contact with the silver wire electrode inside of the tube. Adapted from [87, 6].
2.3 Methods to Record Monophasic Slow Waves

Figure 2.24: Dry suction electrode designs used to record slow wave activity. Shown are: (A) NG, (B - C) peroral, and (D) perrectal designs. (A) Two stainless steel wires were attached to the gastric mucosa, when negative pressure was applied through the side-opened NG tube. (B) Components C, E, P, and SC are a rubber cup, a pair of bipolar electrodes, a metal pipe fitted in the suction tube (ST), and a shielded cable. (C) A 3-channel dry suction electrode design inspired by (B). Three electrodes with tip pellets of sintered Ag-AgCl were used. (D) The dry suction electrode was kept in place on the bowel wall by applying negative pressure through the suction tube and the rubber cup. Adapted from [68, 80, 45, 117].

Dry Suction Electrode

The third type of pseudo-intracellular methods to record monophasic slow waves is through the use of dry suction electrodes and was mainly used on the mucosal surfaces of the GI tract. Air was used as the medium to introduce the suction in previous electrode designs. Both unipolar and bipolar designs were proposed in the literature and reviewed in this section. In 1970, monophasic slow waves were recorded from gastric mucosa using the unipolar nasogastric (NG) suction electrode shown in Figure 2.24(A) [68]. Two stainless steel wires with diameters of 0.25 mm were attached to the mucosa and then negative pressure was applied through the side-opened NG tube to achieve contact.
A bipolar peroral suction electrode, shown in Figure 2.24(B), was used to record monophasic slow waves from gastric mucosa [80]. Two stainless steel wires with diameters of 0.6 mm were insulated except the very tips. The electrode was attached to the mucosa by introducing negative pressure through the tube connected with the rubber cup. In 1986, a unipolar electrode design was inspired by this suction electrode design and is shown in Figure 2.24(C) [45]. Three electrodes with tip pellets of sintered Ag-AgCl were used. Monophasic slow waves were recorded from stomach mucosa by using this electrode design.

In 1974, a unipolar suction electrode was designed for rectosigmoid slow wave recording [117]. The design is shown in Figure 2.24(D). One to three stainless steel electrodes with diameters of 0.25 mm were attached to a polythene suction tube. The rubber cup was with a diameter of 5 mm. The electrodes were kept in place onto the bowel wall by applying negative pressure through the suction tube and the rubber cup. Monophasic slow waves were recorded by this design. Pressure change around the recording site was also recorded with the open-end tube on the other side of the suction tube.

A multi-channel unipolar NG tube suction electrode array was designed to record slow waves from gastric mucosa in 2012 [112]. It was based on the electrode design shown in Figure 2.24(B). Eight suction electrodes were installed on an NG tube with a diameter of 4.5 mm. The inter-electrode distance was 1.5 cm. Suction was applied through the NG tube and the rubber cups, to hold the electrodes in place. Single-channel monophasic slow waves were reported. An X-ray photo of the experimental is shown in Figure 2.25.

### 2.3.3 Contactless Methods

An emerging approach to reliably acquire monophasic action potentials is optical mapping, which has been widely used in both the cardiac and neural fields [41, 104, 2, 88]. In brief, the recording surfaces were stained with voltage-sensitive fluorescence dye. The emitted fluorescence was then imaged to record the action potentials. Recently, this optical recording method was introduced as an alternative way to...
access HR activation and recovery mapping by recording monophasic slow waves in the GI field, and the result was promising [127]. The morphology and timing of the recording was consistent with wet suction electrode recordings, as shown in Figure 2.26. However, the signal to noise ratio (SNR) of optical recording was lower than the conventional methods.

### 2.4 Slow Wave Analysis Algorithms in the Gastrointestinal Field

Traditionally, slow wave analysis methods were typically carried out in the following steps: (i) manually classify each fluctuation as slow wave, spike, or noise, (ii) manually find the steepest point (AT) of the activation phase from each slow wave event, (iii) manually group the ATs from all the channels into wavefronts, and find out the slow wave propagation pathway. During the last step, the slow wave propagation pathway is not always identifiable, especially for sparse recordings. Traditional manual methods were originally designed to analyse traditional sparse recordings. However, the analysis of HR recordings is beyond the capability of the traditional methods. To assist the analysis of HR recordings, automated analysis algorithms were developed.

#### 2.4.1 Activation Time Detection Algorithms

In 2008, an automatic analysis algorithm featuring amplitude-sensitive differential operation was designed to identify the AT of each slow wave event from HR recordings by Lammers et al. [71]. This algorithm can be divided into 2 stages: (i) spike removal, (ii) AT detection. For the first stage (Figure 2.27), the voltage offset of the recordings was removed, after which the signals were normalised to 0 - 1. A high-pass filter (30 Hz) was then applied to the normalised signals to extract the high-frequency components. In this step, most of the slow wave events were filtered out. In addition, the noise was eliminated and the spikes were made pronounced using absolute value and running mean operations. The signals were transformed into a series of smoothed peaks indicating spikes. A threshold was calculated by multiplying the median of the running mean with a user-defined detection factor. The segment of the recording where its running mean exceeded the threshold was replaced by blank segment to remove the spike.

In the second stage (Figure 2.28), blanked slow wave signals from the first stage (A) were further processed to identify the AT of each slow wave event. The differential of the input was calculated (B) then inverted (C) to prepare for peak detection of the AT. The amplitude-sensitive differential of the input was
calculated (D) to enhance slow wave events, which usually had larger amplitude and differential values compared to the noise. After which, a similar approach from the first stage was applied to minimise the contribution of the noise by calculating the absolute value (E), calculating the running mean (F), and subtracting the median of the running mean. A series of smoothed peaks representing the timing of slow wave events were resulted. During the timing of each peak, a peak detector was applied on the prepared signal to identify the maximum point (G), which was the AT of that slow wave event.

In 2010, the falling-edge, variable threshold (FEVT) algorithm was developed to improve the accuracy of existing algorithms against low SNR recordings and validated in processing recordings with different noise levels [36]. The falling-edges were more pronounced after the application of a non-linear energy operator (NEO) transform, as in

$$NEO(t) = y(t)^2 - y(t - 1) \times y(t + 1),$$

(2.1)

where $NEO(t)$ denotes the NEO at the time $t$ of the signal $y$ [63]. The convolution of an edge-detector kernel and the recording was calculated, then multiplied by the output of the NEO transform, and fed into the decision stage. In the decision stage, a time-varying threshold was calculated based on the input and applied to detect the timing of each slow wave event. Similar to the previous algorithm, a 3-point central difference approach was used to detect the AT of each slow wave event.

### 2.4.2 Other Algorithms

**Activation Time Grouping Algorithm**

After the ATs were identified, they were grouped into wavefronts based on their timings. For each wavefront, an isochronal map was generated to visualise the pathway of the slow wave propagation. This approach is termed HR slow wave activation mapping. A search and sorting routine was developed to track the pathway of slow wave propagation [69]. In addition, an algorithm called REGROUPS was proposed to automatically group ATs into wavefronts [35]. In this algorithm, region growing and automatic seed selection methods were used. In addition, a suite called GEMS was developed by designing a graphic user interface and integrating several algorithms to provide a pipeline of HR slow wave activation mapping [126].

**Slow Wave Propagation Velocity Estimation Algorithms**

Several methods exist to quantify the *in vivo* gastric slow wave propagation velocity. These methods were already established in the cardiac field and were adapted for the GI field [58, 11]. After ATs were grouped into wavefronts, gastric slow wave velocities were calculated using a smoothed finite-difference approach [96]. An alternative method was also developed for estimating slow wave velocities by determining the time-delay between unipolar recordings via cross-correlations [94]. This approach was more efficient than the smoothed finite-difference approach as extensive manual review was often needed for identifying and grouping activation times accurately.
Figure 2.27: Flowchart and intermediate results of a GI spike removal algorithm. The upper pane is the flowchart, while the lower pane is the intermediate results. The segment of the recording where its running mean exceeded the threshold was replaced by blank segment (F) to remove the spike. Adapted from [71].
Figure 2.28: Flowchart and intermediate results of a GI AT detection algorithm. The upper pane is the flowchart, while the lower pane is the intermediate results. The peaks caused by sudden changes in removing spikes from the first stage, were marked by asterisks in (E). Reproduced from [71].
Recovery Time Detection Algorithm

Compared to the automatic analysis algorithms for identifying AT, methods to identify RT are less common. In 2015, an automatic analysis algorithm for identifying RT was developed [97]. By using wavelet decomposition, the derivative of the slow wave recording was computed. The RT was identified as the peak of the derivative. An example is shown in Figure 2.29. This algorithm was able to identify RT from recordings with a wide range of noise levels.

2.5 Summary

In this chapter, an overview of the GI electrophysiology was discussed, followed by an overview of GI slow wave morphology. In addition, the methods used to record slow wave were outlined, including several previous designs. Finally, the algorithms used to analyse the recordings were reviewed.
Chapter 3

Monophasic Slow Wave Recording Electrode Designs

Aspects of this chapter have been published in: Han, H., Cheng, L. K., and Paskaranandavadivel, N. (2022). High-resolution in vivo monophasic gastric slow waves to quantify activation and recovery profiles. Neurogastroenterol. Motil., e14422 [49].

Monophasic slow waves contain information about the activation and the recovery slow wave phases during normal and abnormal propagation patterns. Currently, only sparse suction electrodes are available for monophasic slow wave recording, and therefore spatial information is not utilised. Thus, there is a need to capture monophasic slow waves in HR to study the spatial information and to understand dysrhythmias. In this chapter, three types of multi-channel electrode arrays (bipolar contact, dry suction, and wet suction) were designed, manufactured, applied to record HR monophasic slow waves from the pig stomach, and validated with HR FPC electrode arrays.

3.1 Methods

3.1.1 Bipolar Contact Electrode Design and Manufacture

As mentioned in Section 2.3.2, contact electrodes have been widely used in the cardiac field to record monophasic action potentials. In general, these historical designs were similar. They all utilised a bipolar electrode scheme. Pressure was applied on the active electrode which was in contact with the recording surface. The reference electrode was either gently placed on the adjacent surface or in contact with the adjacent surface via a conductive medium. Based on these similarities, the following single and multi-channel bipolar contact electrodes were designed.
Single-Channel Bipolar Contact Electrode

The design drawing of a single-channel bipolar contact electrode design is shown in Figure 3.1(A). This design was based on a previous bipolar contact electrode (Figure 2.21). The electrode was designed using SOLIDWORKS 2017 SP0.0 (Vélizy-Villacoublay, France).

A round piece with a diameter of 25 mm was cut from a 10 mm thick acrylic board using a Trotec Speedy 300 laser engraver (Marchtrenk, Austria). At the centre on one side of the round piece, a hollow recess of 5 mm and a diameter of 20 mm was then engraved. On the other side of the round piece, two holes of 1 mm in diameter were cut at the centre for the active electrode and at 5 mm off the centre for the reference electrode. Two grooves of 2 mm wide and 1 mm deep was engraved for routing of the wires connected to the electrodes.

Perfluoroalkoxy-coated silver wire (788000, A-M Systems, WA, USA) was used to make the electrodes. This silver wire had a bare diameter of 0.635 mm and a coated diameter of 0.762 mm. Silver wires of lengths 26 mm and 18.5 mm were used as the active and reference electrodes respectively. The coating was removed for 0.5 mm from the end of the active electrode wire. The remaining coating was kept to minimise crosstalk between the two electrodes. For the reference electrode wire, the entire coating of 18.5 mm was removed. The surface of both wires were chloride by immersion into 4% sodium hypochlorite for 5 min.

The end of the active electrode wire, was inserted into the middle hole until 1 mm above the wall of the hollow on the other side of the round piece. The reference electrode wire was inserted into the other hole until 2.5 mm below the wall of the hollow on the other side of the round piece. The other end of each wire was bent and positioned in the groove. The electrodes were held in place with epoxy resin adhesive. Copper wires connected to the recording device were soldered to both of the electrodes and were protected by a piece of heat shrink. A photo after this step is shown in Figure 3.1(B). A porous sponge was made to match the shape of the hollow and then installed into the recess. The active electrode protruded through the sponge and was thus not in contact with the sponge. Whereas the reference electrode was kept inside the sponge, thus was in contact with the sponge as shown in Figure 3.1(C).

Multi-Channel Bipolar Contact Electrode Array

The design drawing of the multi-channel bipolar contact electrode array is shown in Figure 3.2(A). The design and manufacture process was similar to that of the single-channel bipolar contact electrode. In addition, the size of the acrylic part was 47.5 × 47.5 × 10 mm. The inter-electrode distance was 22.5 mm. The length of each silver wire was adjusted based on the length of the ditch for each hole. Photos of the electrode array without and with sponges assembled are shown in Figure 3.2(B - C).

3.1.2 Dry Suction Electrode Design and Manufacture

In this section, the designs of dry suction electrodes are presented, which includes a suction pump and proportional-integral-derivative (PID) controller to maintain constant negative pressure.
Figure 3.1: Single-channel bipolar contact electrode design drawing and photos. Components A and R are the active and the reference electrodes individually. (A) The design drawing. (B) A photo of the electrode without sponge assembled. (C) A photo of a completely assembled electrode. The reference electrode was covered by the sponge. (D) A photo of the electrode applied in a pig study.
Figure 3.2: Multi-channel bipolar contact electrode array design drawing and photos. The electrodes A and R are the active and the reference electrodes individually. (A) The design drawing. (B) A photo of the electrode array without sponges assembled. (C) A photo of a completely assembled electrode array. Reference electrodes were covered by sponges. (D) A photo of the electrode array applied in a pig study.
3.1 Methods

Figure 3.3: Single-channel dry suction electrode design drawing and photos. (A) The design drawing. (B) A photo of a single-channel dry suction electrode. (C) A photo of the electrode applied in a pig study. 3D printing was used for manufacture.

Single-Channel Dry Suction Electrode

The design drawing of the single-channel dry suction electrode design is shown in Figure 3.3(A). The electrode was designed using SOLIDWORKS 2017 SP0.0 and manufactured using Ultimaker 2+ printer (Ultimaker, Utrecht, the Netherlands). The material used in three-dimensional (3D) printing was polylactic acid.

A silver wire was soldered to a female Bayonet Neill-Concelman (BNC) connector. The silver wire was then inserted through the 3D printing component. After which, the female BNC connector was fitted into the wide end of the 3D printing component and was attached to it using epoxy adhesive. The silver wire was then trimmed to match the length of the 3D printing component. The recording device was connected via a male BNC connector and a copper wire. A photo of the electrode is shown in Figure 3.3(B).
Multi-Channel Dry Suction Electrode Array

The design drawing of the multi-channel dry suction electrode array is shown in Figure 3.4(A). The manufacture process was similar to that of the single-channel dry suction electrode. Due to the complexity of the design, a Form 2 printer (Formlabs, MA, USA) was used for the 3D printing. The material used to print this part was Tough Resin (Formlabs, MA, USA). The inter-electrode distance was 5 mm.

Silver wires mentioned in Section 3.1.1 were used as electrodes in this design. Air was used as the medium of suction in this design, hence the crosstalk between channels was negligible. The coating was removed before the wires were inserted into the 4 off-centre holds on top of the 3D printing component. After inserting, the silver wires were trimmed to match the height of the 3D printing component as shown in Figure 3.4(B). The other ends of the silver wires were connected to the recording device using copper wires.
3.1 Methods

Figure 3.5: The (A) flowchart and (B) a photo of the pressure control system. A PID controller was used to maintain constant negative pressure.

**Pressure Control System**

The flowchart of a pressure control system is shown in Figure 3.5(A). A pressure sensor (NXP Semiconductors, Eindhoven, the Netherlands) that was capable of sensing as low as -115 kPa and a microcontroller (Microchip Technology, AZ, USA) were used to measure the pressure and to implement the PID controller. The output of the pressure sensor (0 - 5 V) was sampled by a 10-bit analogue digital converter embedded in the microcontroller. The difference between the measured pressure and the target pressure was input into the PID controller as an error signal. The microcontroller then generated an appropriate pulse width to control the suction pump to adjust the pressure accordingly. The code of the microcontroller was developed in the BASCOM compiler (MCS Electronics, Almere, the Netherlands).

3.1.3 Wet Suction Electrode Design and Manufacture

In addition to dry suction electrodes, wet suction electrodes were also used to record monophasic slow waves in previous studies. Saline solution was used as the medium of the suction. Suction pumps were not used in the following designs as PID system had time lags in attaining pressure, thus was not able to control pressure in an effective manner. Instead, the suction was applied using a syringe before recording. In this section, the design and manufacture process of single and multi-channel wet suction electrodes were described.

**Single-Channel Wet Suction Electrode**

A single-channel wet suction electrode is shown in Figure 3.6(A). The manufacturing process was based on the instructions previously described [62]. In brief, a piece of silver wire with a diameter of 0.254 mm (A-M Systems, WA, USA) was used as the electrode within the microelectrode holder (672443, A-M Systems, WA, USA). A capillary glass tube with an inner diameter of 0.86 mm and an outer diameter of
Figure 3.6: Single-channel wet suction electrode. (A) Assembled single-channel suction electrode. Components 1 - 6 are: a stopcock, flexible tubing, a capillary glass tube, a microelectrode holder, a female 2 mm pin connector, and a copper wire to the recording device. (B) A photo of the single-channel wet suction electrode applied in a pig study.

1.5 mm (628000, A-M Systems, WA, USA) was trimmed such that the silver wire electrode tip would be level with the capillary glass tube tip when installed. The glass tube was then installed onto the holder around the silver wire electrode. The suction port of the holder was connected to a stopcock via a piece of flexible tubing (Tygon S3 E-3603, Saint-Gobain, La Défense, Courbevoie, France). The silver electrode wire was connected to the mapping system via a female 2 mm pin connector (105-0702-001, Cinch Connectivity Solutions, IL, USA) and a copper wire.

**Multi-Channel Wet Suction Electrode Array**

A design drawing and an experimental application of the multi-channel wet suction electrode array are shown in Figure 3.7(A) and (B). Multi-channel suction electrode arrays were created by integrating multiple single-channel suction electrodes using an acrylic grid frame.

An acrylic grid frame capable of incorporating 5 × 5 channels was designed in SOLIDWORKS 2019 SP0.0. The holes on the acrylic grid frame were designed to accommodate the outer diameter beneath the suction port of the microelectrode holders. An inter-electrode spacing of 10 mm was used to allow space for the suction ports and the flexible tubing. The acrylic board was 10 mm thick and was laser cut using the laser engraver described in Section 3.1.1.

Flexible tubing was attached to the suction port of each microelectrode holder. Then, the microelectrode holders were inserted into the frame. After which, the microelectrode holders were equally divided into 5 suction bundles. Within each suction bundle, a stopcock was used to control the suction of all the microelectrode holders by joining the free ends with cross connectors (X0-1, Eldon James, CO, USA).

The silver wire of all the microelectrode holders was trimmed to the same length. Similar to the manufacture process of single-channel wet suction electrode, the capillary glass tube was trimmed and installed. Finally, each electrode was connected to the recording device.
3.1 Methods

Figure 3.7: Multi-channel wet suction electrode array. (A) Design drawing. As in Figure 3.6, components 3 - 5, and 7 are: a capillary glass tube, a microelectrode holder, a female 2 mm pin connector, and an acrylic grid frame of 5 × 5 channels. (B) A photo of a 5 × 5 multi-channel wet suction electrode array applied to the gastric serosa.

3.1.4 Experimental Methods

Ethical approval for experimental studies was granted by the University of Auckland Animal Ethics Committee. The experimental methods and animal care were performed as previously described [31]. In brief, the pigs were anaesthetised and vital signs were continuously monitored during the experiment. A mid-line laparotomy was performed to gain access to the gastric serosal surface. Various types of monophasic slow wave recording electrodes were then placed on the gastric serosa. In addition, conventional HR FPC electrode arrays were applied to validate these monophasic slow wave recording electrodes. For HR FPC electrode arrays, warm saline-soaked gauze was placed over the arrays. Bipolar contact electrode arrays, dry suction electrode arrays or wet suction electrode arrays were prepared and placed using the methods described in the following sections. After the electrodes were placed, the wound edges were then approximated to minimize cooling and drying. Data were recorded using a passive ActiveTwo recording system (BioSemi, Amsterdam, the Netherlands) at 512 Hz. The reference electrodes were placed on the hindquarter thigh. The pigs were euthanised at the end of the experiments while under anaesthesia.

Bipolar Contact Electrode Array

Before a bipolar contact electrode array was placed, the sponges were fully soaked with warm saline solution. Photos of experimental applications of the single and the multi-channel bipolar contact electrodes are shown in Figures 3.1(D) and 3.2(D).

Dry Suction Electrode Array

Flexible tubing (Tygon S3 E-3603, Saint-Gobain, La Défense, Courbevoie, France) was used to connect the suction ports of all the channels to a suction pump before a dry suction electrode array was placed. The
electrode array was then placed on the serosa and was held in place by a clamp stand. The suction pump was then turned on and controlled by a PID controller to provide and maintain the negative pressure of -41 kPa, which was enough to invaginate a portion of the serosa into the electrode. Photos of experimental applications of the single and the multi-channel dry suction electrode array is shown in Figure 3.3(C) and 3.4(C).

**Wet Suction Electrode Array**

Firstly, the electrode was placed in gentle contact with the serosa and was held in place by a clamp stand. Unlike the dry suction electrode arrays, the following steps were used instead of a suction pump to apply negative pressure to each channel of the wet suction electrode array: (i) a syringe was filled with warm saline solution and was used to fill the capillary glass tubes via the stopcocks, (ii) suction was applied until a portion of the serosa invaginated into the capillary glass tubes, (iii) the stopcocks were then closed to maintain the negative pressure. Photos of experimental applications of the single and the multi-channel wet suction electrode array are shown in Figures 3.6(B) and 3.7(B).

### 3.1.5 Signal Processing and Slow Wave Analysis

Raw experimental recordings were first down-sampled to 30 Hz for computational efficiency. Then, baseline drift was removed by a Gaussian moving median filter of 20 s, followed by a Savitzky-Golay filter (polynomial order: 9, window width: 1.7 s) of 0 - 1.98 Hz to remove the high-frequency noise [95]. Signal processing, data analysis, and method development were all performed in MATLAB R2019b (MathWorks, Natick, USA). The recordings from the monophasic slow wave recording electrodes were visually classified as having slow waves with a monophasic morphology. If a slow wave event had the maximum potential value greater than its absolute minimum potential value, it was determined as a monophasic slow wave event.

### 3.2 Results

The 3 types of monophasic slow wave recording electrodes and conventional FPC electrode arrays were applied to record gastric slow waves for 47.0 ± 19.7 minutes per subject in pig studies (n = 17, 40.5 ± 3.0 kg). The overall results are shown in Table 3.1. Bipolar contact electrodes recorded monophasic slow waves from 2 out of 3 pig studies (67%). Dry suction electrodes recorded monophasic slow waves from 3 out of 5 pig studies (60%). In comparison, wet suction electrodes recorded monophasic slow waves from 12 out of 14 pig studies (86%). In summary, wet suction electrode design outperformed the other 2 designs in terms of the ability to record monophasic slow waves.

#### 3.2.1 Bipolar Contact Electrode

Three pig studies were conducted to record monophasic slow waves using single-channel bipolar contact electrodes (3 studies) and multi-channel bipolar contact electrode arrays (2 studies). Monophasic slow
3.2 Results

<table>
<thead>
<tr>
<th>Electrode Type</th>
<th>Bipolar Contact</th>
<th>Dry Suction</th>
<th>Wet Suction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Pig Studies</td>
<td>3</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Number of Studies Where Monophasic Slow Wave Signals Were Acquired</td>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Percentage of Studies Where Monophasic Slow Wave Signals Were Acquired</td>
<td>67%</td>
<td>60%</td>
<td>86%</td>
</tr>
</tbody>
</table>

Table 3.1: Summary of experiments conducted with 3 types of electrodes.

Figure 3.8: Examples of bipolar contact electrode recordings. A $2 \times 2$ bipolar contact electrode array and an HR FPC electrode array were placed on the serosa as shown on the left. The recordings of a bipolar contact and 3 FPC channels are shown on the right. The positions of corresponding channels are indicated by (A) red and (B) green rectangles. The recordings of the active and the reference electrodes from the bipolar contact electrode channel are shown as the first 2 traces and the corresponding bipolar signal is shown as the third trace. The slow wave propagation direction is shown as the blue arrow. The ATs and RTs were identified as plus and cross symbols on the traces.

Example recordings illustrating antegrade (normal) slow wave propagation from corpus to antrum are shown in Figure 3.8. The location and the recording of the bipolar contact electrode array are shown in Figure 3.8(A), while the location and the recording of the FPC electrode array are shown in Figure 3.8(B). In this example, monophasic slow waves were recorded by the bipolar contact electrode array. The amplitude of monophasic slow waves was 95% lower than biphasic slow waves. However, RTs can be identified reliably from monophasic slow waves. In comparison, no RT could be identified in this example from biphasic slow waves. In addition, the monophasic slow wave recordings were generally of less ventilator noise than the unipolar biphasic slow wave recordings due to the subtraction of the reference electrode recording. In Figure 3.8(A), similar noise was captured by both the active and the reference electrodes, and was then eliminated by the subtraction, which revealed the monophasic slow waves.
3.2.2 Dry Suction Electrode

Five pig studies were conducted to record monophasic slow waves using single-channel dry suction electrodes (2 studies) and multi-channel dry suction electrode arrays (4 studies). Monophasic slow waves were recorded from 1 of the 2 studies (50%) using single-channel dry suction electrodes, while they were recorded from 2 of the 4 studies (50%) using multi-channel dry suction electrode arrays.

Example recordings illustrating antegrade (normal) slow wave propagation from corpus to antrum are shown in Figure 3.9. The location and the recording of the FPC electrode array are shown in Figure 3.9(A), while the location and the recording of the dry suction electrode array are shown in Figure 3.9(B). In this example, monophasic slow waves were recorded by the dry suction electrode array. The amplitude of monophasic slow waves was 12% higher than biphasic slow waves. For biphasic slow wave recordings, no RT was identifiable. Whereas in monophasic slow wave recordings, all the RTs were clearly identifiable by the sharper downstrokes. However, the morphology of the monophasic slow wave was not ideal. Movement artifacts were introduced from the intermittent operation of the suction pump, due to the porosity of these electrodes. In addition, PID system had time lags in attaining pressure, thus was not able to maintain pressure in an effective manner.

3.2.3 Wet Suction Electrode

Fourteen pig studies were conducted to record monophasic slow waves using single-channel wet suction electrodes (7 studies) and multi-channel wet suction electrode arrays (7 studies). Monophasic slow waves were recorded from 5 of the 7 studies (71%) using single-channel wet suction electrodes, while they were recorded from 7 of the 7 studies (100%) using multi-channel wet suction electrode arrays.
Figure 3.10: Examples of wet suction electrode recordings. Two HR FPC electrode arrays and a multi-channel wet suction electrode array were placed on the serosa as shown on the left. The recordings of 3 channels from each electrode array are shown on the right. The positions of corresponding channels are indicated by (A and C) green and (B) red rectangles. The slow wave propagation direction is shown as the blue arrow. The ATs and RTs are identified as plus and cross symbols on the traces.

Example recordings illustrating antegrade (normal) slow wave propagation from corpus to antrum recorded by both the FPC and the multi-channel wet suction arrays are shown in Figure 3.10. The locations and the recordings of the 2 FPC electrode arrays are shown in Figure 3.10(A) and (C), whereas the location and the recording of the wet suction electrode array are shown in Figure 3.10(B). In this example, the recordings from the FPC and the suction electrode arrays showed biphasic and monophasic morphologies, respectively. The amplitude of monophasic slow waves was 105% higher than biphasic slow waves. RTs were identifiable from the monophasic slow wave recordings of all wet suction electrode channels. In comparison, four out of six channels had identifiable RTs from the biphasic slow wave recordings. In addition, the suction provided a stable contact between wet suction electrodes and recording sites. Thus, monophasic slow wave recordings were generally of less ventilator noise than biphasic slow wave recordings.

3.3 Discussion

All of the 3 types of electrode designs described in this chapter were able to record monophasic slow waves. As described in Section 2.3.2, they are termed pseudo-intracellular methods, as they were all able to approximate intracellular potentials using extracellular recording methods. The developed electrodes all utilised some method to affect the recording tissue. For example, bipolar contact electrodes applied positive pressure to the tissue by the protruding active electrodes, whereas both the dry and the wet suction electrodes applied negative pressure to invaginate tissue into the suction tubes. In general, all of the 3 types of electrodes slightly injured the recording surface to record monophasic slow waves. At the recording
sites, there were small indentations in the tissue, which will be quantified using histology in the future. This observation was consistent with the literature that monophasic slow waves can be recorded when the recording surface is injured as described in Section 2.2.

All the electrode types were applied in in vivo pig studies to record propagating monophasic slow waves, where the activation and the recovery profiles were captured with high-fidelity. The designs were verified against conventional FPC electrode arrays, which were used in pig studies to record biphasic slow waves. Comparisons were conducted among the electrode types in terms of the ability to record monophasic slow waves. Wet suction electrode design was concluded as the best design, which was the most reliable and recorded the highest quality monophasic slow waves.

In the cardiac field, a pronounced early repolarisation, or J-wave as indicated on the electrocardiogram, is related to cardiac disorders, such as Brugada syndrome and is a marker for idiopathic ventricular fibrillation [8, 79]. Similar functional basic studies are required in the GI field through the use of monophasic recordings to uncover the relevance of a distinct early repolarisation in health and disease. APD (Section 2.2.1) has been defined across the heart and its dispersion has been associated with cardiac arrhythmias [24, 10]. Alteration to the normal cardiac electrical restitution curve has been used to hypothesise mechanisms that lead to cardiac arrhythmias [9, 108]. However, in the GI field, the information of ARI has not been fully studied. Furthermore, the GI electrical restitution has not been defined to date.

3.4 Summary

In this chapter, three types of HR monophasic slow wave recording devices were designed, manufactured, and validated against conventional HR FPC electrode arrays. The ability to record monophasic slow wave from pig stomachs was compared across the three types of electrode designs. The wet suction electrode design was the most reliable. Thus, this design was concluded as the best among the three and was hence decided as the final design of the monophasic slow wave recording device in this thesis. Recordings from HR wet suction electrode arrays were used in the analysis in the following chapters.
Chapter 4

Quantification of Gastric Slow Wave Velocity Using Bipolar High-Resolution Recordings


Slow wave propagation dynamics could be used to discriminate dysrhythmic and normal gastric activity (Section 2.1). In Chapter 3, novel electrode designs have been built for slow wave recordings and algorithms to define metrics of interest such as the velocity profile are necessary for data analysis. In particular, velocity could be an objective measure, which was proposed as a biomarker for dysrhythmic activity [84]. Various approaches exist to estimate velocity from HR recordings, which include polynomial approach, finite difference approach, and time delay approach (Section 2.4.2) [37, 96, 94]. In the GI field, manual marking is typically necessary for velocity estimation, in particular for finite difference approach, and polynomial approach. In this chapter, a novel bipolar velocity estimation (BVE) method was developed to detect the wavefront velocity of slow waves from biphasic HR recordings without the need for manual review. This method was inspired by a cardiac study where bipolar analysis of data was used [81]. The method was applied to synthetic and experimental data and was validated against the standard “marking-and-grouping” framework.

4.1 Methods

Ethical approval for experimental studies was granted by the University of Auckland Animal Ethics Committee. The experimental methods and animal care were performed as described in Section 3.1.4. HR FPC electrode arrays were used to acquire in vivo unipolar slow wave recordings. The recordings were filtered as described in Section 3.1.5 before further analysis.
4.1.1 Synthetic Signal Generation

Synthetic unipolar HR signals were generated using experimental unipolar slow wave recordings from in vivo pig studies. The first principal component of experimental slow wave events from a 10-minute single-channel recording was defined as the noise-free slow wave signal event [92] and was used to define 3 synthetic HR data sets [47] (10 × 10 channels of 4 mm spacing): (i) linear wavefront pattern (oriented at 45°), (ii) pacemaker wavefront pattern, and (iii) colliding wavefront pattern.

To mimic the presence of noise in an experimental setting, ventilator (low-frequency) and high-frequency noise were added to the synthetic slow wave signals [46]. Ventilator noise originates from the pig breathing during the experiment, and high-frequency noise is from electrical interference. In brief, simulated ventilator noise was modeled as a sine wave which ranged 0% - 100% of the average slow wave amplitude, with a frequency of 12 cpm. High-frequency noise was simulated as Gaussian white noise with an SNR range of 4 - 60 dB. For each noise type and noise level, 50 noise signals were randomly generated to evaluate the performance of the algorithm.

4.1.2 The Bipolar Velocity Estimation Algorithm

The flowchart of the BVE algorithm is shown in Figure 4.1. The algorithm was divided into 3 main stages: (i) slow wave event likelihood estimation using kurtosis of the signal gradient, (ii) wavefront orientation estimation using bipolar signals, and (iii) computation of wavefront velocity using the bipolar signal morphology.

The filtered signals were segmented using a sliding window of 17 s and a step size of 1 s. To avoid 2 or more wavefronts in a single window, a window length of 17 s was chosen, which accounted for a normal slow wave frequency as high as 3.5 cpm in a pig stomach [31].

Slow Wave Event Likelihood Estimation Using Kurtosis of Signal Gradient

To estimate if slow waves were present in the unipolar segments, two conditional criteria were to be satisfied. The first was an adaptive amplitude range threshold, followed by a kurtosis of signal gradient threshold.

The first threshold condition was based on an observation that a slow wave segment with a very high or low amplitude range was unlikely to include a slow wave event. An adaptive threshold (AdTh) of acceptable signal amplitude range was calculated for each sliding window. AdTh was defined as the range from negative to positive standard deviation (SD) from the mean amplitude range of the signal segments across all channels, as in

$$AdTh = [E(R) - \sigma, E(R) + \sigma]$$

where $R$ represents the amplitude ranges of the signal segments across all channels, $E(\cdot)$ denotes the mean and $\sigma$ denotes the SD of $R$. Any signal segment with an amplitude range within the AdTh was not likely to contain large noise deviations and was used in the next step.

During data analysis, it was observed that a signal segment with slow wave events typically had gradient values of a leptokurtic distribution (has fatter tails than normal distribution), whereas a signal
4.1 Methods

Figure 4.1: Flowchart of the BVE algorithm. The algorithm was divided into 3 main stages: (A) slow wave event likelihood estimation using kurtosis of the signal gradient, (B) wavefront orientation estimation using bipolar signals, and (C) computation of wavefront velocity using the bipolar signal morphology.
Figure 4.2: Kurtosis of signal gradient calculation for experimental recordings: (A) a slow wave signal, and (B) a noisy recording with no slow waves but ventilation artifacts. Kurtosis of the signal gradient was calculated on a moving window of 17 s.

Figure 4.2: Kurtosis of signal gradient calculation for experimental recordings: (A) a slow wave signal, and (B) a noisy recording with no slow waves but ventilation artifacts. Kurtosis of the signal gradient was calculated on a moving window of 17 s.

segment without slow wave events had gradient values close to a mesokurtic distribution (has the same kurtosis as the normal distribution). As a result, the kurtosis of the signal gradient (KuGr) was used to quantify how likely slow wave events were recorded [125], as in

\[
KuGr_{x,y} = \frac{E\left((S_{x,y} - E(S_{x,y}))^4\right)}{E\left((S_{x,y} - E(S_{x,y}))^2\right)^2},
\]

(4.2)

where \(KuGr_{x,y}\) denotes the KuGr of the signal segment from a channel with the coordinates of \((x,y)\) in the electrode array, and \(S'_{x,y}\) denotes the gradient of the signal segment of this channel.

After empirical testing with experimental data, a KuGr value of 20 was selected as the threshold. Two examples of KuGr calculation of experimental recordings are shown in Figure 4.2. When a slow wave event was present in the segment, the KuGr value was over 20 as shown in Figure 4.2(A). However, when a slow wave event was not present, the KuGr value was below 20 as shown in Figure 4.2(B). In the BVE algorithm, if KuGr was over the threshold, it was considered to have a high likelihood of containing a slow wave event, and was considered for further processing. Otherwise, the signal segment was excluded for further processing.

Wavefront Orientation Estimation Using Bipolar Signals

If electrodes were located along a wavefront, they would have similar potential values and therefore the bipolar signal would have a relatively small amplitude, especially when compared to bipolar electrode pairs oriented perpendicular to the travelling wavefront plane [81].

Signal segments were normalised to have an amplitude range of 0 - 1, after which the mean baseline offset of the normalised signal segment was subtracted. To find the wavefront orientation, bipolar signal segments of various configurations were constructed. To maintain spatial resolution and provide sufficient angle resolution, two patch sizes were selected: 3 × 3 electrodes and 2 × 2 electrodes as shown in Figure 4.3. Patches were translated over the electrode array sequentially. The middle channel of the patch was termed
4.1 Methods

Figure 4.3: Patch size configurations for the BVE algorithm. The middle channel marked with C represents the current channel with coordinates (x,y). (A) For the 3 × 3 patch configuration, there was 1 patch around the current channel. (B) For the 2 × 2 patch configuration, there were 4 possible patches around the current channel (identified by different line styles).

the current channel. The electrode pair configurations were designed to have distances as equal as possible to avoid bias in the amplitude range calculation.

There was always a 3 × 3 patch around the current channel. Eight directional bipolar signal segments were calculated using the signal segments of the channels within this patch with directions of 0°, 27°, 45°, 63°, 90°, 117°, 135°, and 153° (angles were reported clockwise to the positive direction of the x axis) as shown in Figure 4.4(A - H). The electrode pair with the largest total KuGr was selected as the pair to represent each direction. If any of the 8 directional bipolar signal segments was not present, the algorithm processed the current channel with a 2 × 2 patch instead.

There were at most four 2 × 2 patches around the current channel as shown in Figure 4.4(I - L). Four bipolar signal segments of different directions were calculated using the signal segments of the channels within these patches (0°, 45°, 90°, and 135°). As with the 3 × 3 patch, the bipolar electrode pair in the 2 × 2 configuration with the largest total KuGr was selected to represent the direction. Directional bipolar signal segments were calculated using available patches at the edges or the corners of the electrode array. All 4 directional bipolar signal segments were required to compute the wavefront orientation, otherwise the orientation was unable to be estimated. The direction of the bipolar signal segment with the smallest potential amplitude range was considered to be aligned with the wavefront.

Computation of Wavefront Velocity Using Bipolar Signal Morphology

Once the wavefront orientation was determined, the algorithm assessed the morphology of the directional bipolar signal segment orthogonal to the wavefront, to estimate the propagation direction and speed. The time delay between the unipolar signal downstrokes (activation phases) of the corresponding channel pair resulted in a positive or negative deflection in the bipolar signal segment as shown in Figure 4.6(C - D). To determine if it was a positive or a negative deflection, a Tukey window of amplitude 1 - 2 was first applied to the bipolar signal segment to minimize boundary effects.

The gradient of the bipolar signal segment was then calculated using central finite differences, after which the steepest upstroke and downstroke points were identified. Based on this information, one of
Figure 4.4: Electrode pair configurations. Each figure represents the electrode pair configuration for the bipolar signal calculation of 1 direction. Within each figure, different colours represent different electrode pair candidates. The electrode pair with the largest total KuGr was selected as the pair to represent each direction. Eight and four directional bipolar signal segments were calculated using the electrode pairs as in (A - H) for the 3 × 3 patch and in (I - L) for the 2 × 2 patch respectively.
the two potential propagation directions was eliminated, resulting in the final estimated direction of the propagation. The time delay between the steepest upstroke and downstroke points was calculated from the gradient of the bipolar signal segment. The estimated speed was computed by dividing the distance between the corresponding electrode pair by the time delay.

If the slow wave frequency was higher than 3.5 cpm, two events would be present in a bipolar signal segment. In such cases, a reversed propagation velocity may be estimated, when the upstroke and downstroke points from different wavefronts were identified. Thus, a threshold of 2 s was applied on the time delay between the upstroke and downstroke points to address this issue. The threshold value of 2 s was selected to account for normal slow wave speed as low as 5.7 mm·s⁻¹ [31]. If the time delay was greater than the threshold, the morphology analysis would be performed again only on the first half of the bipolar signal segment to account for only the first event.

After the calculation of the directions and speeds, interpolation and filtering were introduced to improve the coverage and visualisation. Inverse distance weighting interpolation with a radius of double the inter-electrode spacing (8 mm) was applied on the estimated directions and speeds, once to interpolate, followed by once to extrapolate the edges [111]. Then, the interpolated directions and speeds were filtered with a 2-dimensional Gaussian smoothing kernel with an SD of 0.5 [42].

4.1.3 Visualisation and Performance Measurements

For the synthetic signals, the ATs were analytically defined at a frequency of 3 cpm using methods as previously described [96]. For experimental signals, the standard “marking-and-grouping” framework was applied to identify and group the ATs followed by manual review and correction [126]. For each propagating wavefront, an isochronal map was generated. The isochronal colour bands indicated the area of the wavefront propagation over a given time interval. The slow wave propagation velocities were estimated using the smoothed finite difference method [96], which was used as the gold standard.

The BVE algorithm was applied to both the synthetic and the experimental signals to estimate the wavefront velocity. The velocity vectors estimated by the BVE algorithm were overlaid as arrows on the isochronal activation maps to visualise and validate the results at each channel and for each sliding window. The angle of the arrow indicates the direction, while the length indicates the speed.

The mean absolute direction and speed errors between the velocities from the BVE algorithm and the gold standard were computed for each sliding window with slow wave event. For the synthetic signals, the mean absolute direction and speed error against each type and level of the simulated noise was calculated for each slow wave pattern. For the experimental signals, the mean absolute direction and speed error for each pig was calculated. The overall accuracy of the BVE algorithm was evaluated against experimental signals. The mean absolute direction and speed error across all the pigs was computed and reported as mean ± standard error of the mean. The time used by the “marking-and-grouping” method and the BVE algorithm was measured and compared.
4.2 Results

Synthetic signals of the 3 propagation patterns were generated and the corresponding isochronal activation maps are shown in Figure 4.5. Ten \textit{in vivo} HR recordings from pigs (\(n = 7\), 43 ± 11 kg), with a duration of 5 minutes per recording were used for this study. Four of the ten recordings had normal slow wave patterns, while the other six exhibited dysrhythmic patterns.

4.2.1 The Bipolar Velocity Estimation Algorithm

The BVE algorithm was applied to the synthetic signals and the \textit{in vivo} HR slow wave recordings. Examples of intermediate results from each stage of the algorithm and the overall results are described in the following sections.

Wavefront Orientation Estimation Using Bipolar Signals

An example of wavefront orientation estimation is shown in Figure 4.6(A - B). Eight directional bipolar signal segments were constructed using the unipolar signal segments from the channels within the 3 × 3 patch as shown in Figure 4.6(A). The potential amplitude ranges were calculated and are shown in Figure 4.6(B). The bipolar signal segment of the smallest potential amplitude range was at an orientation of 27°, which was the estimated wavefront orientation. Two potential wavefront directions were identified at 117° and 297°, which were orthogonal to the estimated wavefront as shown in Figure 4.6(A).

Computation of Wavefront Velocity Using Bipolar Signal Morphology

The gradient of the bipolar signal segment that was orthogonal to the estimated wavefront was calculated, which was at 117° (or 297°). The unipolar signal segments from the corresponding electrode pair are shown in Figure 4.6(C). The resulting bipolar signal segment is shown in Figure 4.6(D) and the gradient of the bipolar signal segment is shown in Figure 4.6(E). The maximum and minimum points of the gradient of the bipolar signal segment were identified. The morphology of the deflection caused by the time delay between the unipolar signal downstrokes was analysed. In this example, the minimum point occurred prior...
Figure 4.6: Intermediate results of the BVE algorithm (isochronal level is 2 s). (A) The configuration of the HR electrode array overlaid on the activation map. The 3 × 3 square shows the patch configuration. The dotted line shows the estimated wavefront orientation. The 2 potential propagation directions are shown as arrows. The blue diamond and square show the locations of the active and the reference electrodes in (C). (B) The normalised bipolar potential amplitude range of the 8 directional bipolar segments. The solid bar shows the smallest range. (C) The unipolar signal segments from the electrode pair that was orthogonal to the estimated wavefront. (D) The bipolar signal segment corresponding to (C). (E) The gradient of (D). (F) The interpolated and filtered velocity estimation overlaid on the activation map. The result for the current channel is surrounded by a square.
to the maximum point and therefore the deflection was identified as negative. Thus, the wavefront reached the active electrode before the reference electrode and the slow wave propagation direction was estimated to be $117^\circ$.

The bipolar signal segment that was orthogonal to the estimated wavefront was produced by a pair of electrodes with a distance of about 8.9 mm as shown in Figure 4.6(A), given the inter-electrode distance was 4 mm. The time delay between the slow wave events from this pair of electrodes was 1.2 s and therefore resulted in an estimated speed of $7.4 \text{ mm} \cdot \text{s}^{-1}$.

### 4.2.2 Visualisation

Figure 4.6(F) shows an isochronal map using the “marking-and-grouping” approach along with the velocities from the BVE algorithm with a sliding window which ended at 427 s. The velocities were shown as arrows overlaid on the “marking-and-grouping” generated isochronal activation map. The directions of the arrows were consistent with the isochronal activation map as shown in Figure 4.6(F).

### 4.2.3 Performance Measurements

#### Synthetic Signals

The mean absolute direction and speed errors at all noise levels for each slow wave pattern are shown in Figure 4.7(A - D) and Table 4.1. In general, for both types of synthetic noise, the mean absolute errors increased when the SNR decreased. The linear wavefront pattern was oriented at $45^\circ$, which matched the axis of the bipolar electrodes. Therefore, the BVE algorithm was able to accurately estimate the velocity with minimal error. Thus, the linear wavefront pattern had negligible direction and speed error for all levels of noise investigated. The pacemaker wavefront pattern showed larger errors, while the colliding wavefront pattern showed the largest errors. For the synthetic ventilator noise added signals as shown in Figure 4.7(A - B), the slow wave propagation velocities of the signals became undetectable with a ventilator noise amplitude greater than 75% of the average slow wave amplitude. For the synthetic high-frequency noise added signals as shown in Figure 4.7(C - D), the signals became indistinguishable at SNR lower than 12 dB as shown in Figure 4.8. Thus, the SNRs between 60 dB and 12 dB were included in the calculations.

<table>
<thead>
<tr>
<th>Noise Type</th>
<th>Linear</th>
<th>Pacemaker</th>
<th>Colliding</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilator Noise (0%)</td>
<td>0°</td>
<td>5.3°</td>
<td>8.0°</td>
<td>4.4°</td>
</tr>
<tr>
<td></td>
<td>0.04 mm·s$^{-1}$</td>
<td>0.8 mm·s$^{-1}$</td>
<td>1.8 mm·s$^{-1}$</td>
<td>0.9 mm·s$^{-1}$</td>
</tr>
<tr>
<td>Ventilator Noise (70%)</td>
<td>0°</td>
<td>7.5°</td>
<td>18.2°</td>
<td>8.6°</td>
</tr>
<tr>
<td></td>
<td>0.01 mm·s$^{-1}$</td>
<td>1.2 mm·s$^{-1}$</td>
<td>3.1 mm·s$^{-1}$</td>
<td>1.4 mm·s$^{-1}$</td>
</tr>
<tr>
<td>High-Frequency Noise (60 dB)</td>
<td>0°</td>
<td>9.4°</td>
<td>14.5°</td>
<td>8.0°</td>
</tr>
<tr>
<td></td>
<td>0.04 mm·s$^{-1}$</td>
<td>1.0 mm·s$^{-1}$</td>
<td>2.0 mm·s$^{-1}$</td>
<td>1.0 mm·s$^{-1}$</td>
</tr>
<tr>
<td>High-Frequency Noise (12 dB)</td>
<td>0.1°</td>
<td>10.5°</td>
<td>18.7°</td>
<td>9.8°</td>
</tr>
<tr>
<td></td>
<td>0.2 mm·s$^{-1}$</td>
<td>1.1 mm·s$^{-1}$</td>
<td>2.3 mm·s$^{-1}$</td>
<td>1.2 mm·s$^{-1}$</td>
</tr>
</tbody>
</table>

Table 4.1: Mean absolute direction and speed errors for synthetic signals for 4 noise levels.
Figure 4.7: Absolute direction and speed error results in comparison to the gold standard. (A - B) The error results against synthetic ventilator noise. The mean error increased from 4.4° and 0.9 mm·s\(^{-1}\) (0%) to 8.6° and 1.4 mm·s\(^{-1}\) (70%). (C - D) The error results against synthetic high-frequency noise. The mean error increased from 8.0° and 1.0 mm·s\(^{-1}\) (60 dB) to 9.8° and 1.2 mm·s\(^{-1}\) (12 dB). Note that the amplitude of the high-frequency noise ranged from 0.2% (60 dB) to 146.4% (4 dB) of the average slow wave amplitude. (E - F) The error results against experimental recordings. The number of sliding windows measured for each pig is shown as n. The mean errors across all the pigs were 19.2 ± 1.7° and 2.0 ± 0.2 mm·s\(^{-1}\). The central mark of each box represents the median. The bottom and top edges of the boxes represent the 25th and 75th percentiles. The whiskers extend to the most extreme data points that are within 1.5 times the interquartile range away from the bottom or top of the box.
**Experimental Signals**

The mean absolute direction and speed errors for each pig are shown in Figure 4.7(E - F). Pig 2 had the largest deviation as it was a case where the FPC electrode array did not have sufficient contact with the tissue and therefore slow waves were not captured by a number of channels. Pigs 1, 3, 4, and 5 exhibited dysrhythmic slow waves, which generally showed larger deviations than the normal slow wave activity (Pigs 6 and 7). The mean absolute direction and speed errors across all the pigs were $19.2 \pm 1.7^\circ$ and $2.0 \pm 0.2 \text{ mm} \cdot \text{s}^{-1}$.

To process a 5 minute HR (256 channels) slow wave recording, the “marking-and-grouping” method took 4 minutes without manual review and correction. This time increased to over 1 hour by introducing manual review and correction to ensure the accuracy for normal slow wave propagation patterns. For dysrhythmic and dynamic slow wave propagation profiles, which included conduction blocks and ectopic pacemakers, a much longer time was required for review. However, the BVE algorithm processed the same data set in $8.7 \pm 1.9$ s (mean ± SD).

**4.3 Discussion**

In this paper we developed the BVE algorithm, verified the approach with synthetic signals and validated it against the standard “marking-and-grouping” framework using *in vivo* HR experimental slow wave recordings. In the BVE algorithm, bipolar signals were constructed from HR unipolar recordings to compute the local directional information of the propagating wavefront. Gastric slow wave velocities were estimated rapidly using the BVE algorithm with minimal errors, from all the synthetic and experimental signals.

Bipolar signals have not been used in GI *in vivo* HR recordings and more information could be gained using this approach. In particular, the BVE algorithm can be used to detect regions of high velocity in...
gastric HR recordings, which could help identify the presence of dysrhythmias in a rapid manner [84]. The automated nature of the BVE algorithm will be significant for long term studies and chronic recordings, which are necessary to relate symptoms and GI function to bio-electrical slow wave activity [91, 120]. Bipolar slow wave recordings will also motivate new HR bipolar electrode array designs, such as electrode arrays with non-uniform density [74, 107]. There has been increasing interest to measure bio-electrical activities on the body surface and surface laplacian techniques have been commonly used to enhance the signal [40]. This method could be translated into a custom hardware electrode array as has been designed for surface electrocardiology and electroenterogram [13, 39]. In the future, the BVE algorithm can be applied to monophasic recordings to estimate the velocity of the activation phase and the recovery phase.

In comparison to the cross-correlation velocity method [94], the BVE velocity error was slightly higher (by 1 mm·s$^{-1}$, 19°), but was 3 times more computationally efficient. In the BVE algorithm the KuGr of the unipolar recording was used as a metric to denote the likelihood of containing slow wave events, which is a novel metric that can assist with bad channel and slow wave event detection. This is similar to the application of kurtosis to assess signal fidelity for gastric slow wave analysis in real time [19].

Additional features could be incorporated to implement the BVE algorithm in real time. For example, causal linear filters can be used to enable real time processing and visualisation [21]. By extending the algorithm with the ability to group and classify the estimated velocities, diseased tissue location or the orientation of muscle activation around the organ axis could be detected in real time. New techniques such as the cardiac omnipolar mapping technique can be translated for GI applications, benefiting the BVE algorithm to provide more accurate detection results [75]. The BVE method focuses exclusively on the activation phase to estimate wavefront velocities. In future, the velocity of the slow wave recovery phase wavefront will also be computed. It is anticipated that the dispersion in the activation and the recovery wavefronts could play a role in the mechanics of dysrhythmias [93].

During the third stage of the BVE algorithm (computation of wavefront velocity using bipolar signal morphology), the algorithm may not be able to estimate a velocity. This occurs when part of a slow wave event is contained in a sliding window and the maximum and minimum gradient points may not represent the deflection caused by the time delay between the downstrokes of the unipolar signal segments. In these cases, the algorithm would result in an incorrectly estimated velocity. A range of SNRs from 4.23 - 24 dB have been reported by studies using FPC electrode arrays [90, 34]. Low SNRs could occur due to technical faults and/or physiological aspects. An example of a technical fault is when the connector is not soldered correctly, and a physiological aspect is when slow waves are colliding or if there is a conduction block in the tissue. The signals in that particular region of the electrode array will have poorer SNR.

In future studies, in vivo experimental recordings from different species and different parts of the GI system, such as the intestine and the colon, will be used to validate the BVE algorithm. The parameters will need to be adapted for the varying frequencies and velocities for each of the GI organs. In particular, the sliding window size, the kurtosis signal gradient threshold condition, and the time difference threshold will need to be optimised.
4.4 Summary

In this chapter, a novel BVE algorithm was developed and validated to estimate the gastric slow wave propagation velocity. A key advantage of this method is that it can rapidly estimate slow wave propagation velocities, enabling real time assessment of propagation dynamics. Application of BVE during or after an intervention such as pacing [4] or ablation [1] will allow for wavefronts of interest to be rapidly determined and could aid in the understanding of slow waves in health and disease.
Chapter 5

Detection of Monophasic Slow Wave Activation Time Using Wavelet Decomposition


The timing of the depolarisation of the cell can be captured using the AT in extracellular recordings (Chapter 2). AT identification is essential for AT analysis, RT identification, pacing studies, ablation, and for related electrophysiological investigations [36, 97, 4, 1]. Accurate AT identification enables reliable slow wave analysis. There are various detection approaches, including threshold-based approaches [71]. The electrodes were built in Chapter 3 to record slow waves. For the next step, analysis methods are required. Chapter 4 focused on velocity estimation without detection, whereas this chapter aimed to improve the detection accuracy. In the GI field, several automated algorithms have been developed to detect the biphasic slow wave AT which use threshold-based methods (Section 2.4.1). Wavelets are mathematical decomposition techniques which have been used widely on bio-electrical signals to extract discriminative features for automatic diagnosis [103, 3]. In addition, wavelets have been shown to have combined properties of data smoothing and differentiation which are useful for detection [76, 60]. In this chapter, a novel method termed the variable threshold wavelet (VTW) algorithm was developed to detect the AT of monophasic slow waves. The accuracy of the VTW algorithm was compared to a commonly used varying threshold algorithm.

5.1 Methods

5.1.1 The Variable Threshold Wavelet Algorithm

The VTW algorithm is divided into 2 parts: (i) detection of when a slow wave event has travelled under an electrode, (ii) calculation of the accurate placement of the AT within each slow wave event. For the first part, the VTW algorithm utilised the NEO method in the FEVT algorithm (Section 2.4.1) [36]. For
Figure 5.1: Wavelet decomposition using algorithme à trous. In which, \( y(t; n)^{wl} \) and \( y(t; n)^{wh} \) denotes the low-frequency part and the high-frequency part of the \( n \)th level wavelet decomposition. The low-frequency part of the current wavelet decomposition level was used as the input of the next level.

the second part, the AT of each monophasic slow wave event was marked at the time of the most positive gradient within the activation phase. Wavelet decomposition was used as the derivative algorithm for the VTW algorithm, which has been shown to have combined properties of data smoothing and differentiation [76, 60]. The first derivative of signal \( y(t) \) was calculated as in

\[
\frac{dy(t)}{dt} = \frac{y(t; n)^{wh}}{2^{\frac{\ln 2}{n}}}, \tag{5.1}
\]

where \( y(t; n)^{wh} \) denotes the high-frequency part of the wavelet decomposition of \( y(t) \) with a decomposition level of \( n \). Algorithme à trous (Figure 5.1) was used in this study to calculate the spline wavelet decomposition with a decomposition level of 6 [30, 93]. In brief, the signal \( y(t) \) was filtered by a high-pass filter and a low-pass filter to generate \( y(t; 1)^{wl} \) and \( y(t; 1)^{wh} \). After which, \( y(t; 1)^{wl} \) was fed into the next pair of filters to calculate the wavelet decomposition of the next level. The maximum derivative points were marked based on the results of the wavelet derivative decomposition, which represent the AT. A flowchart of the VTW algorithm is shown in Figure 5.2.

5.1.2 Performance Measurements

Ethical approval for experimental studies was granted by the University of Auckland Animal Ethics Committee. Experimental methods and animal care were performed as described in Section 3.1.4. A single-channel wet suction electrode with a 1.2 mm glass capillary tube was applied to record monophasic slow waves from pig stomachs. Recordings were filtered using the methods described in Section 3.1.5.

Two kinds of simulated noise were added to the \textit{in vivo} recorded raw signals using methods described in Section 4.1.1 to mimic experimental recordings of various SNRs, and to compare the performances of the algorithms against various levels of noise. In addition, for the high-frequency noise simulation, white noise was added to the slow wave signal to create an SNR range of -4 to 60 dB. Sample signals are shown in Figure 5.3.

Two algorithms were applied to identify the AT: the modified FEVT algorithm and the VTW algorithm. The original FEVT algorithm (Section 2.4.1) was developed to detect the ATs (downstrokes) of biphasic
5.1 Methods

Figure 5.2: Flowchart of the VTW algorithm. The left part of the flowchart detects when a slow wave event has travelled under an electrode, while right detects the AT within each event.

Figure 5.3: Examples of ventilator noise added and high-frequency noise added data. (A) shows the raw data (red trace) and the summation of raw data and ventilator noise of 10% average slow wave amplitude (blue trace). (B) shows the raw data (red trace) and white noise added data with an SNR of 50 dB (blue trace).
slow wave signals. In this study, the FEVT algorithm was modified to mark the maximum derivative points on upstrokes to detect the ATs of monophasic signals. The key difference between the 2 algorithms is the method used to estimate the derivative of the signal. The FEVT algorithm used a 3-point central difference approach, whereas the VTW algorithm used a wavelet derivative approach.

Reference ATs were identified by applying the modified FEVT algorithm and the VTW algorithm on signals with the absence of noise and was used for sensitivity calculation as in

\[
\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}
\]  

(5.2)

and positive predictive value (PPV) calculation as in

\[
\text{PPV} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}.
\]  

(5.3)

where a true positive was achieved where an AT was identified within ±1 s period around the corresponding reference marker.

Sensitivity and PPV are inversely correlated [36]. Thus, the area under the curve \( A_{roc} \) was computed as in

\[
A_{roc} = \text{Sensitivity} \times \text{PPV}.
\]  

(5.4)

The \( A_{roc} \) of AT identification with monophasic signals was calculated and averaged for each noise level to assess the performances of both algorithms. A higher \( A_{roc} \) value means better overall performance. The performance difference between the VTW and FEVT algorithms was expressed in percentage improvement metric (PIM) calculated as in

\[
PIM = \frac{A_{roc}^{VTW} - A_{roc}^{FEVT}}{A_{roc}^{FEVT}}.
\]  

(5.5)

5.2 Results

Ten in vivo slow wave signals from pigs (\( n = 7, 36.8 \pm 5.8 \text{ kg} \)) were recorded for a duration of 3 - 5 minutes. A case where the VTW algorithm performed better than the FEVT algorithm is shown in Figure 5.4. In this case, the FEVT algorithm misidentified the second AT, whereas the VTW algorithm marked it correctly. The time difference between the markers from both algorithms was 3.7 s. This showed that the FEVT algorithm was sensitive to high-frequency noise. As a result, the accuracy of the FEVT algorithm was lowered when processing high-frequency noisy signals. This result suggests that with the presence of high-frequency noise, the 3-point central difference derivative approach is not as accurate as the wavelet derivative approach for AT identification.

Figure 5.5(A) shows the \( A_{roc} \) results of the AT detection with the addition of ventilator noise. The FEVT and the VTW algorithms performed similarly with equal accuracy. Figure 5.5(B) shows the results for the signals with additional high-frequency noise. Over all noise levels, the mean value of PIM was
5.3 Discussion

In this study, we introduced an algorithm to detect the AT of monophasic slow wave signals. Based on the comparison conducted, the VTW algorithm had better overall performance than the FEVT algorithm, against both ventilator and high-frequency noise added to slow waves. The VTW algorithm provided more stable and accurate identifications, especially in the presence of high-frequency noise. This makes it more suitable for analysing low SNR recordings. For slow wave signals with ventilator noise, the VTW algorithm did not introduce noticeable improvement, but ventilator noise can be efficiently removed by using ventilator removal algorithm [90] before automatic AT identifications.

Accurate identification of the AT by the VTW algorithm allows for electrophysiology studies to be undertaken more efficiently. Manual review and correction of the inaccurate algorithm outcomes can be reduced, which also minimize the time needed to process and analyse a large amount of data, such as with HR GI recordings [31] and long term recordings [72].

However, the VTW algorithm was only validated for monophasic gastric slow wave recordings from pigs. Verification of the VTW algorithm on other GI organs, such as intestine and colon, are required before application, as the morphology and frequency of slow wave signals in those organs differ from...
Figure 5.5: Performance measurement results of both the algorithms. (A) shows the performances on ventilator noise added signals. The curves almost overlay each other, which illustrates similar performances. (B) shows the performances on high-frequency noise added signals. For all SNR levels, the VTW algorithm had better performance than the FEVT algorithm.

gastric slow waves. Due to the inter-species differences [17], validation on other species is also necessary. As wavelet derivative approach is insensitive to high-frequency noise, it has already been verified and validated with biphasic slow wave RT identification [97]. The deviation in accuracy is minor when the SNR of the signal is below 3 dB. Future studies will focus on applying this algorithm to biphasic slow wave AT detection and monophasic slow wave RT identification.

5.4 Summary

In this chapter, a novel algorithm termed the VTW algorithm was developed, featuring wavelet decomposition to compute the derivative, to detect the AT of monophasic slow waves. The performance of the VTW algorithm was compared against an existing FEVT algorithm. Varying levels of synthetic noise representing ventilator noise and high-frequency noise were added to in vivo slow wave recordings. Sensitivity, PPV, $A_{roc}$, and PIM of AT identification accuracy were calculated. Compared to the existing FEVT algorithm, the VTW algorithm achieved similar performance in identifying the AT of slow waves against ventilator noise. In the presence of high-frequency noise, the VTW algorithm improved the $A_{roc}$ of the existing FEVT algorithm by 11.1%. This VTW algorithm was applied in the following chapters to automatically identify ATs and RTs during slow wave analysis.
Chapter 6

Monophasic Gastric Slow Waves to Quantify Activation and Recovery Profiles

Aspects of this chapter have been published in: Han, H., Cheng, L. K., and Paskaranandavadivel, N. (2022). High-resolution in vivo monophasic gastric slow waves to quantify activation and recovery profiles. Neurogastroenterol. Motil., e14422 [49].

Monophasic cardiac action potentials contain vital recovery information to help explain the genesis of cardiac arrhythmias (Section 1.2). In the GI field, most investigations have focused on biphasic extracellular recordings [72, 83, 31], whereas, there have been limited studies that have focused on slow wave recovery analysis. There is interest in defining the mechanism of abnormal activation, which requires recovery analysis. Multi-channel wet suction electrode arrays (Chapter 3) were built to provide the recovery phase of slow waves. Detection and quantification methods were also developed to support recovery analysis investigations (Chapters 4 and 5). In this chapter, multi-channel wet suction electrode arrays were used to record HR monophasic gastric slow waves and were validated against conventional FPC electrode arrays. The phases of monophasic slow waves were quantified. ATs and RTs of monophasic gastric slow waves were analysed to show that additional information, including recovery phase spatial profile and ARI profile, can be obtained from monophasic slow waves compared to biphasic slow waves.

6.1 Methods

Experimental methods and animal care were performed as described in Section 3.1.4. HR wet suction electrode arrays (Section 3.1.3) and contact FPC electrode arrays (192 - 224 channels with 4 mm spacing) were placed adjacent to each other in gentle contact with the gastric serosa. Recordings were filtered using the methods described in Section 3.1.5.

The ATs of the recordings from the FPC and the wet suction electrode arrays were automatically marked followed by manual review. For biphasic slow wave recordings, ATs were marked at the time of the most negative gradient within the activation phase of each slow wave event using the FEVT algorithm (Section 2.4.1). For monophasic slow wave recordings, ATs were marked at the time of the most positive
gradient within the activation phase of each slow wave event using the VTW algorithm described in Chapter 5 [46]. The RTs were marked at the maximum gradient for biphasic slow wave signals or the minimum gradient for monophasic slow wave signals in the period 1 to 7 s after ATs, using the VTW algorithm. The time range was based on previous studies [93, 33]. Finally, manual review and correction were performed to ensure the accuracy of the markers. Biphasic and monophasic slow wave events were extracted by segmenting the filtered recordings with a window of 13 s (from 4 s before to 9 s after each AT). The baseline offset of each signal segment was then removed.

### 6.1.1 Categorisation of Monophasic Slow Wave

For each of the monophasic signal segment, the amplitude of the slow wave event and the amplitude of the early repolarisation were measured. In Figure 6.1, the blue line denotes the monophasic signal segment whereas the red line denotes the early repolarisation. In this example, the amplitude of the slow wave event was measured between the peak and the baseline of the slow wave event at 1.1 mV, whereas the amplitude of the early repolarisation was measured between the peak and the trough of the early repolarisation at 0.6 mV. Each segment was then categorised into 1 of the 2 groups. If the early repolarisation amplitude of the slow wave event was greater than 50% of the slow wave event amplitude, this slow wave event was categorised as monophasic slow wave morphology 1. Otherwise, this slow wave event was categorised as monophasic slow wave morphology 2. Thus, the example shown in Figure 6.1 was categorised as monophasic slow wave morphology 1. The percentage prevalence of each group was calculated for each pig. The overall mean and SD prevalence of each morphology was calculated across all pigs.
6.1 Methods

6.1.2 Average Morphology, Amplitude, Activation to Recovery Interval, and Gradient

For each pig, four types of average morphologies were calculated: (i) biphasic slow wave, (ii) monophasic slow wave (containing both morphology 1 and 2), (iii) monophasic slow wave morphology 1, (iv) monophasic slow wave morphology 2. The overall average morphology was generated by calculating the mean and the SD across all the pigs. The amplitude of each slow wave event was computed by subtracting the maximum potential amplitude by the minimum potential amplitude within a window from 1 s before to 4 s after the AT. The time range was defined based on the observation that both the trough potential and the peak potential of a slow wave event were occurred within this range. The ARI for each slow wave event was computed by measuring the time difference between the AT and the corresponding RT. The gradients at the AT and the RT of each slow wave event were calculated using a three-point central difference approach.

For each pig, the mean amplitude, ARI, AT gradient, and RT gradient were calculated for each morphology group. The overall average amplitude, ARI, absolute AT gradient, and absolute RT gradient of each morphology group were then generated by calculating the mean and the SD across all the pigs. Absolute gradients were used because the slopes for the AT and the RT had opposite signs for biphasic and monophasic signals. One-way ANOVA and Tukey’s honest significance tests were then conducted between biphasic and monophasic, and between monophasic morphology 1 and morphology 2 groups, respectively. A p-value of 0.05 was used to determine statistical significance.

6.1.3 Coverage Loss Percentage Calculation

Since the signals were recorded simultaneously by the FPC and the wet suction electrode arrays, ATs of biphasic and monophasic slow wave recordings were manually clustered together, into wavefronts based on the timing. Three wavefronts were then selected for each pig and included in the following analysis. For each wavefront, the coverage of each electrode type was calculated as

\[
AT \text{ Coverage} = \frac{\text{Number of Channels with an Identifiable AT}}{\text{Total Channel Number}} \times 100\% \quad (6.1)
\]

and

\[
RT \text{ Coverage} = \frac{\text{Number of Channels with an Identifiable RT}}{\text{Total Channel Number}} \times 100\%. \quad (6.2)
\]

For each electrode type, a loss percentage between the AT and the RT coverages was calculated for each wavefront as

\[
\text{Coverage Loss Percentage} = \frac{AT \text{ Coverage} - RT \text{ Coverage}}{AT \text{ Coverage}} \times 100\%. \quad (6.3)
\]

After which, the overall average coverage loss percentage of each electrode type was calculated by computing the mean and the SD across all the pigs using the method described in Section 6.1.2.
6.1.4 Activation and Recovery Map Generation

In experimental recordings, slow waves may not be recorded by some electrodes, due to loss of electrode array contact or degradation of the electrode. To compensate for these electrodes, within each wavefront and each electrode array, the ATs and RTs were interpolated for each of these electrodes using inverse distance weighting interpolation with a radius of twice the inter-electrode distance (8 mm for FPC electrode arrays and 20 mm for wet suction electrode arrays) [111]. Finally, the ATs and RTs were linearly interpolated with a unified spacing across all the electrode arrays to improve visualisation.

The interpolated ATs and RTs were visualised using isochronal activation and recovery maps to assess the slow wave propagation pathways. The isochronal colour bands indicated the area of the wavefront propagation per a certain time interval. For each electrode array, a pair of activation and recovery maps were generated for each wavefront.

6.2 Results

HR biphasic and monophasic slow waves were recorded by FPC and wet suction electrode arrays individually, from 8 pigs (41.3 ± 2.3 kg), each for a duration of 5 minutes. An average of 1145.9 ± 584.9 slow wave events per pig were analysed for all the pigs. Normal antegrade slow wave patterns were observed in 3 out of 8 pig studies, whereas the other 5 exhibited dysrhythmic patterns with slow wave frequencies ranging from 2 to 4 cpm.

6.2.1 Categorisation of Monophasic Slow Wave and Average Morphology

Across all experimental studies, the overall prevalence of monophasic slow wave morphology 1 and 2 was 37 ± 25% and 63 ± 25%. The average morphologies of biphasic slow wave, monophasic slow wave, monophasic slow wave morphology 1, and monophasic slow wave morphology 2 are shown in Figure 6.2. The average biphasic slow wave morphology included a gradual upstroke, a sharp downstroke, and a sharp upstroke, followed by a gradual upstroke back to the baseline. The average monophasic slow wave morphology consisted of a sharp upstroke, a sharp downstroke (early repolarisation), and a gradual upstroke, followed by a plateau and a sharp downstroke back to the baseline. The average morphology of monophasic slow wave morphology 1 and 2 was similar to the average monophasic slow wave morphology, other than the amplitude of the early repolarisation. There was greater variability in the morphology of the monophasic slow wave compared to the biphasic slow wave as shown in Figure 6.2(A - B) (the maximum value within 1 SD of the mean was 0.6 mV vs 0.3 mV). There was less variation in monophasic slow wave morphology 1 compared with morphology 2 as shown in Figure 6.2(C - D) (the maximum value within 1 SD of the mean was 0.2 mV vs 0.6 mV).
Figure 6.2: Average morphology of (A) biphasic slow wave, (B) monophasic slow wave, (C) monophasic slow wave morphology 1, and (D) monophasic slow wave morphology 2. The mean morphology is shown as the black line, and 1 SD from the mean is shown as the grey shaded area. The ATs and the RTs are identified as green plus and red cross symbols.
6.2.2 Average Amplitude, Activation to Recovery Interval, Activation Time Gradient, and Recovery Time Gradient

Box plots of the results of the average amplitude, ARI, AT gradient, and RT gradient are shown in Figure 6.3. In summary, between the biphasic and the monophasic groups, there were no differences in amplitudes and absolute AT gradients as shown in Figure 6.3(A) and (C) (1.6 ± 0.5 mV vs 1.6 ± 0.5 mV, p = 0.7977 and 0.5 ± 0.2 mV·s⁻¹ vs 0.6 ± 0.2 mV·s⁻¹, p = 0.1256). However, the ARIs from the biphasic slow waves were shorter than those from the monophasic slow waves as shown in Figure 6.3(B) (3.2 ± 0.8 s vs 4.4 ± 0.9 s, p = 0.0199). In addition, the absolute RT gradients of monophasic slow waves were significantly larger than those of the biphasic slow waves as shown in Figure 6.3(D) (0.5 ± 0.1 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹, p = 0.0410). In other words, monophasic slow waves had similarly steep activation phases and steeper recovery phases when compared to biphasic slow waves. For both the biphasic and the monophasic slow waves, the activation phases were easily identifiable due to the large gradient around 0.55 mV·s⁻¹. Monophasic recovery phases resulted in similar absolute gradient values to activation phases and facilitated accurate detection of this phase. Between the monophasic morphology 1 and 2 groups, similar ARIs were detected as shown in Figure 6.3(B) (4.3 ± 1.0 s vs 4.4 ± 0.9 s, p = 0.8498). However, the monophasic morphology 2 had significantly larger amplitude, AT gradient, and RT gradient than the morphology 1 as shown in Figure 6.3(A), (C), and (D) (1.8 ± 0.5 mV vs 1.1 ± 0.2 mV, p = 0.0038, 0.8 ± 0.2 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹, p < 0.0001, and 0.5 ± 0.1 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹, p = 0.0030).

6.2.3 Coverage Loss Percentages, Activation Maps, and Recovery Maps

The results of the coverage loss percentage are shown in Figure 6.4. The coverage loss percentage of wet suction electrode arrays was significantly lower than that of FPC electrode arrays (4 ± 6% vs 43 ± 11%). Identifiable RTs were not recorded by 43% of FPC electrodes on average, when identifiable ATs were recorded by the same electrode. This percentage decreased to 4% on average, when using wet suction electrodes. Two pairs of activation and recovery maps from 2 pigs are shown in Figure 6.5. From the activation maps of both pigs, the slow wave propagation directions can be identified from both FPC and wet suction electrode array maps, indicating antegrade (normal) slow wave propagations. However, for the recovery maps, a larger portion of FPC electrodes were unable to record identifiable RTs while recording identifiable ATs, compared to the wet suction electrode arrays. Therefore, the spatial recovery maps of the FPC electrode arrays provided decreased coverage of RT points in the array and were unreliable for spatiotemporal analysis. In comparison, the recovery maps of the wet suction electrode arrays had comparable coverages to the activation maps as seen in Figure 6.5. The pathways of the recovery profiles from the wet suction electrode arrays in Figure 6.5 were recognisable as antegrade slow wave propagations, which were consistent with the corresponding activation profiles. In general, these recovery maps from wet suction electrode arrays were able to provide more useful information about the propagation dynamics than those from FPC electrode arrays.
6.2 Results

Figure 6.3: Box plots quantifying the (A) amplitude, (B) ARI, (C) AT gradient, and (D) RT gradient, for biphasic slow waves (grey), monophasic slow waves (violet), monophasic slow wave morphology 1 (green), and monophasic slow wave morphology 2 (pink). The number of pigs included in each calculation is shown as n. *: p < 0.05.
Figure 6.4: Comparison of coverage loss percentages between the FPC electrode arrays and the wet suction electrode arrays. The coverage loss percentage of FPC electrode arrays was over 10 times higher than that of wet suction electrode arrays. The number of pigs included in each calculation is shown as n.

6.3 Discussion

In this chapter, the HR multi-channel wet suction electrode array was applied in in vivo pig studies to record propagating monophasic slow waves, where the activation and recovery profiles were captured with high-fidelity. Conventional FPC electrode arrays were used in pig studies to record biphasic slow waves. The average morphologies of slow wave recordings from both electrode types were compared, with the wet suction electrode array showing a more identifiable recovery phase with a higher gradient. In addition, monophasic slow wave events from the wet suction electrode array were categorised into 2 groups based on their morphological characteristics, after which their amplitudes, ARIs, and gradients were compared.

The morphologies of the biphasic and the 2 monophasic slow wave groups were similar to the various types of slow waves recorded in a previous in vitro cat study [15]. In this study by Bortoff, slow waves of 3 morphology types were recorded using needle electrodes under different experimental conditions: (i) slow waves of biphasic morphology were recorded in the bath at 1 mm above the jejunum serosa, (ii) slow waves of monophasic morphology 1 were recorded with light pressure onto the jejunum serosa, and (iii) slow waves of monophasic morphology 2 were recorded when the electrodes penetrated the jejunum serosa. The pressure between the electrode and the recording site was concluded as the reason for various recording morphologies. It was therefore hypothesised that the 2 distinct monophasic morphologies in this study were related to the difference in the pressure applied. Future studies with controlled and uniform pressure will be used to further understand the genesis of the waveforms.

Another approach to reliably acquire the recovery phase of an action potential is through optical mapping, which has been widely used in both the cardiac and the neural fields [41, 104, 2, 88]. Recently, this use of optical mapping was applied on the stomach to capture monophasic slow waves [127]. The morphology and timing of the recorded potentials were consistent with conventional gastric monophasic
Figure 6.5: Activation and recovery maps with the corresponding positions of the electrode arrays. The isochronal interval used is 1 s. The maps within the same row were generated from the same wavefront and considered as a pair. Activation maps are shown on the left, whereas corresponding recovery maps are shown on the right. (A) The positions of the electrode arrays for (B - E). (F) The positions of the electrode arrays for (G - J). The AT or RT was not identifiable for the channels marked with a white dot and was interpolated.
slow wave recordings. However, the SNR of optical recording was lower than the conventional surface contact electrode methods. The wet suction electrode could be used in tandem with optical mapping to better improve the understanding of slow waves propagation.

The leap from single-channel contact electrodes to HR FPC electrode arrays allowed the generation of activation maps showing the activation phase propagation patterns in normal and diseased cases. Abnormal slow wave activation propagation patterns have been recorded from diseased cases, whereas ordered slow wave activation propagation patterns have been recorded from subjects without gastric disorders [31, 82, 5]. It is reasonable to hypothesise that the recovery phase propagation patterns differ between normal and diseased cases and play a critical role in maintenance of these normal or abnormal slow wave propagations [93]. It is anticipated that with the introduction of this HR wet suction electrode array for recovery phase mapping, the ARI and the RAI can be quantified and the gastric electrical restitution can be defined across the organ with the use of pacing or drugs. Although the importance of ARI, RAI, and gastric electrical restitution is not yet known, it is anticipated to provide useful information about abnormal slow wave propagations and to explain the mechanisms that lead to the genesis and maintenance of gastric dysrhythmias, similar to the cardiac field (Section 1.2) [24, 10]. The use of multi-channel wet suction electrode array will help in dysrhythmia analysis and assessment of therapeutics, by enabling the ability to monitor these electrophysiology metrics in HR. For example, during pacing or ablation strategies [22, 1], the slow wave recovery phase could be used to determine the accurate timing of impulses or the precise location for ablation. In the future, HR wet suction electrode arrays can also be applied to other parts of the GI system such as the intestines and colon to capture monophasic signals for slow wave activation and recovery analysis.

6.4 Summary

In this chapter, HR wet suction electrode and FPC electrode arrays were applied in pig studies to record monophasic and biphasic slow waves. Monophasic slow wave events were categorised into two groups based on their morphological characteristics, after which their amplitudes, ARIs, and gradients were compared. Coverage of activation and recovery maps for both electrode types were calculated and compared. Monophasic slow waves had a more pronounced recovery phase with a higher absolute gradient than biphasic slow waves (0.5 ± 0.1 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹). Between the two groups of monophasic slow waves, there was a significant difference in amplitude (1.8 ± 0.5 mV vs 1.1 ± 0.2 mV), AT gradient (0.8 ± 0.2 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹), and RT gradient (0.5 ± 0.1 mV·s⁻¹ vs 0.3 ± 0.1 mV·s⁻¹). For wet suction and conventional FPC electrode arrays, the recovery map coverage in comparison to the activation map, decreased by 4 ± 6% and 43 ± 11% respectively. In conclusion, slow wave recovery phase analysis could be performed more efficiently on the monophasic signals recorded by the HR wet suction electrode arrays, compared to the biphasic signals, due to the more prominent recovery phases and lower coverage loss percentage.
Chapter 7

Monophasic Slow Wave Recording Analysis

ARI measurements are critical to improve our understanding of the mechanisms that are involved in the initiation of gastric dysrhythmias. Studies to date have focused on biphasic slow waves. Only biphasic recordings have used to analyse the spatial properties of recovery profiles. HR suction electrode array (Chapter 3) and analysis methods (Chapters 4 and 5) were proposed and validated against existing methods. In Chapter 6, it was shown that ARI was more reliable with monophasic slow waves than biphasic ones. In this chapter, the characteristics of monophasic gastric slow wave were studied. Regional variations in ARI were quantified across different regions of the stomach. After which, the relation between the ARIs of adjacent wavefronts was investigated. Finally, the relation of ARI and slow wave propagation speed was quantified across the stomach. All of these findings will enable a better understanding of the role that ARI plays in the mechanisms underlying normal and abnormal GI motility.

7.1 Methods

Ethical approval for experimental studies was granted by the University of Auckland Animal Ethics Committee. Experimental methods and animal care were performed as described in Section 3.1.4. HR wet suction electrode (Section 3.1.3) arrays (10 mm inter-electrode distance) were placed in gentle contact with the gastric serosa. Recordings were filtered using the methods described in Section 3.1.5. The ATs and RTs were marked as previously described in Section 6.1.

First, regional variations in ARI across the stomach were calculated. The time difference between each AT and the corresponding RT was calculated as the ARI. For each recording, the mean ARI was calculated. Each recording was categorised into 1 of the 3 groups based on the recording region: (i) upper corpus, (ii) mid-corpus, (iii) antrum. For each group, the overall mean and SD of the ARI were then calculated across all the pigs that had recordings in this region. One-way ANOVA and Tukey’s honest significance tests were then conducted between different regions to assess if there was a difference in ARI. A p-value of 0.05 was used to determine statistical significance.

Secondly, the relation between the ARIs of adjacent wavefronts was studied. The ARI of each channel from each wavefront was calculated and linearly fitted with that of the same channel from the next wavefront. For each linear fit, the slope, the constant, and the p-value were then calculated and compared. In addition, the relation of ARI and propagation speed across the stomach was analysed. For each of
the recordings from the upper corpus and the mid-corpus regions, at the location of each channel, the slow wave propagation speed was estimated using a smoothed finite-difference approach for 3 wavefronts [96]. For each wavefront and each channel, the ARI was then linearly fitted with the corresponding speed. The slope, the constant, and the p-value were then calculated for each linear fit. If the slope was 0 for a linear fit, between the corresponding 2 variables linear relationship can be eliminated. For each recording region, the overall mean and SD of the slope were calculated and the statistical significance in-between was determined.

7.2 Results

HR monophasic slow waves were recorded from pigs (n = 6, 41.2 ± 2.6 kg) for a duration of at least 5 minutes at 1 or more recording regions (upper corpus, mid-corpus, or antrum). The recordings were acquired from the upper corpus (n = 4), the mid-corpus (n = 5), and the antrum (n = 3). Normal antegrade slow wave patterns were observed in 1 out of 6 pigs, while the other 5 exhibited dysrhythmic patterns. The slow wave frequencies of these recordings ranged from 2 to 4 cpm.

7.2.1 Regional Variations in Activation to Recovery Interval Across the Stomach

Representative monophasic recordings for each of the 3 regions are shown in Figure 7.1(A). In this example, the mean ARIs of the 3 traces were 5.1 s (upper corpus), 5.1 s (mid-corpus), and 4.1 s (antrum). The box plot of ARI values for each region is shown in Figure 7.1(B). The ARIs from the upper corpus, the mid-corpus, and the antrum regions were 4.8 ± 0.5 s, 4.7 ± 0.4 s, and 3.5 ± 0.7 s on average. In addition, the ARIs of the antrum region were significantly shorter than the other regions. Whereas there was no significant difference between the ARIs from the upper corpus and the mid-corpus regions.

7.2.2 Relation Between the Activation to Recovery Intervals of Adjacent Wavefronts

Detailed analysis was performed using the methods described in Section 6.1.4 on all the recordings (n = 3) that included a change in the activation propagation pattern and at least 5 wavefronts before and after the pattern change.

Case 1

For the first recording, the activation maps of 10 wavefronts and the location of the electrodes are shown in Figure 7.2(A - J) and (K). Pacemaker and linear slow wave propagation patterns were recorded in this case. In waves A - D, a pacemaker wavefront was observed. On the next wave (E), a linear wavefront was seen, suggesting the the pacemaker moved proximally. On the subsequent wave (F), an ectopic pacemaker was observed which collided with the proximal wavefront. Waves G - H then exhibited a pacemaker profile in the array.
7.2 Results

Figure 7.1: Representative (A) monophasic slow waves and (B) regional variations in ARI across the stomach. Plus and cross signs denote ATs and RTs. The number of pigs included in each calculation is shown as n. ARIs from the antrum region was significantly shorter than the other regions. *: $p < 0.05$. 
Figure 7.2: The (A - J) activation maps and the (K) location of the electrodes of case 1. There was a change in the propagation pattern between (D - E), (E - F), and (F - G). The AT was not identifiable for the channels marked with a white dot and was interpolated using neighbouring points. The isochronal level used in the maps was 1 s.
7.2 Results

Figure 7.3: The (A - C) linear fits between the ARIs from adjacent wavefronts and (D) the slope and the p-value of all the linear fits for case 1. For (A - C), each cross corresponds to an electrode. The linear fits between the ARIs from waves A and B, D and E, and I and J are denoted by solid lines. The dotted lines denote the 95% confidence bounds.

A linear fit was calculated between the ARIs of the same channel from each pair of adjacent waves. Three linear fit calculations are shown in Figure 7.3(A - C). Between waves A and B, D and E, and I and J, the slopes and the p-values of the linear fits were 0.74 and 0.00 (waves A and B), 0.36 and 0.20 (waves D and E), and 0.75 and 0.00 (waves I and J). A summary of the slope and p-value for each pair of waves are shown in Figure 7.3(D). For pacemaker propagation patterns (waves A - D and G - J), the slopes were around 0.8 and the p-values were less than 0.05. Before the change of the propagation pattern, the slope dropped to 0.72 (waves C and D). During the change of the propagation pattern, the slopes were much lower (0.36 between waves D and E) or higher (1.10 between waves F and G) than 0.8. The p-value (0.20) was over 0.05 for the collision wavefront between waves D and E.
Case 2

For a second recording, the activation maps of 10 wavefronts and the location of the electrodes are shown in Figure 7.4(A - J) and (K). Pacemaker and linear slow wave propagation patterns were recorded in this case. In waves A - C and I - J, pacemaker slow wave propagations were observed. However, there was a change in the propagation between waves C and D, D and E, and H and I. In waves E - H, linear propagations were observed. Whereas in wave D, a transaction between pacemaker and linear propagation was recorded.

The ARIs of the same channel from each pair of adjacent waves were fitted to a linear model. Three linear fit calculations are shown in Figure 7.5(A - C). Between waves A and B, D and E, and G and H, the slopes and the p-values of the linear fits were 0.78 and 0.03 (waves A and B), 0.60 and 0.05 (waves D and E), and 0.27 and 0.34 (waves G and H). A summary of the slope and p-value for each pair of waves are shown in Figure 7.5(D). For the first part of pacemaker propagation patterns (waves A - C), similar to case 1, the slopes were around 0.8 and the p-values were less than 0.05. Before the change of the propagation pattern, the slope dropped to 0.71 (waves C and D). During the change of the propagation pattern, the slope was much lower (0.60 between waves D and E) than 0.8. The p-value of the corresponding linear fit was 0.05. For the second part of pacemaker propagation patterns (waves I - J), the slopes and the p-values were around 0.4 and 0.3.

Case 3

For a third recording, the activation maps of 10 wavefronts and the location of the electrodes are shown in Figure 7.6(A - J) and (K). Pacemaker and linear slow wave propagation patterns were recorded in this case. In waves A - E, pacemaker slow wave propagations were observed. However, there was a change in the propagation between waves E and F. In waves F - J, linear propagations were observed.

For each pair of adjacent waves, a linear fit was calculated between the ARIs of the same channel. Three linear fit calculations are shown in Figure 7.7(A - C). Between waves B and C, E and F, and G and H, the slopes and the p-values of the linear fits were 0.75 and 0.00 (waves B and C), 0.26 and 0.21 (waves E and F), and 0.45 and 0.01 (waves G and H). A summary of the slope and p-value for each pair of waves are shown in Figure 7.7(D). For pacemaker propagation patterns (waves A - E), similar to case 1 and 2, the slopes were around 0.8 and the p-values were less than 0.05, except between waves C and D. Between waves C and D, the slope and the p-value were 0.24 and 0.28. During the change of the propagation pattern, the slope was much lower (0.26 between waves E and F) than 0.8. The p-value (0.21) was over 0.05 for the transaction between waves E and F. For the linear propagation after the pattern change (waves G - I), the slopes and the p-values were around 0.4 and 0.05.

Summary of the 3 Cases

Across the 3 cases, the slopes and the p-values between normal pacemaker propagation patterns were around 0.8 and less than 0.05 individually. Before a propagation pattern change, the slope dropped to a lower level (0.72 for case 1, 0.71 for case 2, 0.24 for case 3). In other words, ARI lost linearity across waves before a propagation pattern change. During the propagation pattern change, the slope dropped or
Figure 7.4: The (A - J) activation maps and the (K) location of the electrodes of case 2. There was a change in the propagation pattern between (C - D), (D - E), and (H - I). The AT was not identifiable for the channels marked with a white dot and was interpolated using neighbouring points. The isochronal level used in the maps was 1 s.
Figure 7.5: The (A - C) linear fits between the ARIs from adjacent wavefronts and (D) the slope and the p-value of all the linear fits for case 2. For (A - C), each cross corresponds to an electrode. The linear fits between the ARIs from waves A and B, D and E, and G and H are denoted by solid lines. The dotted lines denote the 95% confidence bounds.
Figure 7.6: The (A - J) activation maps and the (K) location of the electrodes of case 3. There was a change in the propagation pattern between (E - F). The AT was not identifiable for the channels marked with a white dot and was interpolated using neighbouring points. The isochronal level used in the maps was 1 s.
Figure 7.7: The (A - C) linear fits between the ARIs from adjacent wavefronts and (D) the slope and the p-value of all the linear fits for case 3. For (A - C), each cross corresponds to an electrode. The linear fits between the ARIs from waves B and C, E and F, and G and H are denoted by solid lines. The dotted lines denote the 95% confidence bounds.

\[ ARI_C = 0.75 \times ARI_B + 1.38, p = 0.00 \]

\[ ARI_F = 0.26 \times ARI_E + 3.42, p = 0.21 \]

\[ ARI_H = 0.45 \times ARI_G + 2.55, p = 0.01 \]
7.3 Discussion

Regional variations in ARI were defined across the pig stomach using HR wet suction electrode arrays. In the literature, intracellular recording methods were used to record monophasic slow waves. In an in vitro human study, the ARI was measured at 5.4 ± 1.4 s from upper corpus muscle strips, 3.5 ± 0.6 s increased to a much lower or higher level (0.36 and 1.10 for case 1, 0.60 for case 2, 0.26 for case 3), while the p-value increased beyond 0.05 (0.20 for case 1, 0.05 for case 2, 0.21 for case 3).

7.2.3 Relation of Activation to Recovery Interval and Propagation Speed Across the Stomach

Four recordings from the upper corpus and 4 recordings from the mid-corpus of 6 pigs were used in this analysis. Seven of the 8 recordings exhibited normal slow wave propagation patterns, whereas the other 1 exhibited abnormal slow wave propagation patterns. For both the upper corpus and the mid-corpus regions, the activation, recovery, ARI, and speed maps, the linear fit between ARI and speed, and the location of the electrodes of an example wavefront are shown in Figure 7.8 and 7.9. In this example, the slope of the linear fit was positive (1.12 mm·s\(^{-2}\)) for the upper corpus region (Figure 7.8), whereas it was negative (-4.38 mm·s\(^{-2}\)) for the mid-corpus region (Figure 7.9). In summary, the overall results are shown in Figure 7.10. The antrum region was not included due to not having enough data. The mean slopes were positive (0.20 ± 0.39 mm·s\(^{-2}\)) and negative (-0.71 ± 2.08 mm·s\(^{-2}\)) for the upper corpus and the mid-corpus regions. There was no significant difference between the 2 regions of the stomach.

Figure 7.8: The (A) activation, (B) recovery, (C) ARI, and (D) speed maps, the (E) linear fit between ARI and speed, and the (F) location of the electrodes of an example wavefront from the upper corpus region. In (E), each cross corresponds to an electrode. The slope of the linear fit was 1.12 mm·s\(^{-2}\).
Figure 7.9: The (A) activation, (B) recovery, (C) ARI, and (D) speed maps, the (E) linear fit between ARI and speed, and the (F) location of the electrodes of an example wavefront from the mid-corpus region. In (E), each cross corresponds to an electrode. The slope of the linear fit was $-4.38 \text{ mm·s}^{-2}$.

Figure 7.10: The linear fit slope comparison between slow wave ARI and propagation speed from different regions of the stomach. The number of pigs included in each calculation is shown as $n$. There was no significant difference between the groups.
from mid-corpus muscle strips, and 1.6 ± 0.1 s from antrum muscle strips [100]. The upper corpus had the longest ARIs among the 3 regions, whereas the antrum had the shortest ones. This trend remained consistent compared to the recordings obtained from pig stomachs as presented in this thesis.

The relation between the ARIs of adjacent wavefronts was investigated utilising linear fits in this study. The slope and the p-value of the linear fits were related to the slow wave propagation pattern change. In the cardiac field, the non-uniform shortening of the APD with the heart rate increment was considered as the cause of arrhythmia [89]. Similar to the cardiac field, in this study, the slope and the p-value are 2 parameters that quantify the non-uniformity of the ARI change across the area of the electrode array. Thus, it is reasonable to hypothesise that the 2 parameters are potential indicators of the underlying electrophysiological changes in the stomach.

In addition, the relation of the ARI and the slow wave propagation speed was investigated utilising linear fits for the upper corpus and the mid-corpus in this study. The average slope from the upper corpus was positive, whereas that for the mid-corpus was negative. Furthermore, the variation for the mid-corpus was much larger than the upper corpus. These findings suggest that physiologically a longer ARI indicates a higher slow wave propagation speed because of the positive slope in the upper corpus, whereas a lower slow wave propagation speed because of the negative slope in the mid-corpus, on average. Based on these findings, ARI was related to slow wave propagation speed. The measurement of slow wave propagation speed can be achieved by ARI analysing, which is more computationally efficient and robust especially when the recording coverage is not ideal. Novel therapies that manipulate the ARI to regulate the slow wave propagation can be inspired to treat gastric disorders.

However, there are limitations in this study. First of all, the coverage of the electrode was not ideal. Most of the channels were recording monophasic slow waves, whereas there were still a number of channels that recorded biphasic slow waves, which were excluded in this study. In the future, the improvement of the electrode design will focus on the ability to record consistent monophasic slow waves across the array. Secondly, the stomach was roughly divided into 3 regions. The low resolution of this division did not enable a detailed distribution across the stomach, especially the gradient trend across regions. In future studies, a larger number of regions will be used to categorise the recordings. Thirdly, not all the recordings included in the analysis of the relation between the ARI and the propagation speed were from normal slow wave propagation patterns. The corresponding result may not be effectively related to recording regions. In the future, studies will be conducted to include slow wave recordings from normal propagation patterns only. Finally, the data used in this study were still limited. More data from various subjects, such as diseased animals and obese animals, are needed to enable a better understanding of the pathways that initiate dysrhythmias. In the future, a larger number of cases will be analysed to help understand these relations under abnormal and normal stomach conditions.

7.4 Summary

In this chapter, the methods described in the previous chapters were used to determine the characteristics of monophasic gastric slow waves. Multi-channel wet suction electrode arrays were used to record HR monophasic slow waves from pig stomachs. Based on the recordings from different regions of the stomach,
regional variations in gastric ARI were defined. In addition, the changes in the slow wave propagation patterns were related to the linear fit between the ARIs of adjacent wavefronts. The slope and the p-value of the linear fits could be parameters related to gastric recovery dynamics alterations, which indicated gastric electrophysiological changes including slow wave propagation pattern changes. Finally, the relation of ARI and slow wave propagation speed was studied across the stomach. The slopes of the linear fits were positive in the upper corpus region, whereas negative in the mid-corpus region, on average.
Chapter 8

Conclusions and Future Directions

This thesis presented a novel workflow to record, analyse, and visualise monophasic gastric slow waves, in HR. The development of novel HR wet suction electrode arrays enabled detailed in vivo recovery phase analysis to study normal and dysrhythmic slow wave activity in the stomach. Using this workflow, the characteristics of monophasic slow waves were experimentally investigated.

8.1 Conclusions

8.1.1 Slow Wave Recording Methods

Three types of multi-channel electrode arrays suitable for recording HR monophasic signals were designed and fabricated. Among which, wet suction electrodes utilised saline solution as the medium for the negative pressure. Dry suction electrodes applied negative pressure via air. Whereas bipolar contact electrodes were designed based on a bipolar electrode structure instead of a suction electrode structure, a saline-soaked sponge was used as the reference electrode. Among the three designs, the wet suction electrode design recorded monophasic slow waves from 12 out of 14 pig studies (86%). This rate of success was higher than the other two designs (67% for bipolar contact electrode design and 60% for dry suction electrode design). This novel design enabled the HR recording of monophasic slow waves, which were capable of providing valuable recovery information compared to the biphasic slow waves that were usually recorded in the literature.

8.1.2 Slow Wave Analysis Methods

Velocity is a metric that can be quantified from slow wave propagation and has been related to dysrhythmic activity [84]. The BVE algorithm enabled rapid estimation of slow wave velocities with minimal errors. With experimental data, the BVE algorithm resulted in $19.2 \pm 1.7^\circ$ of direction error and $2.0 \pm 0.2 \text{ mm}\cdot\text{s}^{-1}$ of speed error, when compared to the standard “marking-and-grouping” method. However, the processing time was significantly reduced (8.7 s vs over 1 h for processing a 5 minute HR slow wave recordings of 256 channels). In addition, the VTW algorithm achieved better performance in identifying the ATs of slow waves compared to the commonly used FEVT algorithm. In the presence of high-frequency noise,
the VTW algorithm improved the $A_{roc}$ by 11.1%. In summary, these algorithms improved the efficiency and the accuracy performance of automated slow wave analysis algorithms, thus enabled rapid velocity inspection through a large amount of recording data and reduced the time needed for manual review and correction for slow wave analysis workflow.

**8.1.3 Slow Wave Characteristics**

Compared to biphasic slow waves, monophasic slow waves had a more pronounced recovery phase with a higher absolute gradient. Monophasic slow waves were categorised into two morphology groups based on the amplitude of the early repolarisation. Between the two groups, there was a significant difference in amplitude, AT gradient, and RT gradient. The recovery map coverage in comparison to the activation map, decreased by $4 \pm 6\%$ and $43 \pm 11\%$ for the wet suction electrode array and the conventional contact electrode array, respectively. In addition, based on the recordings from different regions of the stomach (upper corpus, mid-corpus, and antrum), the regional variations in gastric ARI was defined. The slow wave propagation pattern change was related to the linear fit between the ARIs of adjacent wavefronts. Between normal pacemaker propagation patterns, the slopes and the p-values were individually around 0.8 and less than 0.05. ARI lost linearity across waves before a propagation pattern change. The slope dropped or increased to a much lower or higher level, while the p-value increased beyond 0.05 during the propagation pattern change. Finally, the relation of ARI and slow wave propagation speed was studied across the stomach. The slopes of the linear fits were positive in the upper corpus region, whereas negative in the mid-corpus region, on average. These findings highlighted the importance of the recovery information contained in monophasic slow waves. Moreover, abnormal recovery phases have been related to abnormal slow wave propagation patterns.

**8.2 Future Directions**

Characteristics of monophasic slow waves were investigated using the methods described in this thesis. However, there are still possible improvements for hardware, software, and application in the future.

**8.2.1 Hardware**

To improve the suction electrode array design, the spatial resolution can be further enhanced by using smaller microelectrode holders which allow a larger number of channels and smaller inter-electrode spacing. Increased flexibility of the suction electrode can be obtained by replacing the rigid acrylic frame with a flexible frame, to match the contour of the recording surface. Alternatively, frames that match the curvature of the recording surface can be designed and manufactured *ad hoc*. To simplify construction for future designs, suction electrode arrays of fewer channels can be used as modules to build a high channel count array, by introducing attachment between the grid frames. In addition, the syringe can be replaced with a closed-loop system where the pressure is recorded using a sensor and actuated to deliver constant negative pressure using a suction pump.
In the cardiac field, monophasic action potentials have been recorded from endocardium using cardiac catheters (Section 2.3.2). In the GI field, contact surface electrodes have been used to record slow waves from gastric mucosal surface [26, 7]. Suction electrode arrays can potentially be applied to the mucosal surface to capture recovery dynamics in a less invasive manner. Furthermore, the electrode design could be potentially embedded into endoscope, where both the endoscopy images and the HR monophasic slow wave recordings can be captured simultaneously to assist diagnosis. In addition, data can be collected endoscopically from healthy and diseased human stomachs. A better understanding of recovery dynamics will be gained and can be used clinically for diagnosis and to guide therapeutics. In the future, this multi-channel suction electrode array design could be adopted to record HR monophasic action potentials from other bio-electrical organs such as the heart and intestines.

8.2.2 Software

The BVE algorithm can be optimised to work in real time. For example, causal linear filters can be used to enable real time processing and visualisation [21]. The algorithm will then gain the ability to group and classify the estimated velocities, diseased tissue location, or the orientation of muscle activation around the organ axis in real time. New techniques such as the cardiac omnipolar mapping technique, which estimates the propagation velocity using a travelling wave model, can be translated for GI applications, benefiting the BVE algorithm to provide more accurate detection results [75]. Currently, the BVE method focuses exclusively on the activation phase to estimate wavefront velocities. In the future, the velocity of the slow wave recovery phase wavefront will also be computed to access the information about the recovery phase dynamics. In addition, the BVE algorithm could be translated into a custom hardware electrode array, similar to the translation from surface laplacian algorithms to electrode arrays designed for surface electrocardiology and electroenterogram [13, 39].

For the VTW algorithm, as wavelet decomposition had combined properties of data smoothing and differentiation, it has already been applied to biphasic slow wave RT identification [97]. In addition, the VTW algorithm was used to identify the RT of monophasic slow wave in Chapter 6. The performance of the VTW algorithm for monophasic slow wave RT identification will be quantified in the future. Moreover, future studies will focus on applying this algorithm to biphasic slow wave AT identification and reporting the performance.

In the future, these algorithms can be applied to translational studies, which require the algorithms to be reliable over an extended period of time and to handle noise effectively. Optimisations will be implemented for the algorithms to improve the reliability and the performance against noise. In addition, based on the results of the monophasic slow wave analysis, the slope and the p-value of the linear fits between the ARIs of adjacent wavefronts were hypothesised as parameters indicating gastric electrophysiological changes. Algorithms that monitor these parameters can be developed to track the changes in spatial slow wave propagation.
Conclusions and Future Directions

8.2.3 Application

Future studies with controlled and uniform pressure will be used to further our understanding of the genesis of the two monophasic morphologies. In addition, the results from this thesis suggested that the abnormal timing of the slow wave recovery phase was related to abnormal slow wave propagation. Thus, recovery phase mapping for normal and abnormal slow wave propagation patterns will be carried out to study the mechanisms that lead to the genesis and maintenance of gastric dysrhythmias. Furthermore, the GI electrical restitution will be defined by analysing HR monophasic slow wave recordings with the use of pacing or drugs. Studies will be conducted to assess the ability to change the electrical restitution back to normal in diseased stomachs. Functional basic studies are required in the GI field through the use of monophasic recordings to uncover the relevance of a distinct early repolarisation in health and disease.

Recently, optical mapping methods were applied on the stomach to capture monophasic slow waves with lower SNRs than the conventional surface contact electrode methods [127]. The suction electrode could be used in tandem with optical mapping to improve our understanding of slow waves propagation. In addition, HR suction electrode arrays, the BVE algorithm, and the VTW algorithm can also be applied to other species such as human and other parts of the GI system such as the intestines and colon to capture and analyse the activation and recovery phases of monophasic signals in the future. At present, we anticipate this suction recording approach will serve as a critical step in improving our understanding of pathophysiology and validating new tools and therapeutics in an animal model, prior to possible application in future human studies. The key advantage of the presented suction electrode array is the ability to record high-fidelity monophasic slow waves in HR to capture both the activation and recovery phases. A key electrophysiological application that we can now envisage is to define the gastric slow wave electrical restitution spatially across the organ. After defining the gastric restitution profiles, parameters for novel therapeutics or devices (such as dosage for drugs, stimulation protocols for pacing) can be optimised in pre-clinical animal models for multiple motility disorders. The use of suction arrays on human serosal studies has a variety of logistical and ethical challenges. Histology study will be conducted to assess the damage produced by the electrodes, similar to the previous histology study in the GI field [1]. However, the application of suction techniques to the mucosal surface of the stomach may be a viable approach. For example, mucosal surface suction recording can be conducted endoscopically for human applications. For both the algorithms, the parameters will need to be adapted for the varying frequencies and velocities for each of the species and organs.

8.3 Concluding Remarks

It is commonly accepted that slow waves are critical for the coordination of GI motility. Thus, assessing slow wave recordings enabled a new way to observe the GI motility as well as an alternative approach to understand GI disorders. The methods in this thesis improved upon existing approaches for analysing slow wave recordings and presented novel electrode designs to record HR monophasic slow waves, which contained additional recovery information compared to conventional biphasic slow waves. These approaches provided a method to access the recovery information of slow waves. In addition, the results
in this thesis presented the morphological characteristics of monophasic slow waves and the relation between abnormal recovery timing and abnormal slow wave propagation. These findings emphasised the importance of the slow wave recovery information. Further investigation into the recovery phase of slow waves will provide a better understanding of the mechanisms involved in dysrhythmias and functional disorders, and aid in critical development of novel diagnostics such as pharmaceuticals, ablation, and electroceuticals [1, 4, 22].
Appendix A

Publications and Presentations

The following publications and presentations were produced during the course of this thesis.

A.1 Peer-Reviewed Publications


A.2 Conference Abstracts


A.3 Oral Presentations


Han, H., Cheng, L. K., Avci, R., and Paskaranandavadivel, N. Detection of slow wave propagation direction using bipolar high-resolution recordings. *42nd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Montreal, Canada, 2020*

Han, H. High-resolution refractory analysis of slow waves to understand dysrhythmic activation. *ABI Research Forum 2021, Auckland, New Zealand, 2021*
References


References


