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This is the accepted version of the following article published in *Neurogastroenterology & Motility*, which has been published in final form at at: <http://onlinelibrary.wiley.com/>  
<http://dx.doi.org/10.1111/nmo.12136>

doi: 10.1111/nmo.12136

Please cite this article as:

Spencer, N. J., Nicholas, S. J., Sia, T. C., Staikopoulos, V., Kyloh, M. and Beckett, E. A. (2013), By what mechanism does ondansetron inhibit colonic migrating motor complexes: does it require endogenous serotonin in the gut wall?. *Neurogastroenterology & Motility*, 25: 677–685.

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By what mechanism does ondansetron inhibit colonic migrating motor complexes:  
does it require endogenous serotonin in the gut wall ?

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## Abstract

**Background:** 5-HT<sub>3</sub> antagonists, such as ondansetron (Zofran), retard colonic transit and provide effective relief of symptoms of diarrhea-predominant irritable bowel syndrome (IBS), but the mechanism by which ondansetron retards transit is unclear. What is clear is that the frequency of colonic migrating motor complexes (CMMCs) is reduced by ondansetron, which could account for reduced transit. Our aim was to determine whether acute depletion of 5-HT from enteric neurons would disrupt spontaneous CMMCs; and determine whether the sensitivity of ondansetron to reduce CMMC frequency would change in a 5-HT depleted preparation. **Methods:** Mice were injected with reserpine 24 hours prior to euthanasia to deplete neuronally synthesized 5-HT. Mechanical recordings were made from proximal and mid-distal regions of isolated whole mouse colon. Immunohistochemical staining for 5-HT was used to detect neuronal 5-HT. **Key Results:** Reserpine depleted all detectable 5-HT from enteric nerves. In whole colons, with mucosa and submucosal plexus removed, the frequency and amplitude of spontaneous CMMCs was no different between groups treated with or without reserpine. Surprisingly, in mucosa and submucosal plexus-free preparations, ondansetron was equally, or, significantly more effective at inhibiting CMMC frequency when compared to control preparations (containing 5-HT). Reserpine pretreatment had no effect on the sensitivity of ondansetron to inhibit CMMCs. **Conclusions & Inferences:** Endogenous 5-HT in enteric neurons (or the mucosa) is not required for the spontaneous generation or propagation of CMMCs. Furthermore, the primary mechanism by which ondansetron inhibits CMMC frequency is not mediated via the mucosa, submucosal plexus, or 5-HT in myenteric neurons.

## INTRODUCTION

5-HT<sub>3</sub> antagonists have been prescribed in clinics for effective relief of symptoms in patients with diarrhea-predominant irritable bowel syndrome (D-IBS) and chronic diarrhea, where they reduce colonic transit and contractile activity, leading to increased water absorption (1, 2). Whilst it is clear that the primary effect of these antagonists is to prolong gastrointestinal (GI) transit in humans and laboratory animals, the site and mechanism by which they act in the body to inhibit motility and transit is still largely a matter of speculation.

Colonic migrating motor complexes (CMMCs) are one of the major types of motor activity that exist in the large bowel of mammals and are thought to provide a major propulsive force underlying transit of content (3, 4). CMMCs have been recorded from a variety of mammals, but increasing interest is focusing on the isolated mouse colon because CMMCs can be preserved and studied *in vitro* (5-12) and their underlying myoelectric correlates (5, 13-15). There have been major advances in the past 2 years regarding the role of endogenous 5-HT in the generation CMMCs; see: (16, 17) for perspectives. Early studies hypothesized that 5-HT release from the mucosa was critical for spontaneous CMMC generation in the mouse colon (18), since it was thought that removal of the mucosa abolished spontaneous CMMCs. However, when these studies were repeated; and by using direct recordings of 5-HT release, it was found that careful removal of the mucosa abolished all release of 5-HT, but actually did not prevent the generation of spontaneous CMMCs (19). These observations are consistent with recent findings from other laboratories (20, 21) where genetic or pharmacological ablation of 5-HT synthesis from the mucosa has no effect on intestinal or colonic transit times, nor gastric emptying in live mice (20). Whilst it is clear that mucosal 5-HT is not required for spontaneous (19) or evoked (22) CMMCs, the role of neuronal 5-HT in CMMC generation is poorly understood.

A major step forward came about when ondansetron was applied to mucosa-free preparations of mouse colon (19). It was found that despite the absence of the mucosa and submucosal plexus,

ondansetron was still found to potently reduce CMMC frequency (19). This suggested that the mechanisms by which ondansetron slows CMMC frequency was independent of the mucosa. Interestingly, a recent study showed that GI-transit was significantly slower when neuronal 5-HT was genetically ablated by targeting the enzyme tryptophan hydroxylase 1 (TPH1) (21). This suggested that neuronal 5-HT may play an important role in control of GI-transit. However, genetic deletion of the TPH1 enzyme also lead to major developmental and neurochemical changes in enteric nerves (21), which could also reduce transit. This prompted us to investigate the effects of acutely depleted 5-HT in enteric neurons, without the confounding effects induced by genetically deletion of the TPH1 gene. Our aim was to determine if depletion of neuronal 5-HT impaired CMMCs and if not, does depletion of 5-HT affect the sensitivity of ondansetron to reduce CMMC frequency.

## **METHODS**

### ***Preparation of tissues***

Adult mice (C57BL/6) between 30–120 days of age were euthanized by inhalation overdose using isoflurane followed by exsanguination, in a manner approved by the Animal Welfare Committee of Flinders University. The entire colon was removed and placed in room temperature Krebs solution which was constantly bubbled with carbogen gas (95% O<sub>2</sub>/ 5% CO<sub>2</sub>). The entire was incised along the longitudinal axis (mesenteric border) and two stainless steel hooks (see Fig.1) used to pierce one of the circumferential edges of the colon, so that mechanical recordings could be made from the circular muscle layer. These preparations contained intact mucosa and submucosal plexus (Fig.1A). The second type of preparation used had the entire mucosa and submucosal plexus removed (Fig.1C), as described previously (19).

### ***Mechanical recordings from the circular muscle during spontaneous CMMCs***

We recorded the force generated by the circular muscle layer during each spontaneous CMMC, using an isometric recording transducer (Grass (FT-03C; Grass, Quincy, M.A., U.S.A) connected via fine suture thread to hooks that pierced the muscle wall (Fig.1A). Mechanical recordings were made under isometric conditions, using force transducers that were connected to two custom made

preamplifiers (Biomedical engineering, Flinders University) and then to a Powerlab (model: 4/30; AD Instruments, Bella Vista, N.S.W, Australia). Labchart version 6.0 (AD Instruments, Australia) was used for analysis of data.

### ***Technique to deplete endogenous 5-HT from the enteric nervous system***

The enteric nervous system was depleted of endogenous 5-HT using the technique first demonstrated by (23). This involves a single intraperitoneal injection of reserpine (at a concentration of 5mg/Kg) between 18-24 hours prior to euthanasia. After this period, there is no 5-HT immunoreactivity remaining in the enteric nervous system. Because reserpine does not deplete 5-HT from the mucosa, we then employed our recently published method whereby of removing the mucosa, submucosa and submucosal plexus by sharp dissection from the colon (24). This allows us to test whether acute depletion of 5-HT from enteric nerves and the absence of the mucosa and submucosal plexus impaired colonic motility.

### **Immunohistochemistry**

Isolated segments of guinea-pig distal colon were fixed by pinning sheet preparations of colon under constant tension in a Sylgard lined Petri dish (Dow Corning Corp., Midland, MI, USA) and immersing overnight in Zamboni's fixative (5% Formaldehyde and 15% saturated picric acid in 0.1M phosphate buffer; pH 7.2) at 4°C. Preparations were then cleared in dimethyl sulfoxide (10 min immersion, repeated three times), tissue was washed in phosphate buffered saline (PBS); (0.2M sodium phosphate buffer, pH 7.2) and a whole mount of the myenteric plexus and longitudinal muscle was prepared by removing the mucosa, submucosa and circular muscle with the aid of a dissecting microscope. Goat 5-HT antisera (Immunostar, Cat: 20079) was applied at 1:1500 overnight at room temperature then washed 3 x 10 mins in PBS. Tissue was then incubated for a further 2 hours in secondary antisera (Donkey anti Goat CY3; Jackson Immunoresearch Laboratories Inc) at 1:400 then washed 3 x 10 mins in PBS and mounted in bicarbonate- buffered glycerol (pH 8.6).

### ***Measurements and Statistics***

Measurements of the peak amplitude and interval between each spontaneous CMMC were measured from isometric mechanical recordings, as was the interval between each CMMC contraction. Data in the results section are presented as means  $\pm$  S.E.M. The use of “n” in the results section refers to the number of animals on which observations were made. Data sets were considered statistically significant if P values  $< 0.05$  were reached. Student’s unpaired t-tests and one way ANOVA were used to comparison of data.

### ***Drugs and Solutions***

The Krebs solution used contained (in mM): NaCl, 118; KCl, 4.7; NaHPO<sub>4</sub>.2H<sub>2</sub>O, 1.0; NaHCO<sub>3</sub>, 25; MgCl<sub>2</sub>.6H<sub>2</sub>O, 1.2; D- Glucose, 11; CaCl<sub>2</sub>.2H<sub>2</sub>O, 2.5. Ondansetron hydrochloride, tetrodotoxin and reserpine were obtained from Sigma Chemical Co. St. Louis. Mo. U.S.A.

## **RESULTS**

### ***General observations. – effects of removal of the mucosa and submucosal plexus***

Spontaneous CMMCs were recorded from isolated sheet preparations of whole mouse colon, as previously described (19, 22). CMMCs occurred with a mean interval between contractions of  $170.9 \pm 26.2$  sec; with a mean amplitude of  $4.2 \pm 1.1$  mN in the proximal colon,  $1.5 \pm 0.3$  mN in the mid-distal colon (Fig. 2; N=10). 5-HT immunoreactive nerve fibres were always detected in freshly-fixed whole colons of control mice (Fig.2Ai & Aii). Removal of the mucosa and submucosal plexus (Fig.2) did not prevent the spontaneous generation of CMMCs, but significantly reduced their frequency to a mean interval of  $138.2 \pm 9.2$  sec (Fig. 3; P<0.05; N=10), consistent with previous studies (19, 22). The amplitude of CMMCs in the colon were also significantly reduced to  $2.1 \pm 0.3$ g in the proximal and mid-distal regions of colon  $0.5 \pm 0.1$ g (P<0.05; N=10).

### ***Effects of acute depletion of endogenous 5-HT from the myenteric plexus and removal of the mucosa and submucosal plexus on CMMC activity***

Since removal of the mucosa and submucosal plexus does not prevent the spontaneous generation of CMMCs in mouse colon (19), we were particularly interested in whether acute depletion of neuronal 5-HT would abolish CMMCs, or alter their characteristics. To test this, we injected control mice with a subcutaneous injection of reserpine (5mg/Kg) 24 hours prior to euthanasia (23). 24 hours after reserpine injection mice were euthanized and the whole colon removed, fixed and stained for 5-HT. In preparations treated with reserpine, no detectable 5-HT was ever found in the myenteric plexus, consistent with previous reports (23) (Fig.3Ai & Aii). Interestingly, in preparations that had their mucosa and submucosal plexus removed, we found no differences in CMMCs in animals that had been treated with reserpine, or not treated. The mean interval between CMMCs in reserpine-treated mucosa-free preparations was  $143.0 \pm 2.1$  (N=5) and this was not significantly different from CMMCs that occurred in mucosa-free colonic preparations that were not treated with reserpine ( $138.2 \pm 9.2$ sec; N=5). The amplitude of CMMCs in the proximal colon of reserpine-treated animals, with mucosa and submucosal plexus removed was  $2.9 \pm 0.8$ mN,  $0.6 \pm 0.1$ mN in the mid colon and  $0.5 \pm 0.1$ mN in the distal colon (N=10). These characteristics were no different from those recorded in preparations that had not been injected with reserpine ( $P > 0.05$ ; N=10).

### ***Effects of ondansetron on CMMCs in control mouse colon***

Antagonists of 5-HT<sub>3</sub> receptors have been shown by a number of laboratories to reduce the frequency of spontaneous CMMCs in mouse colon (19, 25) and migrating complexes in the small intestine (26, 27). Recently, we showed that ondansetron still potently inhibited CMMCs in mice that had their mucosa and submucosal plexus removed (19), suggesting that 5-HT<sub>3</sub> receptor antagonists inhibit CMMCs by mechanisms that are independent of mucosa or release of 5-HT from the mucosa. Since experiments above revealed that CMMCs were still recorded in the colon devoid of all 5-HT in the enteric nerves and in the absence of the mucosa, we were particularly interested in whether ondansetron would still be effective in inhibiting CMMCs in these preparations. Interestingly, It was therefore of particular interest to us to determine whether ondansetron would still inhibit spontaneous

CMMCs, in mice that had been injected with reserpine to deplete neuronal 5-HT; and which also had their mucosa and submucosal plexus removed. To do this, we euthanized mice 24 hours after injection of reserpine, then removed their mucosa and submucosal plexus. Immunohistochemical staining for 5-HT confirmed the absence of 5-HT in all reserpine-treated mice (Fig.3) and H&E staining confirmed the absence of the mucosa and submucosal ganglia (Fig.1). To test the effects of ondansetron, we applied consecutive incremental concentrations of the antagonist from  $10^{-9}$ M to  $10^{-5}$ M, where each concentration was perfused for 45 minutes to the colon. In control preparations of colon, it was found that ondansetron significantly reduced the frequency of CMMCs, consistent with previous studies (N=5;  $P<0.05$ ). CMMCs in control animals were always abolished at  $3\mu$ M (N=8).

Interestingly, in preparations of whole colon lacking mucosa and submucosal plexus, ondansetron caused a significantly greater inhibitory effect on CMMC frequency at 100nM and 500nM compared to control preparations (that contained 5-HT in enteric nerves and had mucosa and submucosal plexus intact) (Fig. 3 & 4; N=5). We then compared reserpine-treated animals that also had their mucosa and submucosal plexus removed, with colonic preparations that had not been injected with reserpine, but had their mucosa and submucosal plexus removed. It was found there was no difference between the concentrations of ondansetron ( $10^{-9}$ M to  $10^{-5}$ M) required to inhibit CMMC frequencies in preparations had their mucosa and submucosal plexus and had been treated with reserpine or not (Fig. 3;  $P>0.05$ ; N=6). A concentration response curve of increasing concentrations of ondansetron was plotted against percentage inhibition of CMMC frequency. In control animals, the EC<sub>50</sub> concentration of ondansetron to cause 50% reduction in CMMC frequency was  $6.4 \times 10^{-7}$ M (Fig. 4). In mucosa and submucosal plexus-free preparations the EC<sub>50</sub> was actually lower at  $3.8 \times 10^{-8}$ M and in reserpine-treated mucosa was  $4.2 \times 10^{-8}$ M (Fig.4).

#### ***Tests for non-specific effects of ondansetron on mouse colonic circular muscle***

To determine whether the inhibitory effects of ondansetron on CMMCs were mediated via enteric nerves, or other non-neuronal cell types; such as smooth muscle cells, we investigated whether ondansetron could mediate any effects on circular muscle activity in the presence of TTX. To test this,

we applied TTX (1 $\mu$ M) to the mouse colon and recorded changes in spontaneous myogenic contractions over a three minute recording period. Myogenic circular muscle contractions in the colon occurred at a mean frequency of 5.8  $\pm$  1.2/3 minutes in control; 6.1  $\pm$  0.6/3 minutes in TTX; and 6.5  $\pm$  0.4/3 minutes in ondansetron. No differences were detected ( $P > 0.05$ ; One way ANOVA; N=6). Since no effects were seen at 3 $\mu$ M ondansetron, and CMMCs were always blocked at 3 $\mu$ M, there was no need to test for non-specific effects at lower concentrations. The fact that ondansetron had no effect on the colon (in TTX) at 3 $\mu$ M suggests that ondansetron acted on enteric neurons to mediate its inhibitory effects.

## **DISCUSSION**

There are two major findings of the current study. Firstly, acute depletion of neuronal 5-HT from the myenteric plexus, together with removal of the mucosa and submucosal plexus, does not prevent the generation of spontaneous CMMCs in isolated whole mouse colon. Secondly, in preparations of mouse colon that had their mucosa and submucosal plexus removed, ondansetron was found to reduce the frequency of spontaneous CMMCs as effectively, or, significantly more effectively ondansetron (at 100nM and 500nM) when compared to control animals that contained normal levels of 5-HT. Interestingly, in preparations of whole colon with mucosa and submucosal plexus removed, there was no difference in the effectiveness of ondansetron in inhibiting CMMCs in preparations that had been treated or not with reserpine. These data lead to the fundamental conclusion that the major mode of action of ondansetron to inhibit the CMMC frequency is not via the mucosa, submucosal plexus, or 5-HT containing myenteric neurons. These findings have ramifications for the development of therapeutic agents that modulate serotonergic transmission in the GI-tract.

### ***Previous hypotheses regarding the role of 5-HT in the generation of CMMCs***

It was once thought that the mucosa or release of 5-HT from the mucosa was important for spontaneous CMMC generation, since it was shown that antagonists of 5-HT<sub>3</sub> receptors abolished CMMCs and removal of the mucosa was suggested to abolish CMMCs (18). However, more recently

other studies, using real time amperometry, have demonstrated that removal of the mucosa abolished all detectable 5-HT release, but did not prevent spontaneous CMMCs (19); an observation verified by other investigators (Dr. H. Sjövall, University of Gothenburg, Personal communication). Furthermore, perhaps more interestingly, in our previous study, when the mucosa and submucosal plexus were removed from the colon, ondansetron was still found to potently inhibit CMMCs (19). This showed that in mucosa-free preparations, the mechanism by which 5-HT<sub>3</sub> antagonists inhibited CMMCs must have involved processes independent of the mucosa, or 5-HT release from the mucosa (19). Since mucosa-free preparations still contained 5-HT in enteric neurons, one possibility was that ondansetron acted on enteric neurons to suppress the frequency of CMMCs. The current study tested this notion, by depleting 5-HT acutely from enteric neurons using reserpine. The major finding of our study is that acute depletion of endogenous 5-HT does not prevent spontaneous CMMC generation or propagation; and that 5-HT<sub>3</sub> receptor blockade still inhibits the frequency of CMMCs even after 5-HT is depleted from enteric neurons and the entire mucosa is removed. These conclusions lead us to the conclusion that activation of 5-HT<sub>3</sub> receptors by endogenous 5-HT is not a prerequisite for the spontaneous generation of CMMCs.

#### ***How do 5-HT<sub>3</sub> antagonists inhibit CMMC generation in 5-HT depleted segments of colon ?***

Since ondansetron potently inhibits CMMCs, it has been assumed that activation of 5-HT<sub>3</sub> receptors by endogenous 5-HT plays a major role in CMMC generation. We rationalized that if endogenous 5-HT does indeed play a major role in CMMC generation, then removal of endogenous 5-HT would cause similar disruptions to CMMCs, as those caused by 5-HT antagonists. Clearly, this did not occur. In fact, depletion of neuronal 5-HT with reserpine had no effect on CMMC frequency or amplitude in mucosa-free preparations (Fig. 4). Furthermore, if ondansetron acted to inhibit CMMCs by blocking the action of 5-HT on 5-HT<sub>3</sub> receptors, then one would expect ondansetron would be ineffective in causing the same inhibition of CMMCs in colonic preparations devoid of endogenous 5-HT. Again, this did not happen. A major finding of this study was that ondansetron was equally, or, more effective in inhibiting and blocking CMMCs in preparations that had their mucosa and submucosal plexus removed and were depleted of neuronal 5-HT. This raises the fundamental

question as to how and where do 5-HT<sub>3</sub> antagonists act to inhibit or block CMMCs in colonic preparations depleted of 5-HT ? The most likely answer rides in the knowledge that both the 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors can be constitutively active. This means that the ion channels activated by these receptors can open in the absence of any endogenous ligand (i.e. 5-HT). There is abundant evidence that the ligand-gated 5-HT<sub>3</sub> receptor displays constitutive activity (28). This would mean that blocking 5-HT<sub>3</sub> receptor, in the absence of endogenous 5-HT could close these channels in enteric neurons. We hypothesize that blocking these constitutively active receptors could change the background excitability of enteric neurons involved in the intrinsic neural circuitry that underlies CMMC generation. It is possible, but seems unlikely that ondansetron exerted non-specific effects on the mouse colon to inhibit CMMCs, since ondansetron is highly selective for the 5-HT<sub>3</sub> receptor; and the inhibitory effects we detected were observed in the nanomolar to low micromolar range, which is consistent with the  $K_i$  and  $EC_{50}$  for ondansetron. Also, the fact that in the presence of TTX, ondansetron at high concentrations (3 $\mu$ M) had no effect on myogenic contractility suggests that ondansetron acted on enteric neurons to mediate inhibitory effects on CMMC frequency.

***Is there a functional role for endogenous serotonin in motility patterns of the large intestine ?***

It is well accepted that the majority of serotonin (>95%) in the body is synthesized within the gut wall. However, identifying the functional role of this concentration of endogenous serotonin in the generation of different colonic motor patterns has been difficult to ascertain. We rationalized that if endogenous 5-HT plays a major role in the generation of colonic motor patterns, such as peristalsis or CMMCs, then one would presume that removal or depletion of endogenous 5-HT would have major consequences to these motor patterns. At present, no data exists to support this presumption. On the contrary, substantial evidence has recently been presented that removal of mucosally derived 5-HT has minor, or no effects on peristalsis (24) and CMMCs (19). Furthermore, genetic or pharmacological depletion of mucosal derived 5-HT has no effect on motility in vivo (20). At present, we are unable to provide a clear answer on what might be the functional role of endogenous serotonin is on colonic motility. Our existing conclusions (26) that 5-HT<sub>3</sub> receptor activation is important for spontaneous CMMC generation is incorrect. Instead, the findings of the current study

clearly show ondansetron is no less effective at inhibiting CMMCs, in mucosa-free preparations that also have neuronal 5-HT depleted. In terms of a functional role for 5-HT, whilst it is not important in naïve bowel, it is entirely possible that during inflammatory bowel disease states an upregulation of various serotonergic signaling pathways may underlie or contribute to changes in gastrointestinal motility.

## CONCLUSIONS

The findings of this study show that acute depletion of 5-HT from enteric neurons and the removal of the mucosa and submucosal plexus does not prevent the cyclical generation of spontaneous CMMCs in isolated mouse whole colon. Interestingly, the effectiveness of ondansetron at inhibiting the frequency of CMMCs is unaffected by acute depletion of 5-HT in the enteric nervous system. In fact, we show that ondansetron (at 100nM and 500nM) was actually significantly more effective at inhibiting CMMC frequency compared to control preparations containing 5-HT. Our findings show that the primary mechanism by which ondansetron reduces CMMC frequency is mediated independently of endogenous 5-HT in the mucosa, or the submucosal and myenteric plexuses. These findings have major implications for future development of therapeutic agents designed to modulate the actions of 5-HT<sub>3</sub> antagonists in the large bowel.

## Acknowledgments

We would like to acknowledge the National Health & Medical Research Council of Australia (NH&MRC) for funding provided to NJS (#10257566).

## FIGURE LEGENDS

**Figure 1.** Diagrammatic representation of the two types of preparations used for mechanical recordings of spontaneous CMMC activity in isolated whole mouse colon. Recordings were made simultaneously from proximal and mid-distal colon using a claw to penetrate the muscle layers. B, H&E staining shows full thickness mouse colon. C, Preparations of colon were studied that had

mucosa and submucosal plexus removed from the whole length colon. D, H&E staining confirmed the absence of mucosa and submucosal ganglia.

**Figure 2.** Effects of ondansetron on colonic migrating motor complexes recorded from isolated whole mouse colon. Ai & Aii, shows immunohistochemical staining for 5-HT in the myenteric plexus. 5-HT was present in small proportion of axons in myenteric ganglia and internodal strands. B, shows effects of ondansetron at incremental increases in concentration from  $10^{-9}$ M to  $10^{-5}$ M on spontaneous CMMCs recorded from intact whole colon (containing mucosa). Each concentration of ondansetron was applied for 30 minute increments. CMMCs were abolished at  $10^{-6}$ M in this preparation.

**Figure 3.** shows immunohistochemical staining for 5-HT in a preparation of colon that was removed from a reserpine-treated mouse. The mucosa and submucosal plexus were sharp dissected away. 5-HT was not detected in any axons in myenteric ganglia or internodal strands. B, shows effects of ondansetron at incremental increases in concentration from  $10^{-9}$ M to  $10^{-5}$ M on spontaneous CMMCs recorded from intact whole colon with entire mucosal layer and submucosal plexus removed. Note, spontaneous CMMCs occur in reserpine-treated preparations devoid of mucosa and submucosal plexus. Each concentration of ondansetron was applied for 30 minute increments and showed a progressive reduction in the frequency of occurrence of CMMCs. CMMCs were abolished at a lower concentration than in control preparations containing 5-HT.

#### **Figure 4**

Concentration response curve showing the percentage inhibition of CMMCs in control preparations (containing mucosa and non-reserpinized) compared with mucosa-free preparations (non-reserpinized) and mucosa-free preparations that were also reserpinized. The data shows that at 100nM and 500nM there was a significantly greater decrease in frequency of spontaneous CMMCs in mucosa-free preparations and mucosa-free (reserpinized) preparations compared to control preparations. There was, however, no difference between the sensitivity of ondansetron in mucosa-

free preparations and mucosa-free (reserpinized) preparations, suggesting that acute depletion of neuronal 5-HT has no effect on the ability of ondansetron to reduce CMMC frequency.

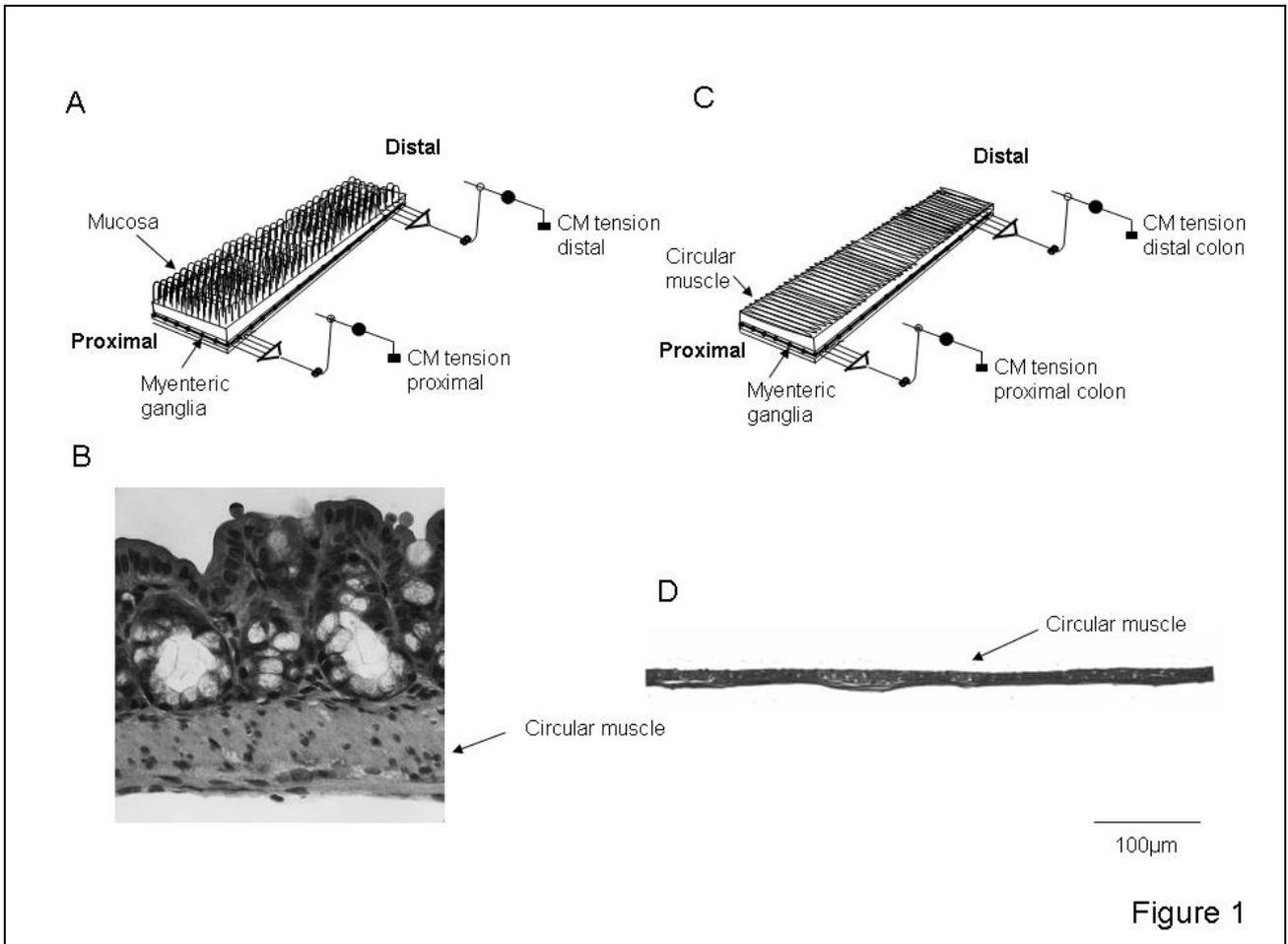
### Figure 5

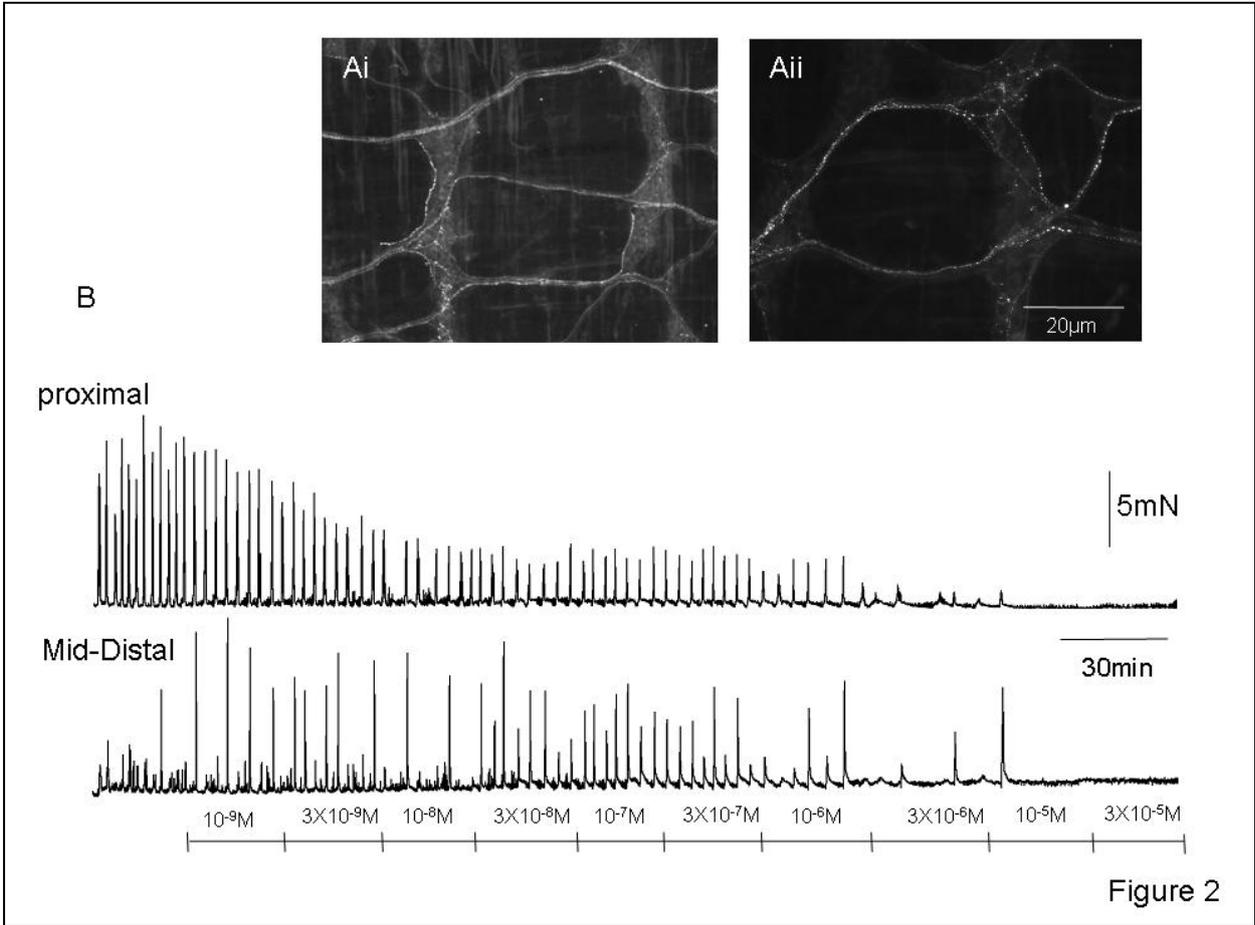
Effects of ondansetron on the amplitudes of CMMCs recorded from control preparations, mucosa-free preparations and mucosa-free (reserpinized) preparations. Overall, ondansetron reduced the mean amplitude of CMMCs in the proximal colon of all three types of preparations. Interestingly, ondansetron had no effect on the amplitude of CMMCs recorded from either of the three preparations in the mid-distal region of colon.

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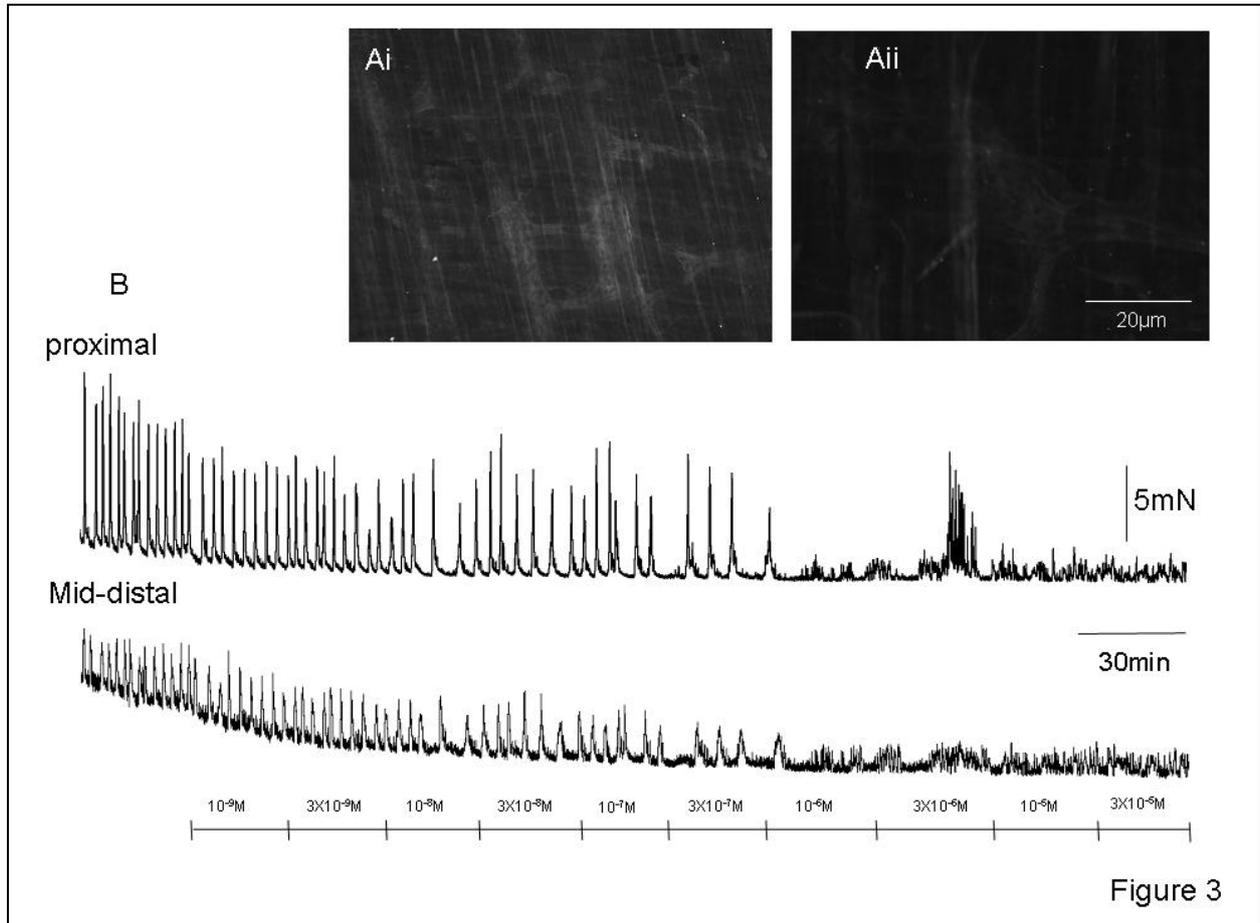
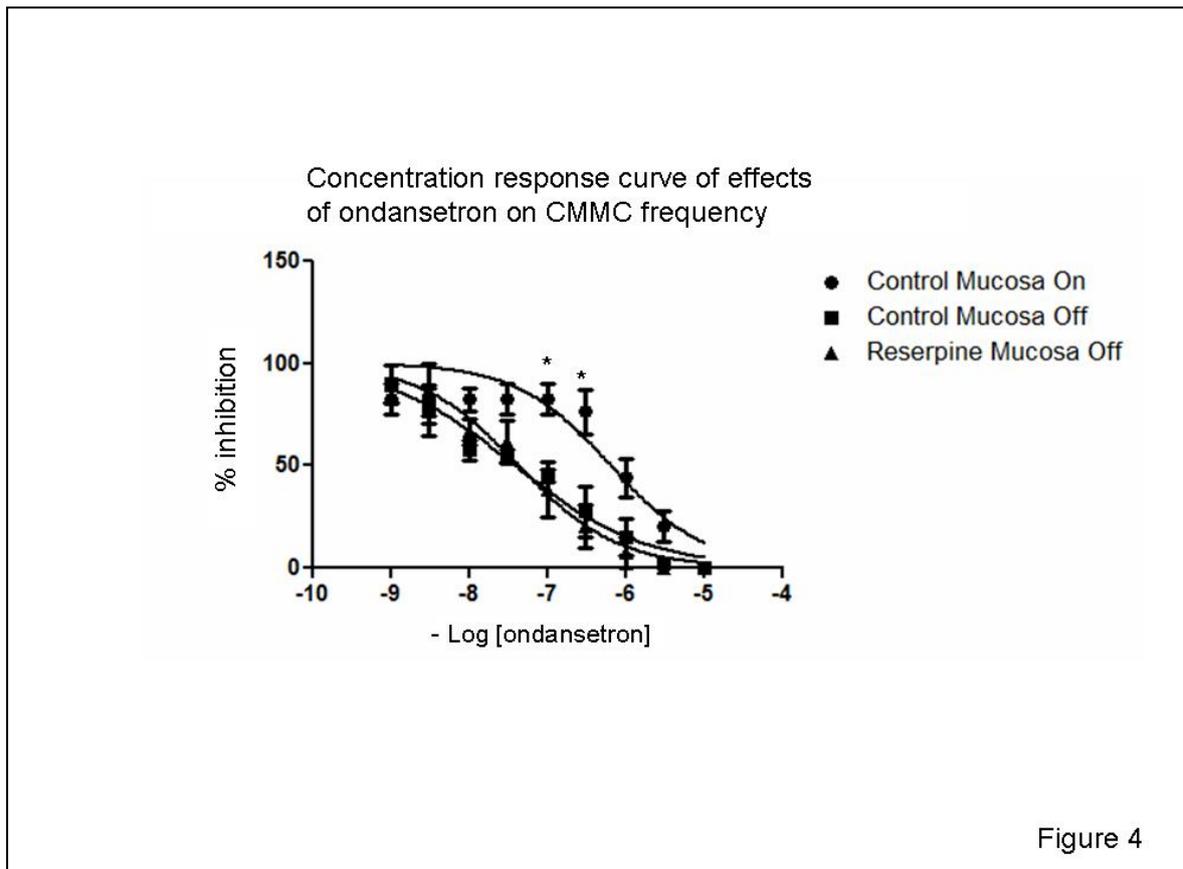


Figure 3



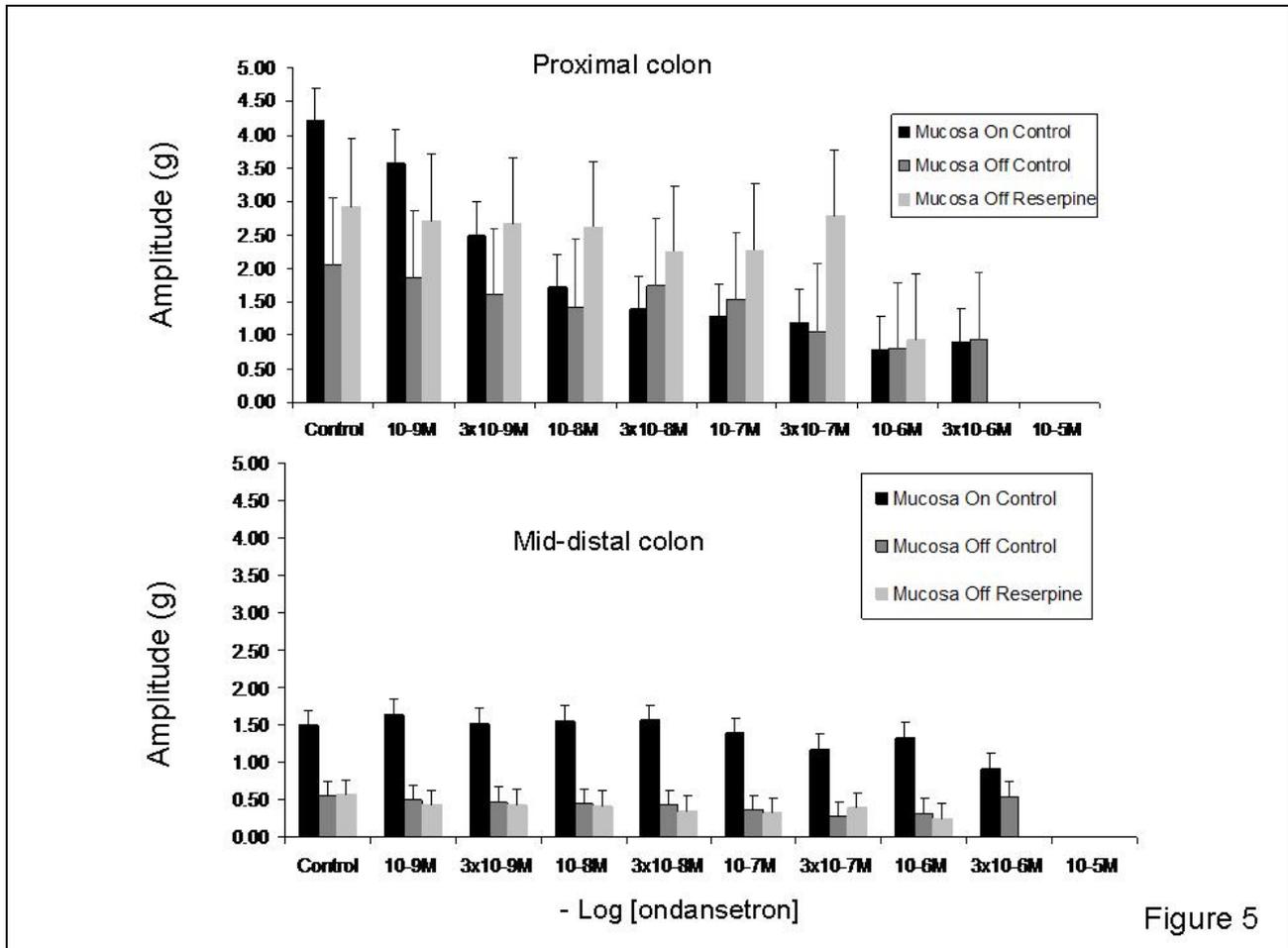


Figure 5