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REGULAR ARTICLE

Morphologies and distributions of 5-HT containing enteroendocrine cells in the mouse large intestine

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Abstract
Serotonin (5-HT)-containing gastrointestinal endocrine cells contribute to regulation of numerous bodily functions, but whether these functions are related to differences in cell shape is not known. The current study identified morphologies and localization of subtypes of 5-HT-containing enteroendocrine cells in the mouse large intestine. 5-HT cells were most frequent in the proximal colon compared with cecum and distal colon. The large intestine harbored both open (O) cells, with apical processes that reached the lumen, and closed (C) cells, not contacting the lumen, classified into O1, O2, and O3 and C1, C2, and C3 cells, by the lengths of their basal processes. O1 and C1 cells, with basal processes sometimes longer that 100 μm, were most common in the distal colon. Their long basal processes ran against the inner surfaces of the mucosal epithelial cells and were strongly immunoreactive for 5-HT; these processes are ideally placed to communicate with the epithelium and to react to mechanical forces. O2 and C2 cells that had similar but shorter basal processes were also most common in the distal colon. O3 and C3 cells had no or very short basal processes. The O3 open type 5-HT cells were abundant in the proximal colon, particularly at the luminal surface, where they could release 5-HT into the lumen to act on luminal 5-HT receptors. Numerous O3 type 5-HT cells occurred in the lower (submucosal) region of the crypts in all segments and might release 5-HT to influence cell renewal in the crypt proliferative zones.

Keywords 5-HT · Serotonin · Enteroendocrine cells · Enteric hormone · Large intestine

Introduction

5-HT cells, called enterochromaffin cells because of their reactions with chrome salts, were the first type of enteroendocrine cell (EEC) to be identified. For many years, they were thought to be a single cell type (Vialli and Erspamer 1933), with a single function within the gastrointestinal tract, the initiation of muscle contractions or peristaltic reflexes (Bülbring and Crema 1958). This concept has changed dramatically in recent years, with discoveries that 5-HT-containing EEC produce other gut hormones in many different combinations (Reynaud et al. 2016; Fazio Coles et al. 2020), that the 5-HT cells subserve a wide range of functions (Berger et al. 2009; Martin et al. 2017b; Spohn and Mawe 2017), and that they express different receptor repertoires, depending on gut region (Martin et al. 2017a; Lund et al. 2018; Billing et al. 2019). The functions now attributed to enterochromaffin cells include: supplying 5-HT to platelets, activation of propulsive reflexes in intestine and colon, slowing of gastric emptying, enhancement of intestinal inflammation, increase of ionic movement across the mucosal epithelium, mucosal vasodilation, reduction in bone mass, stimulation of pancreatic enzyme secretion, modulation of visceral pain, suppression of appetite, and expulsion of toxins (Berger et al. 2009; Diwakarla et al. 2017; Martin et al. 2017b; Spohn and Mawe 2017).

Another feature that might distinguish EEC, and provide clues to function, is cell morphology, for example

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some EEC being closed cells with no direct connection to the gut lumen and others being open cells whose apical processes are capable of sampling the luminal content. Another specialization is the possession of basal processes by some EEC. These processes may be involved in communication to other EEC, to nearby epithelial cells or to nerve endings.

Materials and methods

Animals

Seven adult C57BL/6 male mice, 8–10 weeks old, were used in this study; tissues from four mice were investigated in Kyoto and tissues from three were investigated in Melbourne. At both sites, the mice were from in-house breeding colonies. Animals were provided standard laboratory chow and water ad libitum. All procedures in Kyoto were approved and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kyoto Institute of Technology. All procedures at the University of Melbourne were conducted according to the National Health and Medical Research Council of Australia guidelines and were approved by the University of Melbourne Animal Experimentation and Ethics Committee.

Tissue preparation

Animals were deeply anesthetized with isoflurane (inhalation) and perfused through the heart with 50 mL of physiological saline and then 100–150 mL of a fixative solution containing 2% paraformaldehyde and 15% saturated picric acid in 0.1 M sodium phosphate buffer (Zamboni fixative, pH 7.3). Some animals were anesthetized with isoflurane and euthanized by cervical dislocation. Segments of the middle part of the cecum, proximal colon 1 cm distal from the cecum, and distal colon 1 cm from the pelvic brim were removed and immersed for 12–18 h in the same fixative at 4 °C. The tissues were passed through dimethylsulfoxide (DMSO) and 0.01 M phosphate-buffered saline (PBS, pH 7.2) for 10 min, each three times, and immersed in PBS containing 30% sucrose and 0.1% sodium azide at 4 °C. Following immersion for 18–24 h, the samples were placed in Tissue-Tek O.C.T. compound (Sakura Finetechnical Co., Tokyo, Japan), immediately frozen with liquid nitrogen, and transversally cut with a cryostat into 100–120-µm frozen sections, for thick section analysis, or 60 µm for confocal analysis.

Immunohistochemistry

Thick 100–120-µm tissue sections were investigated in Kyoto and used for cell shape classification. These were treated for 2–4 h with 0.3% Triton X-100; they were incubated for 30 min with a normal donkey serum (1:10; Jackson ImmunoResearch Laboratories, PA, USA). Sections were then washed 3 times with PBS and incubated for 18–24 h with a polyclonal rabbit anti-5-HT antibody (1: 10,000; 20080, ImmunoStar, WI, USA), followed by incubation for 2 h with a donkey Cy3-labeled anti-rabbit IgG antibody (1:200; ImmunoResearch) or a donkey Alexa 488-labeled anti-rabbit IgG antibody (1:100; Molecular Probes, OR, USA). All incubations were performed at room temperature. The immunostained sections were washed 3 times with PBS and then mounted in 0.5 M sodium carbonate-buffered glycerin (pH 8.2).

Sections of 60 µm thickness were used for confocal analysis in Melbourne. They were incubated with normal horse serum (10% *v*/*v* in PBS with 1% Triton X-100; Life Technologies Australia, Mulgrave, Victoria, Australia) for 1 h at room temperature, then incubated with polyclonal goat anti-5-HT antibody (1:5000; 20079, ImmunoStar, WI, USA) for 3 nights at 4 °C. Sections were washed three times in PBS, 15 min each, and incubated with donkey Alexa 488-labeled anti-goat IgG antibody (1:500; Molecular Probes, OR, USA) overnight at 4 °C. Sections were washed twice with PBS, 10 min each, and quenched with quenching buffer (5 mM copper sulfate and 50 mM ammonium acetate in distilled water, pH 5.0) for 1 h at room temperature, then washed once with PBS and twice with distilled water, 5 min each, followed by Hoechst 33258 solution (10 µg/mL in distilled water; Sigma-Aldrich, Sydney, NSW, Australia) incubation for 45 min at room temperature. Sections were washed 3 times 5 min with distilled water, then mounted on microscope slides in non-fluorescent mounting medium (Dako, Carpinteria, CA, USA). Slides were dried overnight at room temperature prior to imaging.

Images and data analysis

The specimens investigated in Kyoto were examined by a Zeiss fluorescence microscope equipped with appropriate filter sets (Zeiss, Germany), and digital images were captured as TIFF files using the fluorescence microscope connected to a digital CCD camera system (VisualixPro2, Visualix, Kobe, Japan) and analyzed using ImageJ (https://imagej.nih.gov/ij/) to measure areas. Images were processed using Photoshop Elements software (Adobe Inc., CA, USA) to adjust contrast and brightness, and profiles of immunoreactive cells were drawn as silhouettes. Finally, these images were imported into Microsoft PowerPoint software (Microsoft Co., WA, USA) for preparation of figures. For quantitative analysis, 5-HT immunoreactive cells were counted (×20 objective lens) in 10–15 areas selected in 2 or 3 sections from each sample of 4 animals. The numbers of 5-HT cells were calculated as cells per mm^2 area of the mucosa in each segment of the large intestine. Cell counting was performed in the inner (luminal) and outer (submucosal) halves of the crypts in the cecum and proximal colon, and in the inner, middle, and outer thirds of the crypts of the distal colon. The demarcation of the inner, middle, and outer parts is illustrated in the results section. In Melbourne, super-resolution z-stack images were captured by high-resolution confocal microscopy (LSM 880 Airyscan Fast, Zeiss, Sydney, NSW, Australia) with a × 63 oil objective in Zen (black edition) software. The images were processed using Fiji ImageJ, z-stack was maximally projected, and images were converted to RGB color before exporting as TIFF files. Data were analyzed presented as mean ± SD. Statistical analysis to evaluate occurrence frequency of 5-HT cells was performed using Student's *t* test.

Results

A range of shapes of 5-HT immunoreactive cells was observed in the mucosa of the cecum, proximal colon, and distal colon (Figs. 1 and 2). The cells were classified into two types, open (O) and closed (C): the former are cells with apical cytoplasmic processes reaching the lumen and the latter are those without any region open to the lumen (Fujita et al. 1988). Representative open and closed 5-HT cells are shown as silhouettes in Fig. 2 and their characteristics are summarized in Table 1. Open cells were subdivided as follows: open type O1, bipolar cells, each with a long basal cytoplasmic process that was more than twice the somatic length and an apical process open to the lumen (Fig. 1a); O2, bipolar cells with a basal cytoplasmic process less than twice the somatic length and an apical process reaching the lumen (Fig. 1b); and O3, cells with short or no basal processes. O3 cells were further divided into three subtypes (Fig. 1c-A, B, C): O3-A type cells with oval or spindle-like shapes, O3-B those with more than 2 short process (including an apical process), and O3-C those with flask-like or fusiform shapes. Closed cells were divided into the following categories: C1, cells with only a single long basal process more than about twice the somatic length (Fig. 1d); C2, those with only a single basal process that was shorter than twice the somatic length (Fig. 1e); and C3, those with oval or round cell bodies without a basal process or a process opening to the lumen (Fig. 1f).

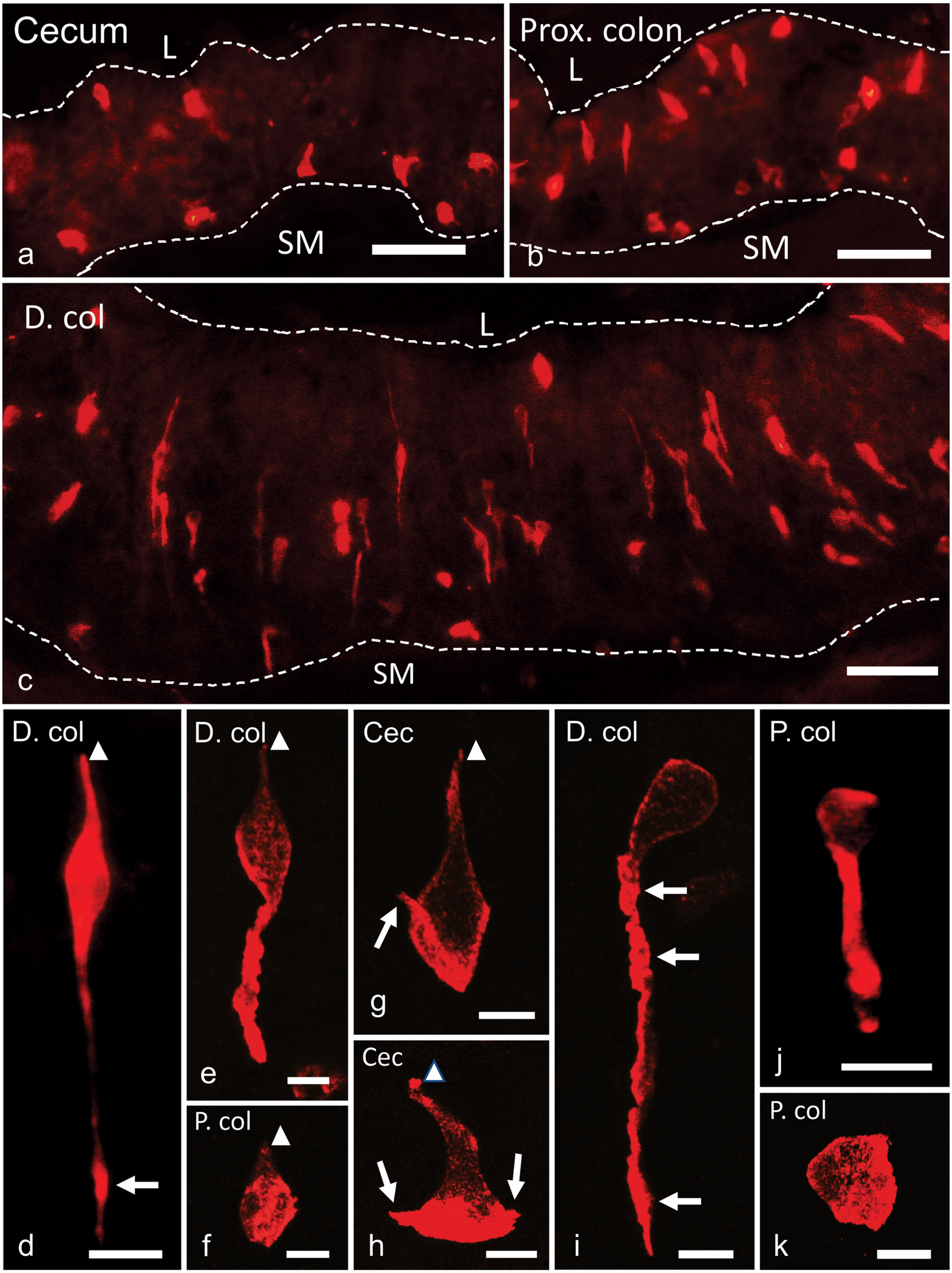
The single processes that emerge from the basal aspects of most O1, O2, C1, or C2 type 5-HT cells generally extended adjacent to the mucosal epithelium towards the submucosa along the basement membrane and never appeared to cross the basement membrane. Immunoreaction was usually found as granular structures in the cytoplasm of the cell bodies and processes of 5-HT cells (Fig. 2), and intense immunoreaction was more commonly localized in the basal processes and the tip region of the apical process than in the soma of open type cells (Fig. 2g, h). Also, swellings similar to neuronal varicosities were often seen at the process terminal or along the processes of O1, O2, C1, or C2 type cells (Figs.1a, b, d and 2d, i).

5-HT cell densities of occurrence

Cell counts revealed densities of occurrence of 242 ± 36, 607 ± 40, and 439 ± 37 cells/mm^2 in the mucosa of the cecum, proximal colon, and distal colon, respectively (Fig. 3a). For 5-HT cell counts, the lengths of the crypts in the cecum (131 ± 11 μm, $n = 4$ mice) and proximal colon (115 ± 10 μm) were divided into the upper (luminal) and lower (submucosal) halves, while the distal colon (crypt length, 190 ± 16 μm) was divided into the inner, middle, and outer thirds and cell numbers in each of these regions were determined (Fig. 3b).

Numbers and distribution of 5-HT immunoreactive cells in the cecum

The numbers and percentages of occurrence of each type of 5-HT cell in the cecum are shown in Table 2 and 4a, and in the upper and lower halves of the crypts in Fig. 5. Open 5-HT cells (77%) were more frequent than closed cells (23%) (Table 3, Fig. 4a) and more cells (62%) were in the lower half of the crypt (Fig. 3b). The percentages of open and closed 5-HT cells were 56% and 44%, respectively, in the upper half, and 90% (open) and 10% (closed) in the lower half (Table 4). The numbers and proportions of each sub-type of 5-HT cell are shown in Table 5 and Fig. 5a. Of all 5-HT cells in the cecum, O3 type cells were most common (83%) (Table 5, Fig. 5a), a majority of which were O3-C type cells (Fig. 2h) that were localized near the deeper part of the crypts. Many O3-B or O3-C cell types and a few cells of the O3-A type were located in the surface epithelium facing the lumen (Fig. 2a). Of closed 5-HT cells, the majority of C1 and C2 type cells projected their single process towards the submucosa. In the lower parts of the crypts, there were a few closed type 5-HT cells and no O1 or C1 type cells (Fig. 5a).


Cecum
L
SM
a
Prox. colon
L
SM
b
D. col
L
SM
c
D. col
d
D. col
e
P. col
f
Cec
g
Cec
h
D. col
i
P. col
j
P. col
k

◂**Fig. 1** 5-HT immunoreactive cells in the mucosa of the mouse large intestine. The dotted lines at the top and bottom indicate the luminal and the submucosal surfaces of the mucosa. **a** Cec. Most 5-HT cells are in the deeper (lower) parts of the crypts. **b** P. col 5-HT cells are more frequent in the outer parts of the crypts. **c** Distal colon. The majority of 5-HT cells are seen in the middle portions of the crypts. **d**–**k** Examples of different types of open (O) and closed (C) cells, see Table 1 for definitions. **d** A bipolar open type (O1) cell with a long thin process with a terminal swelling extending towards the submucosa, and an apical process open to the lumen (arrowhead). **e** A bipolar O2 open cell with a short thick submucosally directed process and an apical process open to the lumen (arrowhead). This image, from a Z-stack, shows the greater intensity of 5-HT staining in the cell process. **f** O3-A open cell with an apical extension (arrowhead) reaching the lumen. **g** O3-B open cell with a short basal process (arrow) and an apical process (arrowhead) at the bottom of a crypt. **h** O3-C open cell with intense immunoreactivity at the tip of an apical process open to the lumen (arrowhead) and basal short processes (arrows) at the bottom of a crypt. **i** C1 closed cell with a single long process extending towards the submucosa, the terminal and proximal parts of which form swollen structures (arrows). **j** C2 closed cell with a single long and thick process directed towards the submucosa in the distal colon. **k** C3 closed cell without any processes seen in the upper region of a crypt. L: lumen, SM: submucosa. Scale bars: 50 µm (**a**–**c**), 5 µm (**d**–**k**)

Numbers and distribution of 5-HT immunoreactive cells in the proximal colon

In the crypts of proximal colon, most cells (76%) were open (Table 3, Fig. 4b), and O3 type cells were the most common (Table 2, Fig. 4b). In contrast to the cecum, 5-HT cells in the upper region of the crypt (63%, 384 ± 46) were more abundant than in the lower portion (37%, 223 ± 11) (Fig. 3b). In the upper halves of crypts, 71% of the cells were open, and in the lower crypt half, open cells were 84% of 5-HT cells (Table 4). The numbers and percentages of each type of 5-HT cells are shown in Table 5 and Fig. 5b. Some of the O2 type 5-HT cells had a submucosally extending process with small swollen regions (Fig. 1b). Of the 5-HT cells in the upper half, 65% (409/626 cells from 4 mice) were O3-A or O3-C type cells (Figs. 1c and 2b), which were embedded in the surface epithelium facing the lumen. C3 type 5-HT cells were the second most common in this region (Table 5, Fig. 5b), some of which extended short basal processes or had no process (Figs. 1f and 2k). Most O3 cells were of O3-C type (Fig. 1c), a large proportion of which were seen near the bottom of the crypts (Fig. 2b). In the proximal colon, there were no cells with long basal processes, i.e., no O1 and C1 type cells (Fig. 5b).

Numbers and distribution of 5-HT immunoreactive cells in the distal colon

In the distal colon, the percentages of open and closed type 5-HT cells were 52% and 48%, respectively (Table 3, Fig. 4c). The numbers and percentages of each type of 5-HT cell are presented in Table 4 and Fig. 4c. Cells were counted in the upper, middle, and lower thirds of crypts (Fig. 5c). The cell number was highest in the mid crypt (48%, 210 ± 48) compared with the upper (27%, 119 ± 17) and lower (25%, 110 ± 15) thirds (Fig. 3b). In the upper third, open type cells were 43% of the total 5-HT cells and closed type cells were 57% (Table 4), but there were a few open or closed 5-HT cells in the surface epithelium facing the lumen (Fig. 2c). In the middle third of the crypt, the percentages of open and closed type cells were 40% and 60%, and in the lower third, open cells were 85% of the total 5-HT cells and closed cells were 15% (Table 4).

The long basal processes of O1 type cells were usually 50–100 µm in length, occasionally beyond 100 µm, and were directed towards the submucosa, following the basal surfaces

Table 1 Morphological classification of 5-HT cells in the large intestine

5-HT cell type	Characteristics
Open	
O1	Bipolar cells with a long basal process (> twice the soma length) directed towards the submucosa and an apical process open to the lumen
O2	Bipolar cells with a submucosally-directed basal process, shorter than that of O1 type cells, and an apical process open to the lumen
O3-A	Oval or spindle-shaped cells with an apical process open to the lumen
O3-B	Cells with more than two processes that are relatively short, including an apical process open to the lumen
O3-C	Flask-like or fusiform cells
Closed	
C1	Cells with only a single long basal process directed towards the submucosa
C2	Cells with only a single long basal process shorter than that of C1 cell types Cells with only a single long basal process shorter than that of C1 cell types
C3	Cells with no or few short basal processes and no process open to the lumen

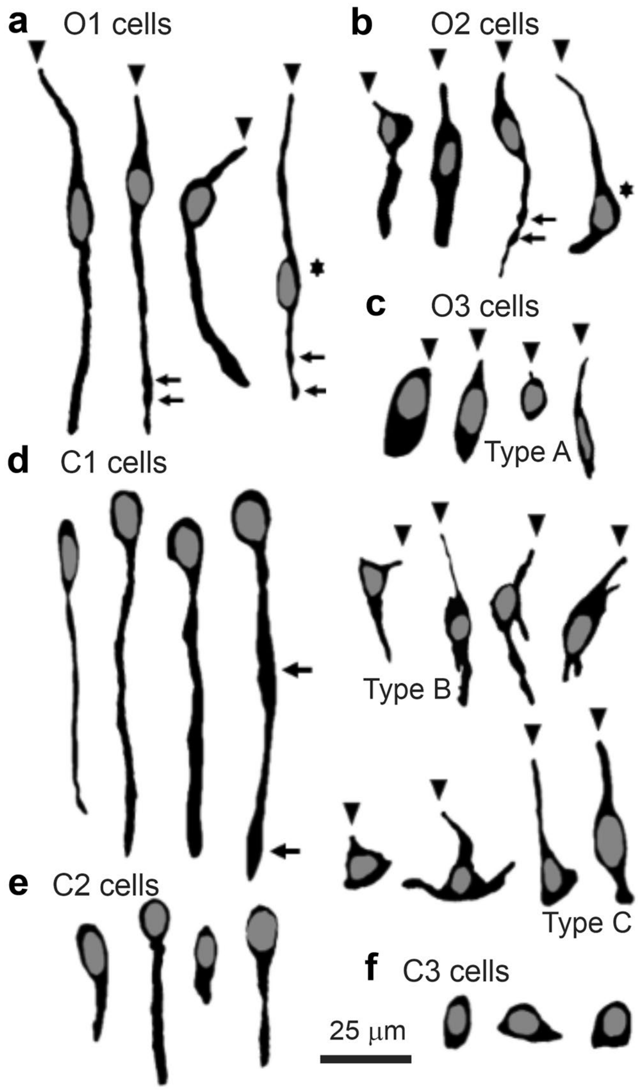


Fig. 2 Morphologies of 5-HT cells in the large intestine depicted as silhouettes. 5-HT cells are divided into open (O; with a process reaching the lumen) and closed (C; no luminal contact) types. **a** O1 type 5-HT cells are bipolar with a long basal process directed towards the submucosa, and an apical process open to the lumen (arrowheads). An occasional cell (asterisk) has a longer apical process. **b** O2 type 5-HT cells are bipolar with submucosally directed processes that are shorter than those of O1 type cells, and apical processes open to the lumen (arrowheads). In some cases, the apical process is longer (asterisk). **c** O3 type cells are subdivided into three classes: O3-A cells are oval or spindle-shaped and often occur in the surface epithelium facing the lumen in the proximal colon. O3-B cells have more than two processes (including an apical process) that are relatively short. O3-C cells have flask-like or fusiform shapes and are usually located near the bottom of the crypt. **d** C1 closed 5-HT cells extend only a single long process towards the submucosa. **e** C2 closed cells possess only a single process, shorter than that of C1 cells. **f** C3 type cells have no process open to the lumen. They are found near the bases of the crypts

of the epithelial cells (Figs. 1a and 2d). The submucosally directed basal process was usually longer than the process opening to the lumen, but in some cells, the apical process was longer (Fig. 1a). The submucosally projected long basal process of many O1 and O2 type cells ended within the middle third although the process of some cells reached the lower third. On the other hand, a number of the C1 cells provided a single long basal process extending to the lower third, but the process of most cells did not extend beyond the middle third. The processes of most C2 cells (Fig. 1e) also appeared to remain within this region.

Most of the O3 type cells that belonged to O3-B or O3-C types (Figs. 1c and 2g) and were located at the bottom of the crypts with some of them having cytoplasmic second projections that appeared to enter between other epithelial cells near the bottom of the crypt (not shown). There were very few O1 and C1 type cells in this region. Most of the C3 type cells (Fig. 1f) were also located near the bottom of the crypts, and some had the basal short processes which extended along basement membrane.

Cells with long processes

The combined proportion of O1 and C1 5-HT cells, relative to the whole population, was approximately 7%. The majority (83%) of these were in the distal colon, where they were 21% of cells, compared with 0.5% of cells in the cecum and none in the proximal colon. The four groups of cells with submucosally directed processes (O1, O2, C1, and C2 cells) represented approximately 26% of 5-HT cells in the large intestine: 26% of cells in the cecum, 3% in the proximal colon, and 58% of cells in the distal colon. These four cell types in the distal colon made up 76% of these types of 5-HT cells in the large intestine.

Discussion

This study has revealed a heterogeneity of 5-HT enteroendocrine cell morphologies in the large intestine, and differences in cell densities between regions and along the crypt axis. The highest density of occurrence of 5-HT cells was in the proximal colon, consistent with Reynaud et al. (2016), although greater cell numbers were found in our study because we employed much thicker sections (up to 100–120 µm), in order to reveal cell processes more effectively.

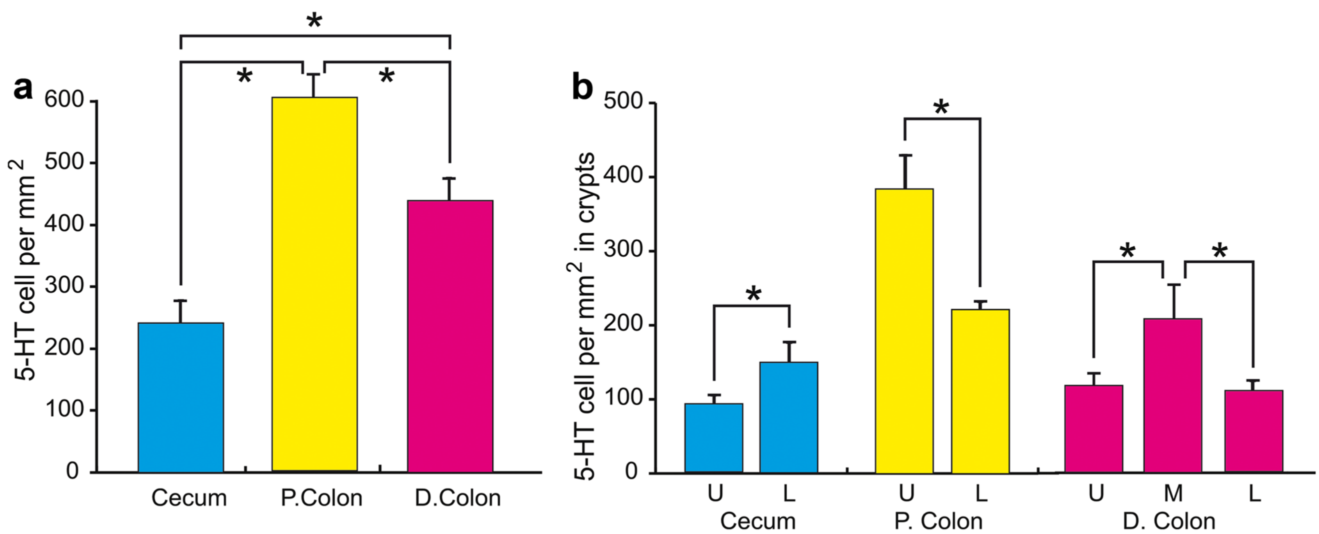


Fig. 3 Distribution of populations of 5-HT immunoreactive cells in the mouse cecum, proximal colon (P. Colon) and distal colon (D. Colon). The proximal colon had the highest cell density (**a**). Numbers of 5-HT cells in the upper (U: luminal), middle (M), or lower (L: submucosal) portions of the crypts (**b**). Data are expressed as mean ± SD; * $P < 0.005$ (*t* test; $n = 4$ mice, cells counted in 10–15 fields at 20× in sections from each mouse)

5-HT cells with long processes

Many cells had very long processes, which are not apparent in other studies that have used thinner sections. The processes were contained within the basement membrane closely adjacent to the bases of enterocytes, as depicted in Fig. 5 and shown previously in an electron microscope study (Kuramoto 2007), showing that the processes were within the inner layer of the basement membrane (lamina lucida) and were covered by the lamina densa (see Yurchenko and Schittny 1990 for review of basement membrane structure). The processes ran along the bases of the epithelial cells and never appeared to cross the lamina densa. Secretory granules were gathered adjacent to the enterocytes (Kuramoto et al. 2007). Examination of the 5-HT cells with long processes in the present study showed that the intensity of immunoreactivity was greater in the process than in the cell body. Thus, the processes of these cells resemble nerve cell processes in which transmitter is concentrated.

Possible functions of the 5-HT cells with basal processes

5-HT cells of the mouse distal colon express the mechanosensitive channel *Piezo2* (Billing et al. 2019), and 5-HT is released on the colon in response to mechanical distortion (Grider et al. 1996). In addition, evidence of 5-HT being released in response to mechanical stimuli, mediated through *Piezo2*, has been found in the small intestine (Wang et al. 2017; Alcaino et al. 2018). This is consistent with earlier studies indicating that EC cells are activated by mechanical stimuli, such as luminal pressure or stretch of the gut wall, to release 5-HT (Bülbring and Lin 1958). The positioning of processes between the lamina densa and the epithelial cells may make them prone to distortion when the mucosa is compressed. In addition, 5-HT cells in the large intestine respond to microbial metabolites (Lund et al. 2018). Because many EC cells of the large intestine cells express both *Piezo2* and microbial metabolite receptors, the same cells may respond to mechanical distortion and microbial products.

Table 2 Numbers (cells/mm^2 of mucosa) and percentages of each type of open and closed 5-HT cells in each segment of the large intestine

	Open type			Closed type		
	O1	O2	O3	C1	C2	C3
Cecum	0.5 ± 0.6 (2%)	26 ± 10 (10%)	170 ± 23 (66%)	0.8 ± 1.0 (0.3%)	38 ± 14 (15%)	21 ± 4 (8%)
P. Colon	0 (0%)	6 ± 8 (1%)	437 ± 30 (75%)	0 (0%)	10 ± 7 (2%)	132 ± 36 (23%)
D. Colon	26 ± 7 (6%)	42 ± 10 (10%)	157 ± 15 (37%)	64 ± 17 (15%)	119 ± 18 (28%)	22 ± 6 (5%)

5-HT released from EC cells initiates peristalsis via intrinsic sensory neurons (Grider et al. 1996), which implies that 5-HT must diffuse from the processes adjacent to the enterocytes to reach nerve endings of intrinsic sensory neurons in the lamina propria. 5-HT also acts on enterocytes to stimulate water and electrolyte secretion (Cooke and Carey 1985; Day et al. 2005), which aligns with the proximity of clumps of secretory vesicles in the basal processes being close to the enterocytes (Kuramoto et al. 2007).

Fig. 5 Frequencies of occurrence and depiction of the different morphological types of 5-HT cells. **a**, **a′** Distributions of 5-HT cells in the upper and lower parts of the crypts in the cecum; **a″** illustrations of the 5-HT cells associated with the mucosal glands in the cecum. **b**, **b′** Distributions of 5-HT cells in the upper and lower part of the crypts in the proximal colon; **b″** illustrations of the 5-HT cells associated with the mucosal glands in the proximal colon. **c**, **c′**, **c″** Distributions of 5-HT cells in the upper, middle, and lower thirds of the crypts in the distal colon; **c‴** illustrations of the 5-HT cells associated with the mucosal glands in the distal colon. Note differences in the scales of the *y*-axes for the cecum, proximal, and distal colon. Data are mean ± SD

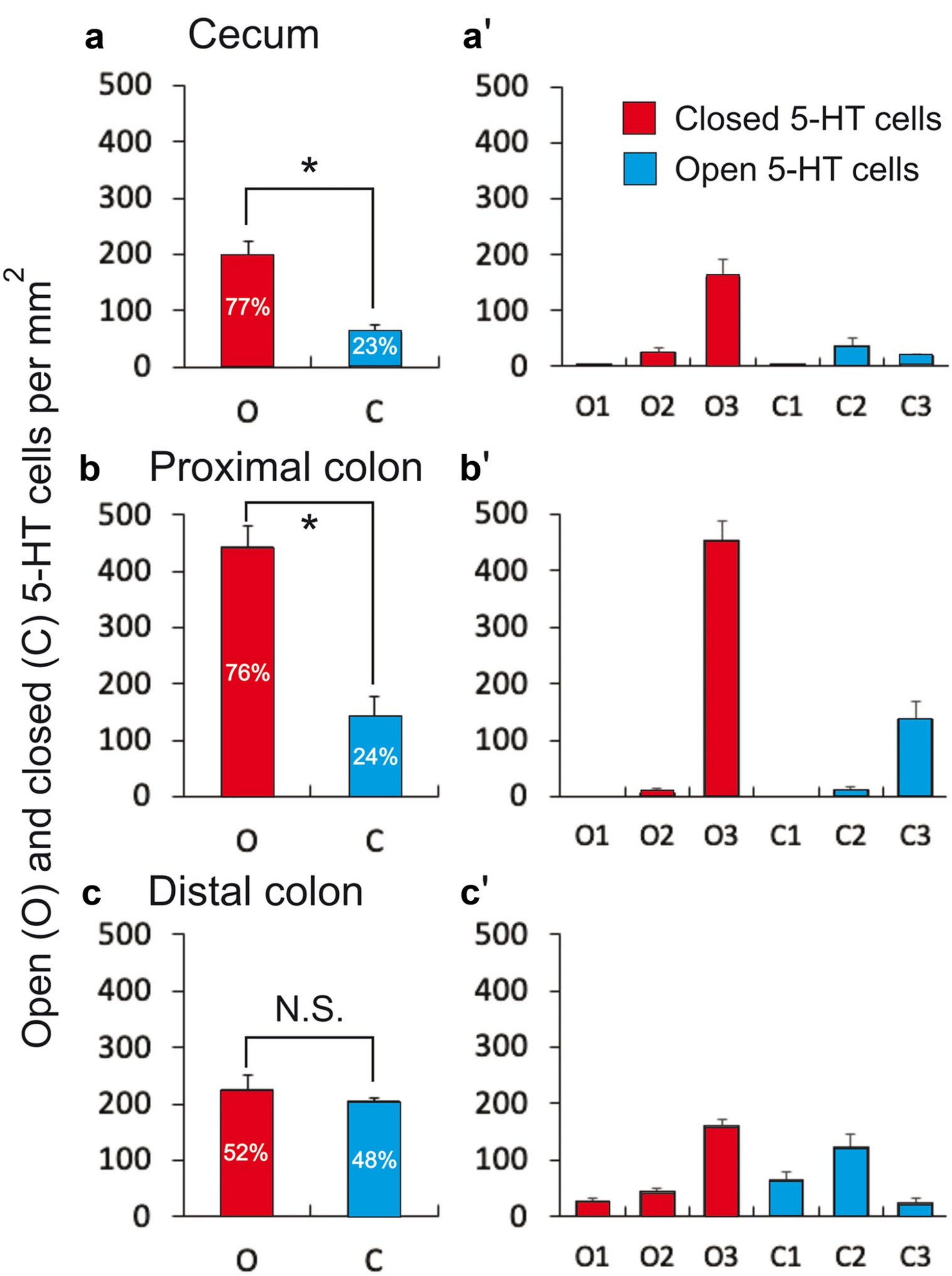


Fig. 4 Numbers of open (O) and closed (C) 5-HT cells in the cecum (**a**), proximal colon (**b**), and distal colon (**c**). At left (**a**, **b**, **c**) is the data for all open and closed cells and at right (**a′**, **b′**, **c′**) for the cell subtypes. Data are mean ± SD; $^*P < 0.005$ (*t* test; $n = 4$ mice, cells counted in 10–15 fields at ×20 in sections from each mouse). N.S. = not significantly different ($P > 0.5$)

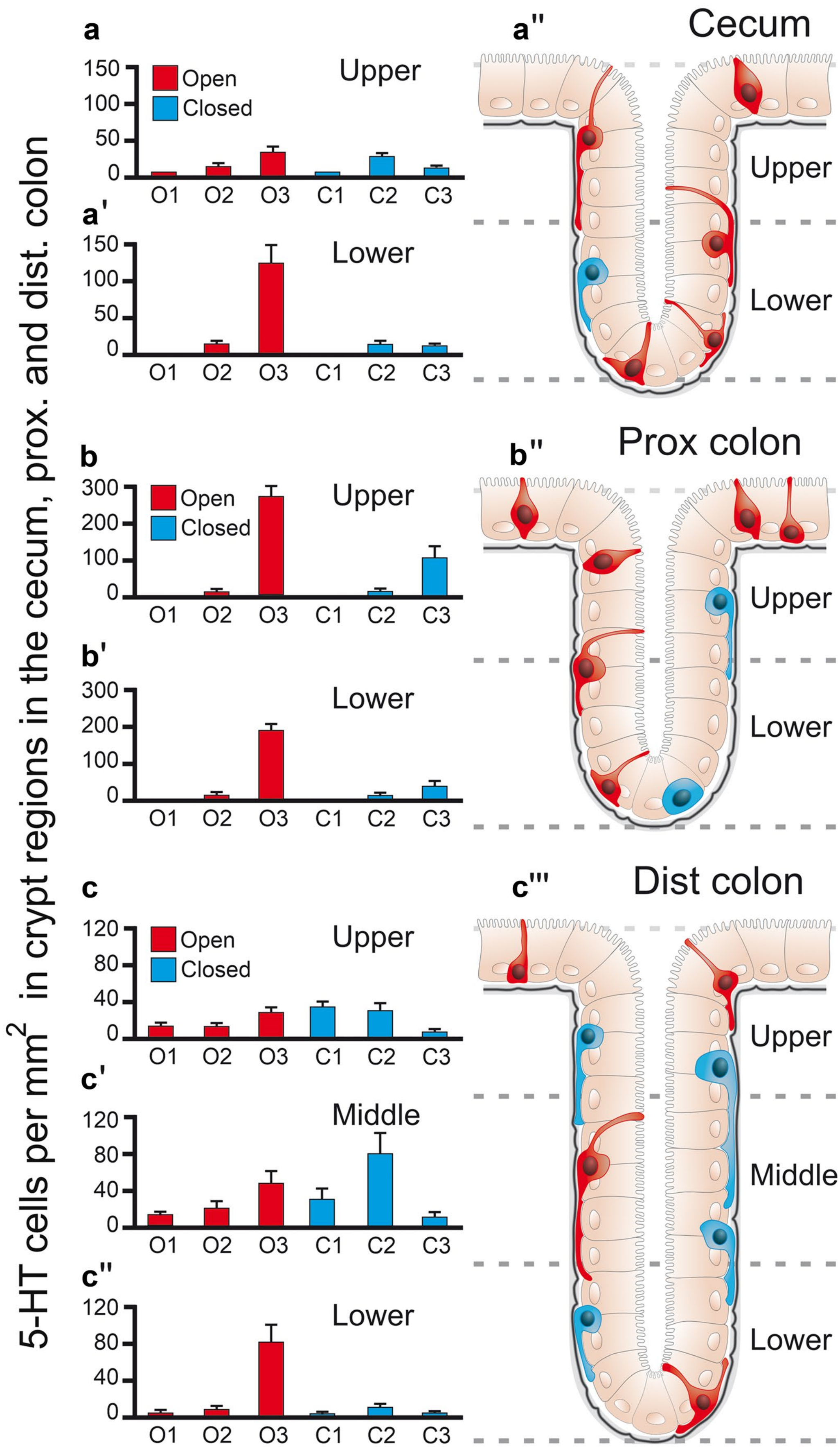

5-HT cells per mm2 in crypt regions in the cecum, prox. and dist. colon
a
Open
Closed
Upper
150
100
50
0
O1 O2 O3 C1 C2 C3
a'
Lower
150
100
50
0
O1 O2 O3 C1 C2 C3
b
Open
Closed
Upper
300
200
100
0
O1 O2 O3 C1 C2 C3
b'
Lower
300
200
100
0
O1 O2 O3 C1 C2 C3
c
Open
Closed
Upper
120
80
40
0
O1 O2 O3 C1 C2 C3
c'
Middle
120
80
40
0
O1 O2 O3 C1 C2 C3
c''
Lower
120
80
40
0
O1 O2 O3 C1 C2 C3
a''
Cecum
Upper
Lower
b''
Prox colon
Upper
Lower
c'''
Dist colon
Upper
Middle
Lower

Table 3 Numbers (cells/mm^2 of mucosa) and percentages of open and closed 5-HT cells in each segment of the large intestine

Region	Cell type	
	Open	Closed
Cecum	197 ± 26 (77%)	60 ± 15 (23%)
Proximal colon	444 ± 36 (76%)	142 ± 35 (24%)
Distal colon	225 ± 25 (52%)	205 ± 5 (48%)

Table 4 Numbers (cells/mm^2 of mucosa) and percentages of open and closed 5-HT cells in upper (luminal), middle, or lower (submucosal) region of each segment of the large intestine

		Open type	Closed type
Cecum	Upper	55 ± 12 (56%)	43 ± 12 (44%)
	Lower	143 ± 18 (90%)	16 ± 6 (10%)
P. Colon	Upper	263 ± 25 (71%)	107 ± 32 (29%)
	Lower	181 ± 15 (84%)	35 ± 16 (16%)
	Upper	50 ± 7 (43%)	67 ± 17 (57%)
D. Colon	Upper	82 ± 10 (40%)	121 ± 18 (60%)
	Lower	93 ± 26 (85%)	16 ± 5 (15%)

Open and closed type 5-HT cells

The terms of 'open (O) type' and 'closed (C) type' for gut endocrine cells arose from investigation of endocrine cells of the gastric body of human and other mammals by Fujita and Kobayashi (1971, 1973), who postulated that the open type cells extend a cytoplasmic process to the mucosal surface to detect the luminal chemical environment, whereas closed cells lie flat on the basement membrane and do not reach the mucosal surface, but respond to mechanical stimuli applied to the mucosal membrane (Kobayashi et al. 1971). More recent work suggests that the basolateral surfaces of EEC exhibit receptors for nutrients and that nutrients may activate basolateral receptors after they are absorbed (Gribble and Reimann 2016). In the large intestine, the majority of 5-HT cells with long processes were of the closed type. As mentioned above, the location of these processes, tight up against the epithelium, may favor them being distorted by pressure against the mucosa. This would support the idea of Fujita and Kobayashi that closed cells could be mechanoreceptors.

Significance of basal processes of enteroendocrine cells

Basal processes have been described for several kinds of gastrointestinal endocrine cells in a range of species. EEC with basal processes include somatostatin cells in the rat and human stomach (Larsson et al. 1979; Hunne et al. 2019); L cells in the distal small intestine and colon, which contain PYY, GLP-1, and GLP-2 (Lundberg et al. 1982; Furness et al. 2013); several cell types in the mouse small intestine (Bohórquez et al. 2011) and 5-HT cells in the rat and human stomach (Hansen and Witte 2008; Fakhry et al. 2019). We suggest that basal processes may have several roles. The processes of somatostatin cells of the gastric antrum extend to gastrin cells, and there is convincing evidence that somatostatin inhibits gastrin release (Schubert and Peura 2008). For the PYY/GLP-1/GLP-2 cells, a target is probably enterocytes which respond to both PYY and GLP-2 (Cox 2016). For some EEC, the processes appear to be sites of endocrine cell innervation, a function similar to dendrites of neurons (Bohórquez et al. 2011), but it is notable that close approaches of nerve fibers to the 5-HT cell processes were not observed by electron microscopy in the colon (Kuramoto et al. 2007). Further investigation of the structural and functional relationships between 5-HT cells with long basal processes in the large intestine and nerve endings and other targets, including other EEC, is necessary to understand the physiological roles of 5-HT signaling by these cells.

Table 5 Numbers (cells/mm^2 of mucosa) and percentages of each type of open and closed 5-HT cells in upper, middle, or lower segment of each segment of the large intestine

		O1	O2	O3	C1	C2	C3
Cecum	Upper	0.5 ± 0.6 (0.5%)	15 ± 6 (16%)	39 ± 13 (40%)	0.8 ± 1.0 (0.8%)	31 ± 12 (32%)	12 ± 7 (12%)
	Lower	0 (0%)	11 ± 9 (7%)	132 ± 15 (83%)	0 (0%)	8 ± 4 (5%)	9 ± 4 (6%)
P. Colon	Upper	0 (0%)	5 ± 6 (1.4%)	258 ± 19 (70%)	0 (0%)	9 ± 7 (2%)	98 ± 30 (26%)
	Lower	0 (0%)	1.3 ± 1.9 (0.6%)	179 ± 14 (83%)	0 (0%)	1 ± 0.8 (0.5%)	34 ± 16 (16%)
D. Colon	Upper	12 ± 5 (10%)	12 ± 1 (10%)	27 ± 3 (23%)	32 ± 9 (27%)	30 ± 9 (26%)	5 ± 1 (5%)
	Middle	14 ± 3 (7%)	23 ± 8 (11%)	46 ± 10 (23%)	31 ± 12 (15%)	79 ± 20 (39)	11 ± 5 (6%)
	Lower	0.8 ± 0.5 (0.7%)	8 ± 3 (7%)	84 ± 24 (78%)	1.3 ± 1.3 (1.1%)	10 ± 4 (9%)	5 ± 2 (5%)

Possible release of 5-HT into the lumen of the proximal colon

A majority of the 5-HT cells in the proximal colon were open. Several studies provide evidence of 5-HT release into the lumen (Kojima and Ikeda 1998; Tsukamoto et al. 2007; Kaji et al. 2015; Ripken et al. 2016). For example, 5-HT is released into the lumen in response to nutrients (Ferrara et al. 1987), acid (Kellum et al. 1983), and short chain fatty acids (Fukumoto et al. 2003), and intraluminal pressure in the rat duodenum increased 5-HT release (Fujimiya et al. 1997). We detected intense immunoreaction in the apical ends of open type 5-HT cells facing the lumen (Fig. 2f, g, h). Likewise, an immunoelectron microscopic study by Fujimiya et al. (1997) demonstrated the localization of 5-HT immunoreactive granules in the apical cytoplasm of EC cells in the rat duodenum. Given that a large number of open 5-HT cells were located in the surface epithelium facing the lumen in the mouse proximal colon, intraluminal release of 5-HT is probable. One of its actions may be to affect the adjacent epithelium. Immunohistochemical evidence for the localization of 5-HT_4 receptors on the apical membranes of surface epithelial cells is consistent with intraluminal 5-HT inducing fluid secretion from the epithelial cells in the rat proximal colon (Kaji et al. 2015).

Predominance of open 5-HT cells without basal processes in the lower regions of the crypts

The predominant location of open cells without basal processes (O3 type 5-HT cells) in the deeper parts of the crypts may be related to an involvement of 5-HT in cell proliferation or renewal in the basal regions of the crypts (Tutton and Barkla 1986; de Bruine et al. 1992; Zachrisson and Uribe 1998; Tackett et al. 2017; Greig et al. 2019). Such a role of 5-HT could contribute to protection of the mucosa from intestinal ischemia and reperfusion injury through increased mucosal growth (Tackett et al. 2019). It is also possible that some O3 type 5-HT cells in the crypts are immature progenitors or precursors of open type 5-HT cells that subsequently differentiate into process-bearing 5-HT that migrate towards the lumen (Tsubouchi and Leblond 1979).

In conclusion, the present study revealed 5-HT cells of different morphologies in the large intestine. In the distal colon, especially, many 5-HT cells with basal processes that were closely adjacent to enterocytes occurred. These could release 5-HT in close proximity to the mucosal epithelial cells. The processes are also positioned where they could respond to mechanical distortion. The abundant open 5-HT cells in the upper crypts of the proximal colon may be involved in intraluminal 5-HT signaling to the adjacent epithelium.

Compliance with ethical standard

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval Procedures were approved by the University of Melbourne Animal Ethics Committee (ethics approval numbers 1613817 and 1814569) and the Kyoto Institute of Technology (approval number 100154). All applicable National and Institutional guidelines for the care and use of animals were followed.

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