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Authors: Damien J. Keating, Nick J. Spencer

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# What is the role of endogenous gut serotonin in the control of gastrointestinal motility?

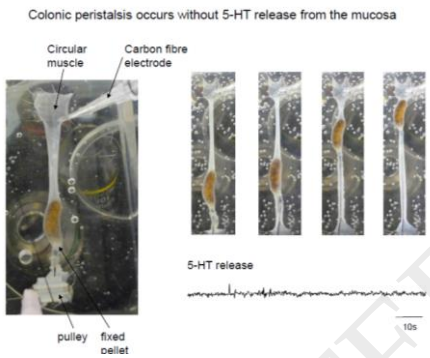
Damien J Keating and Nick J. Spencer*

College of Medicine and Public Health and Centre for Neuroscience, Flinders University of South Australia, Adelaide, Australia

*Corresponding author

Nick Spencer (Ph.D)
College of Medicine and Public Health, School of Medicine, Flinders University, South Australia, Australia
e-mail: nicholas.spencer@flinders.edu.au
PHONE: 61-8-8204 4241

**Graphical Abstract:**




## *Abstract*

In recent years, there have been dramatic changes in our understanding of the role of *endogenous* 5-Hydroxytryptamine (5-HT or serotonin) in the control of gastrointestinal (GI) motility. Whilst it is well accepted that there are numerous types of 5-HT receptors expressed on enteric neurons and that *exogenous* 5-HT potently stimulates GI-motility, understanding the role of endogenous 5-HT in GI-motility has been substantially more difficult to resolve. Recent studies found $5\text{-HT}_3$ and $5\text{-HT}_4$ antagonists have the same effects on peristalsis in colon preparations depleted of endogenous 5-HT. Then, recent work revealed that in mice with genetic mutations to prevent the synthesis of endogenous 5-HT from enterochromaffin EC) cells did not block major neurogenic motor patterns in the gut wall and did not reduce GI-transit in conscious animals, raising doubts about early hypotheses that endogenous 5-HT was critical for neurogenic GI-motility patterns. Indeed, functional evidence now suggests that $5\text{-HT}_3$ and 5-HT4 receptors on enteric nerves display constitutive activity. In summary, recent findings demonstrate that endogenous 5-HT released from the mucosa or enteric neurons is not required for the generation of major

neurogenic motor patterns, at least in the large intestine, but that it likely acts as a modulator of contractile frequency. This review will discuss how and why our understanding of endogenous 5-HT has dramatically changed in the past few years.

**Key words**: enterochromaffin cell, EC cell, peristalsis, colon, colonic migrating motor complex, CMMC, serotonin.

## *Introduction*

It is well accepted that the greatest quantity of serotonin (5-hydroxytryptamine, 5-HT) in the body is synthesized within enterochromaffin (EC) cells in the intestinal mucosa, via the enzyme tryptophan hydroxylase-1 (TPH1). On a much smaller scale, 5-HT is also synthesized (Keating et al., 2013) in approximately 1% of nerve cells bodies in the enteric nervous system, via a different enzyme called tryptophan hydroxylase-2 (TPH2) (Keating et al., 2013). Because such high quantities of 5-HT are synthesized within the gut wall, there have been many studies over the past century that have been directed to understanding the functional role of 5-HT, particularly with regards to the different neurogenic motor patterns in the gastrointestinal (GI) tract(Hu and Spencer, 2018).

Since the early 1950's there had been a gathering of compelling evidence to support the notion that endogenous 5-HT released from the gut wall played an important role in the generation and/or modulation of propagating neurogenic motor patterns along the GI-tract, particularly the small and large intestine(Kadowaki et al., 1996, Dickson et al., 2010, Smith and Koh, 2017). Indeed the corresponding author of this review also published that endogenous 5-HT synthesized within the gut wall likely had a major influence in the generation of gastrointestinal motor patterns in the small and large intestine (Bush et al., 2001). This view has been challenged after recent findings over the past 10 years through the use of newly-developed techniques, including the advent of functional genomics techniques to genetically ablate specific genes of interest. The use of such approaches has provided some changes in our understanding of the role of endogenous 5-HT in GI motility and transit that are contrary to earlier suggestions. This review will highlight the existing doctrine regarding the role of endogenous 5-HT in GI motility, then contrast how these views have been revised in recent years as new and more refined techniques have been used to address

open questions and the differences in key findings and interpretations from different groups in regards to this.

## *What is the role of 5-HT release from the mucosa in regulating GI motility?*

There is no doubt that the greatest quantity of endogenous 5-HT synthesized in the body is within EC cells of the intestinal mucosa and that this source of 5-HT can be potently released in response to an array of stimuli. What has been difficult to ascertain is the relevance of this 5-HT for the generation of neurogenic motor patterns in the gut wall. It was shown in the early in 1950's that high quantities of 5-HT are released from the mucosa at a similar time as peristalsis occurred in the small intestine (Büllbring and Lin, 1957, Büllbring and Lin, 1958, Büllbring et al., 1958). The observation that endogenous 5-HT release occurred at a similar time as peristalsis provided the platform to propose that the release of 5-HT was the trigger that elicited peristalsis. The recordings in these papers were made using high precision liquid chromatography (HPLC), an approach which provides high target specificity but relatively low temporal resolution regarding absolute 5-HT concentrations during contraction. The similarity between release of 5-HT and the generation of peristalsis led these early investigators to conclude that release of endogenous 5-HT from the mucosa played an important role in the generation of peristalsis (Büllbring and Lin, 1958, Büllbring et al., 1958). This was further substantiated by the finding that exogenous bath application of 5-HT applied to the isolated intestine potently activated peristalsis (Büllbring and Lin, 1957, Büllbring and Lin, 1958, Büllbring et al., 1958). Reasonably, these views were widely accepted as doctrine, until more recent technical advances and approaches provided new insights into the functional role of mucosal 5-HT in gut motility.

## *Real time recordings of 5-HT release during intestinal motor activities*

The development of amperometric recording techniques allowed for the first time, the real time release of 5-HT to be recorded from the gut wall. These amperometric recordings were made during neurogenic motor patterns in the small intestine (Bertrand, 2006) and colon (Spencer et al., 2011, Keating and Spencer, 2010), facilitating new insights into the dynamic release patterns of endogenous 5-HT in relation to gut contraction. In such studies it was

possible to correlate the temporal dynamics of 5-HT release from the mucosa with propagating neurogenic motor patterns along the intestine. These experiments revealed some key observations While some contractions underlying peristalsis directly correlated with the release of 5-HT (Spencer et al., 2011, Keating and Spencer, 2010) many peristaltic contractions did not correlate with any detectable release (Spencer et al., 2011, Keating and Spencer, 2010). This lack of consistency between release of 5-HT and peristalsis led to the realization that endogenous 5-HT release from the mucosa may not, in fact be as important to the generation of peristalsis as once thought. It must be acknowledged that many years before the development of real time amperometry, the relevance of endogenous 5-HT release from the mucosa had already been questioned when other investigators had dissected away the mucosa and revealed that the absence of the mucosa did not prevent peristalsis (Ginzel, 1959, Diament et al., 1961, Tsuji et al., 1992). More recent amperometric studies provided additional support that EC cells are actually highly sensitive to mechanical stimuli (Bertrand, 2006) and release 5-HT in response to mucosal compression or contractile events. This is reflected in the statement that "*..the correlation between local motor reflexes and 5-HT release…is caused primarily by the contraction of the smooth muscle and subsequent deformation of the mucosa*" (Bertrand, 2006). More recent studies have now verified that EC cells are indeed mechanically sensitive and express the mechanosensitive ion channel, Piezo2 (Fakhry et al., 2017). Such findings provide evidence to support the notion that mechanical deformation of EC cells, caused by the contraction of the gut wall, is a stimulus for the release of 5-HT from EC cells, rather than 5-HT release being a catalytic step required for the initiation of peristalsis(Bertrand, 2006). This idea was then further supported by findings in the large intestine that endogenous 5-HT release from EC cells could cycle in a similar temporal relationship with peristalsis or CMMCs but that removal of the mucosa abolished all 5-HT release from the mucosa without abolishing peristalsis (Spencer et al., 2011) nor CMMC generation (Keating and Spencer, 2010). Furthermore, peristalsis and CMMCs remain in *TPH1*$^{-/-}$ mice that have lost the ability to synthesize EC cell 5-HT (Heredia et al., 2013). Collectively, such data indicates that the endogenous release of 5-HT from EC cells during a peristaltic contraction or CMMC is a *consequence* not *cause* of these major neurogenic motor patterns. (Fakhry et al., 2017)

## *Mucosal compression and activation of peristalsis – what does it mean?*

Early studies of the peristaltic reflex using compression of the mucosa as a stimulus used a brush to evoke the release of 5-HT and CGRP at the same time as the peristaltic reflex was elicited (Jin et al., 1999, Foxx-Orenstein et al., 1996, Grider et al., 1996). This temporal association led some investigators to hypothesize that "*…5-HT released by mucosal stimulation initiates the peristaltic reflex by activating 5-HT4/5-HT1p receptors"* (Foxx-Orenstein et al., 1996, Grider et al., 1996). Then, in further studies using isolated tubular preparations of guinea-pig colon, the same group proposed that the propagation of fecal pellets along the colon was caused by compression of EC cells, causing the release of 5-HT which activated intrinsic sensory nerve endings that projected into the mucosa and could then activate the myenteric plexus. This was then thought to activate the descending inhibitory and ascending excitatory nerve pathways to the neighboring smooth muscle layers (Jin et al., 1999, Foxx-Orenstein et al., 1996, Grider et al., 1996). Others have also hypothesized in the large intestine that 5-HT release from EC cells in the mucosa is "*critical*" for the cyclical generation of CMMCs (Heredia et al., 2009). Evidence supporting this conclusion was the finding that "*…removing the mucosa appeared to abolish spontaneous CMMCs.."* (Heredia et al., 2009)and, "*the trigger for the CMMC appears to be spontaneous or evoked (ie, a fecal pellet) release of 5-HT from EC cells to stimulate AH neurons*" (Heredia et al., 2009). However, several years later(Heredia et al., 2009), the same group demonstrated that CMMCs still occurred in mice lacking the ability to synthesize 5-HT in EC cells (Heredia et al., 2013). The only change noted for CMMCs in these *TPH1*$^{-/-}$ mice was a reduction in CMMC frequency (Heredia et al., 2013), the same change observed by others when the mucosal layer was dissected away in mouse and guinea-pig colon (Spencer et al., 2011, Keating and Spencer, 2010).(Keating and Spencer, 2010)(Keating and Spencer, 2010)(Keating and Spencer, 2010, Zagorodnyuk and Spencer, 2011)Why the physical removal of the mucosa by one group causes a total loss of CMMCs (Heredia et al., 2009) while CMMCs and peristalsis remain after the same dissection by another group (Keating and Spencer, 2010) remains unknown. The remaining gut motility observed by us in the absence of EC cells is not due to stretch-evoked firing of activation of the myenteric plexus in these preparations, as propositioned by others (Smith and Gershon, 2015), because motility remains in both open sheet and intact tube preparations, and for the propulsion of water, freely-moving pellets, fixed pellets and spontaneously evoked contractions (Keating and Spencer, 2010, Zagorodnyuk and Spencer, 2011). We believe that these key findings proved finally that the cyclical release of 5-HT from the mucosa is indeed a *consequence* of

peristalsis or CMMCs and was not the underlying *cause* of these neurogenic motor patterns (Fig. 1-3). (Keating and Spencer, 2010)(Heredia et al., 2009)

In addition to these experiments discussed above, other groups have undertaken *in vivo* studies on gut motility in the absence of the enzyme tryptophan hydroxylase-1 (TPH1) (Yadav et al., 2010, Li et al., 2011). These studies demonstrated that deletion of TPH1 did not lead to any inhibitory effects on GI-transit in conscious mice (Yadav et al., 2010, Li et al., 2011). This was a particularly important finding, because until this, all other major studies had been performed *in vitro*. These studies therefore also supported the notion that endogenous release of 5-HT from the mucosa was not a prerequisite for *in vivo* transit of content to occur, nor the colonic migrating motor complex in the large bowel *in vitro* (Spencer et al., 2015). Therefore, while EC cell 5-HT is released in response to a number of stimuli (Raghupathi et al., 2013, Zelkas et al., 2015, Martin et al., 2017b, Martin et al., 2017a, Martin et al., 2017c) and while this source of 5-HT plays important paracrine and endocrine roles (Martin et al., 2017c), it acts only as a modulator, not an initiator, of neurogenic motor patterns and gastric transit in the gut wall.

### *Changes in motility when the mucosa and submucosal plexus are removed*

It is important to note that, while peristalsis, CMMCs and colonic transit are not abolished by either the genetic removal of EC cell 5-HT synthesis or upon the removal of the entire mucosa and submucosal plexus, differences were noted in these neurogenic motor patterns compared to intact preparations. For example, in isolated whole mouse colons lacking mucosa and submucosal plexus, CMMCs occurred at a slower frequency, and with greater variability in the intervals between the onset of each contraction (Keating and Spencer, 2010). Also, in the guinea-pig colon, while removal of the mucosa and submucosal plexus did not cause a difference in the peak force of muscle contraction or the duration or interval between peristaltic contractions evoked by natural fecal pellets (Fig. 3C), there was a significant reduction in the velocity of propagation of fecal pellets (Spencer et al., 2011). These effects could potentially be due to the lack of mucosal 5-HT from EC cells (or any other substance released from EC cells), or the lack of the submucosal plexus, or simply the lack of secretions normally present in the lumen that are generated by the mucosa. We speculated that mucosal 5-HT could play a modulatory role to control the frequency and or

velocity of peristalsis (Spencer et al., 2011) and CMMCs (Keating and Spencer, 2010). Future studies using more sophisticated approaches are required to verify these suggestions.

## *Serotonin - A neurotransmitter in the ENS?*

Serotonin is synthesized in about 1% of myenteric neurons (Costa et al., 1996). In addition to 5-HT, it is well accepted that there are about 20 different neurochemicals that can be identified using immunohistochemistry in the ENS. It is important to note however, that the vast majority of neurochemicals that are synthesized in enteric neurons are not neurotransmitters. To satisfy the criteria to be a neurotransmitter, a substance must be synthesized in a neuron, stored within vesicles in that neuron, released from that neuron, act on a postsynaptic membrane and elicit a postsynaptic response (Kandel et al., 2000). The vast majority of enteric neurochemicals have not been demonstrated to evoke a post synaptic effector response (e.g. synaptic potentials) following nerve stimulation. (Wood and Mayer, 1979)

While it is well accepted that enteric neurons are endowed with 5-HT receptors and that exogenous 5-HT potently stimulates enteric neurons (Wood and Mayer, 1979), evidence for a synaptic potential in enteric neurons that is due to *endogenous neuronal* 5-HT has been much more challenging. Electrophysiological recordings from myenteric neurons of human colon (Brookes et al., 1987), mouse colon (Furukawa et al., 1986, Nurgali et al., 2004) and rat intestine (Brookes et al., 1988), (Furukawa et al., 1986, Nurgali et al., 2004, Brookes et al., 1987) have found that all fast synaptic transmission is blocked by hexamethonium. No synaptic potentials have been identified in these species that are mediated by 5-HT. This is perhaps not surprising since 5-HT is synthesized in such a small population of enteric neurons (Costa et al., 1996). Despite these challenges, evidence has been presented that 5-HT may be a neurotransmitter in a small population of guinea pig myenteric (Zhou and Galligan, 1999, Galligan et al., 2000) and submucosal neurons (Monro et al., 2004). A relatively straightforward and direct way to address this issue is to genetically ablate the ability of myenteric neurons to synthesize 5-HT. TPH2 is the rate-limiting enzyme for 5-HT synthesis in neurons, including in the ENS (Neal et al., 2009). Gut motility in $TPH2^{-/-}$ mice is reduced *in vivo* (Li et al., 2011), which may at first glance indicate support for ENS 5-HT to modulate ENS activity and peristalsis. However, major developmental alterations occur embryonically and postnatally in these mice (Li et al., 2011). This is because the primary role of ENS 5-HT is as a growth factor, promoting the development and/or survival of some

classes of late-born enteric neurons. The existence of such ENS abnormalities in *TPH2*$^{-/-}$ mice precludes a definitive insight into whether the *in vivo* changes in gut motility observed in these mice is a because 5-HT is an enteric neurotransmitter that regulates gut motility. The development of an inducible mouse model that enables Tph2 expression to be removed in mature mice may be a suitable approach that circumvents such issues. Nonetheless, although impaired, colonic propulsion and small intestinal transit persist in mice lacking both isoforms of tryptophan hydroxylase (TPH1 and TPH2) and hence all mucosal and neuronal 5-HT (Li et al., 2011). Thus, 5-HT is not necessary for peristalsis *per se*.

### *5-HT antagonists can block peristalsis in preparations depleted of all 5-HT*

Early evidence supporting a functional role for endogenous 5-HT in the generation and propagation of neurogenic motor patterns along the gut was that these motor patterns could be reduced or abolished by antagonists of 5-$HT_3$ or $5HT_4$ receptors (Kadowaki et al., 1996, Grider et al., 1996, Foxx-Orenstein et al., 1996). Recently, these experiments were repeated in the isolated guinea-pig colon that had the mucosa dissected away and all the endogenous ENS 5-HT depleted using reserpine. This treatment blocks storage of 5-HT in vesicles, which normally protect 5-HT from rapid degradation in the cytoplasm by monoamine oxidase. It is this endogenous system that removes 5-HT after reserpine treatment and consequently, mass spectrometry confirmed total depletion of 5-HT in the ENS in such preparations (Sia et al., 2013a, Sia et al., 2013b). Given that the lower detection limit of this approach is ~1nM of 5-HT and that single cell and mucosal 5-HT levels are 2-5 orders of magnitude higher than this concentration, (Raghupathi et al., 2013, Bertrand, 2006), suggestions that some undetected but physiologically significant amounts of 5-HT remain in these preparations (Smith and Gershon, 2015) seems unlikely. Furthermore, the $K_i$ values (corresponds to the dose that produces 50% binding to that receptor) for 5-HT binding to its various receptors are all above 1nM (Murray et al., 2011). Thus if any 5-HT is remaining after this reserpine treatment, it would likely be insufficient to activate its receptors.

In these preparations completely lacking 5-HT, not only did peristalsis still occur robustly, but it was found that 5-$HT_3$ and 5-$HT_4$ antagonists (SDZ-205-557 and ondansetron) still had the same inhibitory effects on peristalsis as they did in intact preparations (Sia et al., 2013a, Sia et al., 2013b). In many cases, the effects of these antagonists were transient, causing a brief suppression, which recovered. In this regard, peristalsis persisted in the presence of

these antagonists (Sia et al., 2013a, Sia et al., 2013b). This showed that endogenous 5-HT was not a requirement for activation of any 5-$HT_3$ and 5-$HT_4$ receptors and, more importantly, that peristalsis could persist after blockade of these receptors. The question was then raised as to why a temporary blockade of peristalsis occurred. One explanation for these findings is that 5-$HT_3$ and 5-$HT_4$ receptors are constitutively active and contribute to the excitability of enteric neurons, but do not require the binding of endogenous 5-HT for their activation. This could readily explain why 5-$HT_3$ and 5-$HT_4$ antagonists have a temporary effect to inhibit peristalsis. Indeed, there is sound evidence that the G-protein coupled 5-$HT_4$ receptor (Berthouze et al., 2005) and the ligand-gated 5-$HT_3$ receptor (Hu and Peoples, 2008) display constitutive activity. We proposed that 5-$HT_3$ and 5-$HT_4$ antagonists reduce the constitutive activity of 5-$HT_3$ and 5-$HT_4$ receptors by acting as inverse agonists. This means that these antagonists reduce the constitutive activity of the 5-$HT_3$ and 5-$HT_4$ receptors, rather than blocking the effects of endogenous 5-HT. While direct evidence supporting the role of these antagonists as inverse agonists remains to be tested, such a concept would meet the role of these drugs that have an inhibitory effect in the absence of endogenous 5-HT (the endogenous ligand).

## *Concluding remarks*

The history of evidence regarding the role of EC cell 5-HT release in initiating GI motility has evolved over the past six decades. We have learnt a lot since the early hypotheses proposed in the 1950's. There is no doubt the notion that endogenous 5-HT is a potent initiator of gut motility was an exciting and appealing concept. However findings using recent advancements in molecular and amperometric technologies from different laboratories have failed to support this notion. The most puzzling question that remains unanswered is what is the function of EC cell-derived 5-HT in gut motility under normal, healthy conditions? It is well accepted that EC-cell derived 5-HT plays an important role in inflammatory bowel disease, or other conditions of intestinal inflammation, where serotonergic signaling pathways are clearly upregulated. However under normal, healthy conditions, endogenous 5-HT from the mucosa appears to play a modulatory, but not essential, role in regulating neurogenic motor patterns.

**Declarations of interest**: None.

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**Figure Legends:**

**Figure 1.**
Removal of the mucosa and submucosal plexus does not prevent distension-evoked peristalsis. A, shows an isolated segment of guinea-pig distal colon that has been inverted so that the serosa faces innermost and circular muscle facing outermost. The entire mucosa and submucosal plexus was sharp dissected away from the preparation. B, in mucosa and submucosa-free dissected preparations (shown in A), natural fecal pellets still propagated along the isolated colon. This showed that the presence of the mucosa and in fact the orientation of the gut wall was unimportant for peristalsis to occur. C, spatio-temporal map from the propagation of the fecal pellet in panel B.

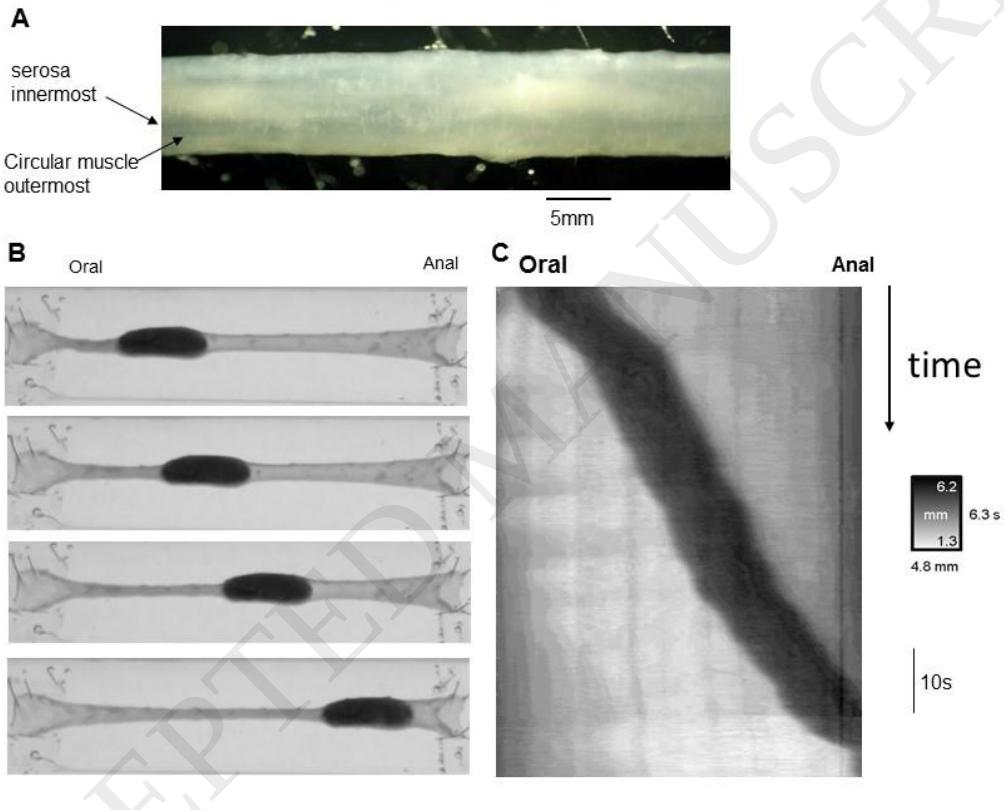


Figure 1

**Figure 2.**
Mucosa-free and submucosal-plexus free colon is able to propel natural fecal pellets. A, preparation of colon lacking mucosa on the innermost surface. B, shows a fecal pellet can propagate along the colon without any 5-HT release.

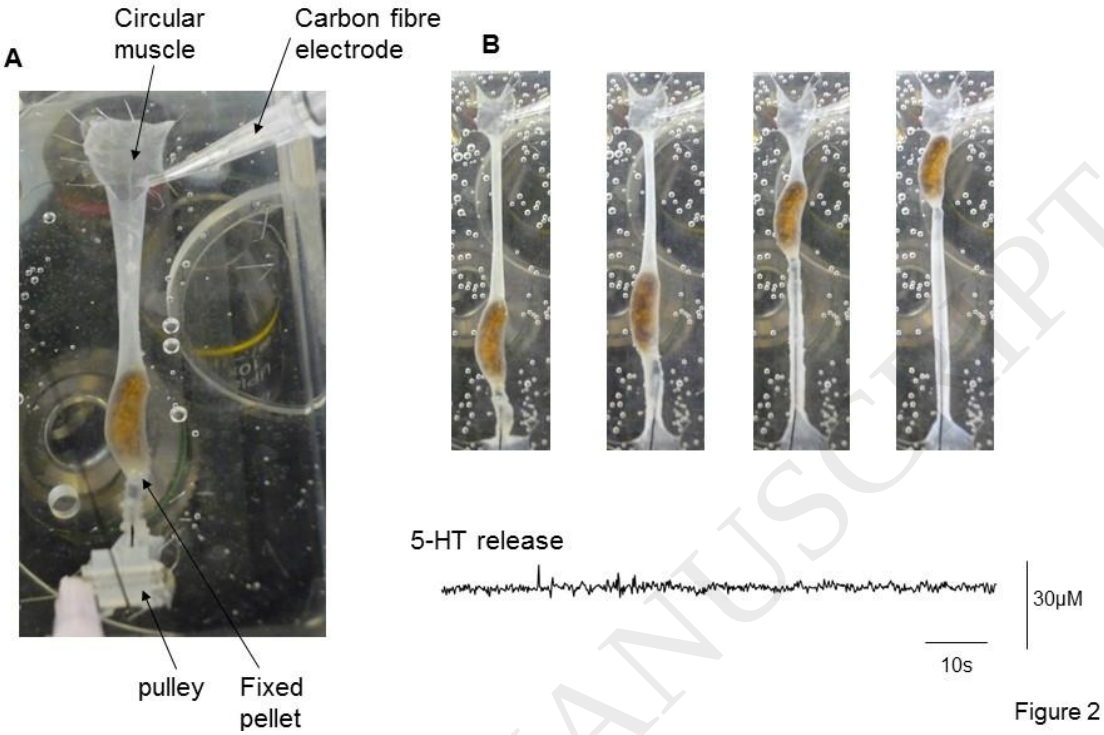


**Figure 3.**
Rhythmic peristaltic contractions evoked by a fixed natural fecal pellet with simultaneous amperometric recording from a mucosa-free isolated distal colon preparation. A, photomicrograph of preparation of colon devoid of mucosa and submucosal plexus. The carbon fibre electrode is positioned above the circular muscle. A cotton thread is attached to the pellet and then to a tension transducer to record circular muscle contractions. B, insertion of a fecal pellet at a fixed location evokes rhythmical peristaltic contractions without any associated release of 5-HT. C, shows in preparations of colon lacking mucosa and submucosal plexus there is no significant difference in the amplitude, half duration and interval.

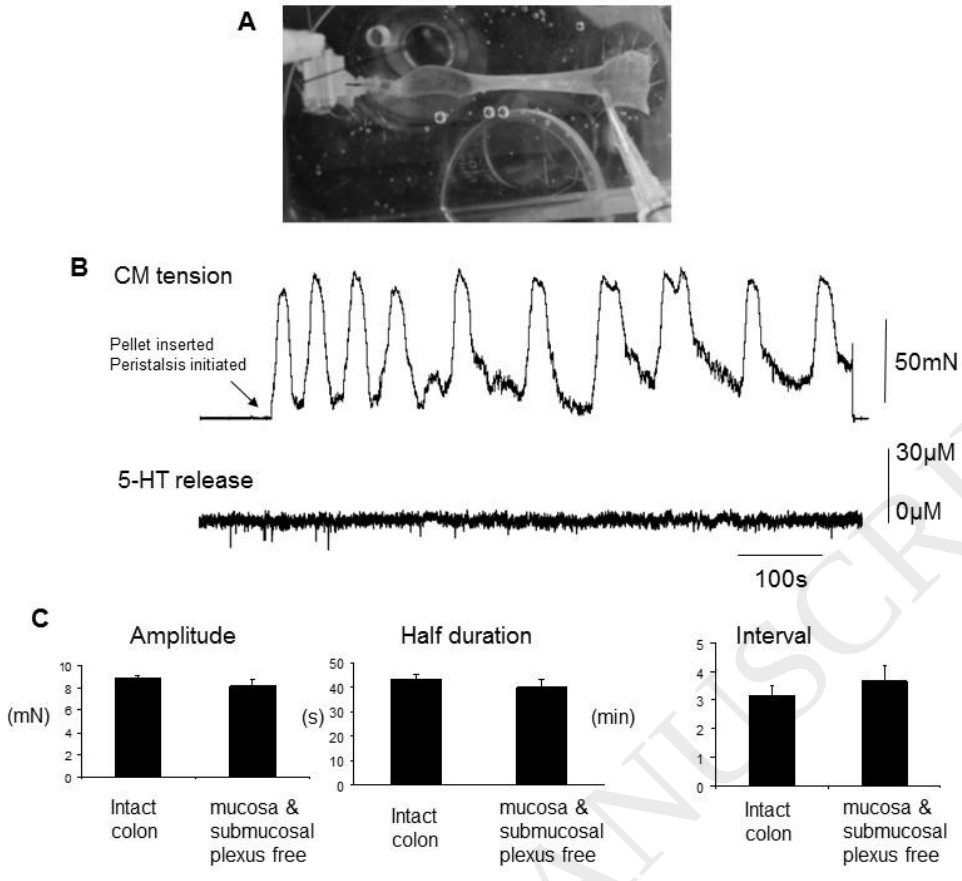


Figure 3