FIRST IN MAN: IDENTIFICATION AND ELECTROPHYSIOLOGICAL CHARACTERISATION OF HUMAN RECTAL AFFERENTS

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INTRODUCTION

• Conscious perception of rectal sensation is important in the maintenance of continence and process of defaecation.

• The desire to defaecate is an important initiating factor in rectal evacuation.
INTRODUCTION

- Communication with higher centres necessary to allow conscious perception.
- Reasonable to assume that the hindgut receives rich extrinsic (in addition to intrinsic) innervation.
INTRODUCTION

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SENSORY NERVE-ENDINGS AND SENSATION IN THE ANAL REGION OF MAN

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The anal region in man (circumanal skin and anal canal) is extremely sensitive, yet a search of the literature reveals that little work, histological or experimental, has been done on its sensory innervation. The histological investigations of Ottaviani (1940) were mainly on small animals and mostly concerned with the rectum. However, he did note that there were nerve-plexuses made up of thick and submucosa at the mucocutaneous junction. He remarked on the presence of occasional Pacinian corpuscles near the anal sphincters. The rectum he found to lack organized endings and networks and to have submucosal nerve-fibres the branches of which were in close relationship to blood-vessels near the epithelium.

The present study was undertaken to investigate

• However, early histology studies failed to identify specific receptors in human visceral tissue.
• Assumed that ‘rectal’ sensation was solely derived from muscle spindles and stretch receptors of pelvic floor musculature.
Recent study recorded nerve activity from nerves supplying the colon and appendix, but **not** rectum.
AIMS

• To record visceral afferent activity from extrinsic nerves supplying the human rectum and colon in vitro; and

• To characterise the response of such nerve traffic to mechanical and chemical stimulation.
METHODS

Specimen procurement

Tissue preparation

Electro-physiological assessment
• Rectal and colonic tissue procured fresh from specimens of patients undergoing colorectal resection.

• Full-thickness sections (1 – 2 cm), with mesentery attached, were obtained from resection margins.

• Tissue samples macroscopically free of disease.
Rectal tissue:

distal portion of anterior resection specimen
METHODS

Colonic tissue:

Proximal end of anterior resection specimen

Distal end of right hemicolecctomy specimen
METHODS

- Tissue transported to laboratory in oxygenated Krebs solution
METHODS

Specimen procurement

- Opened along anti-mesenteric border

Tissue preparation

- Mucosa delicately dissected free from submucosal attachments

Electrophysiological assessment

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• Tissue pinned flat, serosa side up
METHODS

- Mesenteric nerves dissected free from connective tissue
• Between 6 to 10 mesenteric nerves dissected, 1 - 2cm per nerve
METHODS

Specimen procurement → Tissue preparation → Electro-physiological assessment

• Entire preparation transferred to a specialised recording chamber

Nerve recording well (paraffin)

Main chamber (oxygenated Krebs solution)
• Extracellular nerve activity recorded using a silver hook electrode
• 20mN applied via a hook array / pulley to standardise tissue stretch
METHODS

Spontaneous nerve activity → Mechanical stimulation → Chemical stimulation → Mechanical stimulation post chemical stimulation
METHODS

- Nerve activity recorded in **absence of stimulation**
METHODS

- Tissue systematically probed with Von Frey hairs to identify mechanosensitive sites - ‘hot spots’
- ‘Hot spots’ marked with carbon particles
- Von Frey hairs of varying forces applied
  - minimal ‘threshold’ force identified
• Main chamber solution exchanged for:
  – Krebs with ‘inflammatory solution’ (IS), containing 10μM each of histamine, serotonin, bradykinin, prostaglandin E₂
  – Krebs with capsaicin (10μM)
• Tissue systematically **re-probed** to determine:
  - ‘hot spot’ thresholds following IS
  - ‘emergence’ of new hot spots following IS
METHODS

• Nerve activity discriminated and recorded using Spike2 analytical software

• Outcome measures:
  – Presence / absence of nerve activity (i.e. action potentials)
  – Threshold force of mechanical stimulation
  – Peak neuronal discharge rates:
    • Maximum number of action potentials over 1 sec interval
RESULTS

*Tissues Procured:*

8 rectums:
(median 67 yrs [range 38-82], 6 males)
28 rectal nerves studied

10 colons:
(median 73 yrs [range 57-81], 7 males)
30 colonic nerves studied
Spontaneous afferent activity

- 5 sec
- 2 msec

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Spontaneous afferent activity

• Rectal nerves (n = 28):
  – Spontaneous activity recorded in 24 nerves.

• Colonic nerves (n = 30):
  – Spontaneous activity recorded in only 3 nerves.
Response to Mechanical Stimulation

- **Rectal nerves (n = 28):**
  - Punctate mechanosensitive ‘hot spots’ identified in 16 nerves
  - Median threshold 2.0g (range 1.4 – 6.0g)

- **Colonic nerves (n = 30):**
  - Only 1 ‘hot spot’ identified
  - Threshold force = 60.0g
Response to Chemical Stimulation

Spontaneous Activity:

Post Chemical Stimulation:
Response to Chemical Stimulation

Rectal nerves – IS:

- Peak discharge rates increased significantly following IS

Peak discharge rate (spikes/sec)

P<0.001

3 spikes/sec

5 spikes/sec

Pre-IS

Post-IS

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Rectal nerves – Capsaicin:

- Peak discharge rates increased significantly following capsaicin (P<0.001).

<table>
<thead>
<tr>
<th>Pre-capsaicin</th>
<th>Post-capsaicin</th>
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<tr>
<td>3 spikes/sec</td>
<td>6 spikes/sec</td>
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In seven of the 16 nerves with ‘hot-spots’ identified, the threshold decreased post-IS.
DISCUSSION

• First study to functionally demonstrate rectal afferent innervation via extrinsic nerves

• Differences in nerve responses between rectum and colon:
  – ? ‘graded’ sensitivity of rectal but not colonic distension

• Limitations:
  – Inability to correlate neurophysiological findings with bowel symptoms
  – Colon and rectum unpaired samples
CONCLUSIONS

• Successful recording of nerve activity of human extrinsic visceral nerves – first in man.

• Mechanosensitivity to Von Frey probing was demonstrated in rectal but not colonic tissue.

• Chemosensitivity with enhanced mechanosensitivity following exposure to inflammatory mediators.

• Development of *in vitro* model with potential application in disease settings.
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