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Immunohistochemistry and high resolution microscopy of rat gastric nerve fibers and their relationship with enteroendocrine cells

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ABSTRACT

Enteroendocrine cells are important regulators of gastrointestinal, digestive and metabolic function. Here we describe protocols for quantifying the relationship between nerve fibers and enteroendocrine cells in the rat gastric mucosa using immunohistochemistry and high resolution microscopy techniques

MATERIALS

NAME	CATALOG #	VENDOR
Rabbit anti-Gastrin antibody RRID:AB_2762851	8007	
Goat anti-CGRP antibody RRID:AB_2783523	1780	
Rabbit anti-Ghrelin antibody RRID:AB_2767291	RY1601	
Mouse anti-VIP antibody RRID:AB_2783532	V31(asc)	

MATERIALS TEXT

1 Experiments were conducted on Sprague-Dawley rats of 250-350 g. Procedures were approved by the University of Melbourne Animal Ethics Committee. Rats were supplied with food and water ad libitum prior to the experiments.

2 Rats were anaesthetised with a mixture of ketamine (55 mg/kg) and xylazine (9 mg/kg) prior to being perfused transcardially with heparinised phosphate buffered saline (PBS: 0.15M NaCl, 0.01M sodium phosphate buffer, pH 7.2) followed by fixative (2% formaldehyde, 0.2% picric acid in 0.1M sodium phosphate buffer, pH 7.0). The stomach was
removed, dissected, and post-fixed overnight at 4°C in the same fixative, before being cleared with 3 x 10 min washes in dimethyl sulfoxide, 3 x 10 min washes in PBS and then stored in PBS-sucrose-azide (30% sucrose, 0.1% sodium azide in PBS) at 4°C. Tissue was then equilibrated overnight at 4°C in a 1:1 solution of PBS-sucrose-azide and OCT compound
(Tissue Tek, Elkhart, IN, USA) before being embedded and frozen in OCT. Tissue from the antrum and corpus was analysed from 4 animals.

3 Cryosections (50 µm) were cut and placed into PBS as free floating sections in a 48 well plate. The PBS was then aspirated and replaced with blocking solution (10% normal horse serum with 1% Triton-X100 in PBS) and incubated for 3 nights at 4°C. Sections were then incubated in a mixture of 3 primary antibodies from different species for 3 nights at 4°C. Carefully aspirate the antibodies and wash with 3 x 10 minute changes of PBS. Incubate with an appropriate mixture of Alexa fluor labelled secondary antibodies for a further 3 nights at 4°C. Sections were carefully mounted onto slides with ProLong Diamond Antifade Mountant (Life Technologies, Mulgrave, VIC, Australia) using #1.5 thickness coverslips and sealed with nail polish. Samples for which the primary antibodies were omitted were also prepared to investigate background staining and autofluorescence.

4 Free-floating sections for analysis of distances between EEC and nerve fibres were imaged on the LSM880. A low resolution preview scan was obtained in the channel with cell body labelling and then 10 cells from the top half of the mucosa and 10 cells from the bottom half of

the mucosa were chosen per section (2 sections per animal, 4 animals). Each chosen cell was then imaged in all 3 channels as a superresolution z-stack using the fast Airyscan
mode with a 20x oil objective and 6x zoom (70x70µm field of view) through the whole section.

5 Images were deconvoluted using the Zen (Zeiss) three-dimensional Airyscan processing function and then imported into Imaris (Bitplane AG, Zurich, Switzerland) for 3 dimensional analysis. A 3D surface was rendered for each labelled object and the Distance Transformation XTension was used to generate a heat map, assigning a pixel intensity to represent the distance between that pixel and the closest fibre surface in µm enabling us to determine the distance from the edge of the cell to the edge of the closest nerve fibre in the field of view. Results were exported into Excel.