# A FUNCTIONAL ANALYSIS OF GASTROINTESTINAL MOTILITY IN THE GUINEA PIG AND HUMAN

## A thesis submitted in total fulfilment of the requirements of the degree of doctor of philosophy

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# TABLE OF CONTENTS

# THESIS SUMMARY

Gastrointestinal motility results from coordinated contractions and relaxation of smooth muscle layers of the gut wall. Motor patterns mix or propel content along the gastrointestinal tract. The circular and longitudinal muscle layers within the gut wall are comprised of smooth muscle cells and interstitial cells of Cajal (ICC), which work together to make up the myogenic component of gastrointestinal motility. Smooth muscle cells and ICC are electrically and metabolically coupled to themselves and each other by a series of gap junctions. Gap junction coupling has been shown in several cellular systems to be modulated by: pH, $Ca^{2+}$, second messengers, voltage differences and by various neurotransmitters and pharmacological agents. Gastrointestinal motility is also controlled by intrinsic and extrinsic neural inputs. Mechanical and/or electrical recordings of smooth muscle cells from segments of the gut wall have been extensively studied *in vitro* to understand mechanisms underlying gastrointestinal motility. Immediately following setup, many of these preparations display a curious physiological feature: they appear to be completely inactive. These preparations lack tone, they fail to develop spontaneous contractions, they lack excitatory or inhibitory junction potentials, and these preparations fail to respond to electrical or pharmacological stimuli. Responses develop and then stabilise over the next 60-120 minutes. This phenomena is often implicitly reported in the methods section of papers, however the mechanistic causes for the apparent loss of responsiveness is unknown.

One of the aims of this project was to understand the mechanisms that account for the loss of responses after dissection. In chapter 2, the electrical properties of circular smooth muscle cells from specimens of guinea pig ileum and colon were monitored as preparations recovered from dissection. Junction potentials (primarily fast inhibitory junction potentials), resting membrane potential, input resistance and dye coupling were monitored over this period. The role of gap junction coupling in neuromuscular transmission was investigated in chapter 3. In chapter 4, the role of various elements of the setup procedure were studied, in an attempt to highlight which factors activated the uncoupling process. This problem was approached from 2

directions: by testing whether manipulations could evoke a second loss of response, and by testing whether omitting the use of other manipulations reduced the initial loss of responses. In chapter 5 the role of cholingeric interneuronal pathways in generating an ascending contraction in segments of human colon was studied. Few functional *in vitro* studies have considered the role of interneural pathways in human tissue due to limited supply of specimens of an appropriate size required for this analysis. The frequency of spontaneous contractions in narrow segments versus larger segments was also considered. These studies identified two mechanisms that may contribute to the control of gastrointestinal motility *in vitro*. Their physiological significance *in vitro* remains to be determined.

# DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Simona E. Carbone, August 2012

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# PUBLICATIONS AND CONFERENCE PRESENTATIONS

## Refereed journal articles:

**Carbone SE, Dinning PG, Costa M, Spencer NJ, Brookes SJH and Wattchow DA (2013)** 'Ascending excitatory neural pathways modulate slow phasic myogenic contractions in the isolated human colon' *Neurogastroenterol and Motil* In press

**Carbone SE,** Wattchow DA, Spencer NJ and Brookes SJH (2012) 'A loss of responsivenss of circular smooth muscle cells from the guinea pig ileum is associated with changes in gap junction coupline.' *Am J Physiol: Gastrointest Liver Physiol*. Vol. 302, pp: G1434-G1444.

Wattchow D, Brookes S, Murphy E, **Carbone S**, de Fontgalland D, Costa M. (2008) **'**Regional variation in the neurochemical coding of the myenteric plexus of the human colon and changes in patients with slow transit constipation.' *Neurogastroenterol Motil.* vol. 20 no. 12, pp:1298-305.

## Conference proceedings:

Presenting author

**Neurogastroenterology and Motility conference (2012)** SE Carbone, Dinning PG, NJ Spencer, SJH Brookes & DA Wattchow 'Functional evidence of ascending excitatory pathways in the human colon.' (oral presentation)

**VIIth International symposia on ICC (2012)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH 'The responsiveness of smooth muscle cells in the guinea pig ileum is affected by elements of dissection.' (poster presentation)

**Australian Neuroscience Society (ANS) conference (2012)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH **'**Understanding the mechanisms which uncouple smooth muscle cells of the guinea pig ileum following dissection.' (poster presentation)

**Collaborators Day (2011)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH 'A lack of response of smooth muscle cells to electrical and chemical stimuli is associated with changes in gap junction coupling.' (poster presentation)

**Digestive Disease Week (DDW) abstract (2011)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH 'Why smooth muscle preparations are initially unresponsive to chemical and electrical stimuli in vitro: association with changes in gap junction coupling.' (poster presentation)

**ANS abstract (2011)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH **'**Purinergic junction potentials and responses to exogenous ATP by gut smooth muscle cells both require an 'equilbration' period.' (oral presentation)

**Australian Physiological Society (AuPS) conference abstract (2010)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH **'**Gap junction coupling between smooth muscle cells modulates responses to inhibitory motorneurons and exogenous ATP.' (oral presentation)

**ANS abstract (2010)** Carbone SE, Wattchow DA, Spencer NJ, Brookes SJH 'Why gut smooth muscle needs to "equilibrate" before enteric motor innervation becomes fully functional.' (oral presentation)

**ANS abstract (2009)** Carbone SE, Brookes SJH, Wattchow DA and Spencer, NJ 'Mechanisms underlying ascending excitation in the human colon.' (oral presentation)