*Neurogastroenterol Motil* (2007) **19**, 990–1000 doi: 10.1111/j.1365-2982.2007.00986.x

# Changes in colonic motility and the electrophysiological properties of myenteric neurons persist following recovery from trinitrobenzene sulfonic acid colitis in the guinea pig

E. M. KRAUTER,* D. S. STRONG,* E. M. BROOKS,* D. R. LINDEN,*,[1] K. A. SHARKEY*,† & G. M. MAWE*,†

*Department of Anatomy and Neurobiology, The University of Vermont College of Medicine, Burlington, VT, USA
†Department of Physiology and Biophysics, University of Calgary, Calgary, AB, Canada

***Abstract*** *Persistent changes in gastrointestinal motility frequently accompany the resolution of colitis, through mechanisms that remain to be determined. Trinitrobenzene sulfonic acid (TNBS) colitis in the guinea pig decreases the rate of propulsive motility, causes hyperexcitability of AH neurons, and induces synaptic facilitation. The changes in motility and AH neurons are sensitive to cyclooxygenase-2 (COX-2) inhibition. The aim of this investigation was to determine if the motility and neurophysiological changes persist following recovery from colitis. Evaluations of inflammation, colonic motility and intracellular electrophysiology of myenteric neurons 8 weeks after TNBS administration were performed and compared to matched control conditions. Myeloperoxidase levels in the colons were comparable to control levels 56 days after TNBS treatment. At this time point, the rate of colonic motility was decreased relative to controls following treatment with TNBS alone or TNBS plus a COX-2 inhibitor. Furthermore, the electrical properties of AH neurons and fast synaptic potentials in S neurons were significantly different from controls and comparable to those detected during active inflammation. Collectively, these data suggest that altered myenteric neurophysiology initiated during active colitis persists long term, and provide a potential mechanism underlying altered gut function in individuals during remission from inflammatory bowel disease.*

*Address for correspondence*
Dr Gary M. Mawe, Department of Anatomy and Neurobiology, Given D403A, University of Vermont, Burlington, VT 05405, USA.
Tel: +1 802 656 8257; fax: +1 802 656 8704;
e-mail: gary.mawe@uvm.edu
*Received*: 18 April 2007
*Accepted for publication*: 25 June 2007

[1]Present address: Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA.

***Keywords*** *AH neurons, enteric nervous system, inflammatory bowel diseases, intestinal motility, postinflammatory, S neurons.*

## INTRODUCTION

Inflammation of the bowel wall can lead to marked alterations in gut function whether the inflammation is chronic, such as that associated with the inflammatory bowel diseases (IBD) ulcerative colitis or Crohn's disease, or infection from acute bacterial enteritis.[1,2] Diarrhoea and/or constipation are major symptoms of these pathologies, which are indicative of changes in intestinal motility. Frequently, such symptoms can persist after the resolution from the infection or inflammation, or during remission from IBD even when no inflammation is detected from mucosal biopsies.[3,4] Patients with these persistent symptoms of gut dysfunction after an initial bout of infection or inflammation are often diagnosed to have postinfectious/inflammatory irritable bowel syndrome (PI-IBS). The underlying mechanisms for these changes have yet to be completely understood.

Previous studies in a mouse model of PI-IBS have implicated the enteric nervous system (ENS) and/or interstitial cells of Cajal (ICC) as the sites likely responsible for the changes involving dysmotility.[5] Long-term changes to the sensitivity of the extrinsic innervation have also been reported[5,6] and these could also contribute to dysmotility through changes in the reflex sensitivity. We have focused on the ENS in the

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

studies of intestinal inflammation and shown that there are marked alterations in the properties of myenteric and submucosal neurons during inflammation.[7,8] We have also shown persistent changes in the properties of submucosal neurons underlying an altered regulation of secretomotor function.[9] To date, the properties of myenteric neurons have not been assessed after the resolution of inflammation.

Understanding the neurobiology of the ENS has assisted with differentiating between populations of neurons and how altering the properties of these neurons effect motility. Two classes of neurons have been described in the myenteric plexus based on electrophysiological properties. One class is the S neurons, which primarily serve as interneurons and motoneurons, and also includes mechanosensory neurons,[10,11] have a narrow action potential (AP) with a very short after hyperpolarization (AHP) and always receive fast excitatory postsynaptic potentials (fEPSPs). The other class is the AH neurons, which serve as intrinsic sensory neurons and interneurons,[12–15] have a broad AP with a shoulder on the repolarization phase, a long AHP (1–20 s in duration), and almost always receive slow, but not fast, excitatory postsynaptic potentials. During active inflammation properties of these neurons are altered. AH neurons are hyperexcitable and the amplitudes of fEPSPs in S neurons are facilitated,[7,8,16] which are correlated with altered gut function in the guinea pig.[17,18]

The purpose of this study was to determine if the changes observed during active inflammation of the distal colon to intestinal motility and myenteric neurons persist after the resolution from the inflammation. Given that the myenteric plexus is directly involved in generating and coordinating motor activity of the gut, we hypothesized that changes in the properties of myenteric neurons, and associated motility, caused by inflammation from trinitobenzene sulfonic acid (TNBS) persist after the resolution of inflammation.

## METHODS

The University of Vermont Animal Care and Use Committee approved all methods used in this study. Experiments were performed on Hartley guinea pigs (Charles River, Montreal, QC, Canada) of either sex housed in cages with soft bedding. The animals had access to food and water *ad libitum* and were maintained at 23–24 °C on a 12 : 12 h light cycle.

Inflammation was generated in guinea pigs weighing 250–350 g under anaesthesia with isoflurane (induced at 4%, maintained at 1.5% in oxygen) by 0.3 mL of TNBS (25 mg mL$^{-1}$; Fluka, Buchs, Switzerland) in 30% ethanol delivered into the lumen of the colon through a polyethylene catheter inserted rectally 7 cm proximal to the anus. Animals were maintained in a controlled environment for 6 (colitis), 14 (14-day animals), 28 (28-day animals), or 56 (56-day animals) days after TNBS administration. Guinea pigs that lost weight within the first 24–48 h were included in the present study, while animals that did not experience weight loss were assumed to not have undergone acute inflammation, and were excluded from the study. Control animals remained naïve and were matched by weight to the 56-day animals. This control is appropriate as no changes in neuronal properties were detected between saline injected and naïve animals 6 days after TNBS administration.[7] Previously, we have reported that inhibition of cyclooxygenase-2 (COX-2) reverted properties of intestinal motility to normal.[18] In this study, a COX-2 inhibitor, DFU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone, 5 mg kg$^{-1}$; gift from Merck-Frosst, Kirkland, QC, Canada] was subcutaneously administered to the 56-day animals for 4 days prior to euthanasia, and are referred as 56 days/DFU.

At the time of tissue collection, animals were weighed, anaesthetized with isoflurane, and exsanguinated. Macroscopic damage scoring was conducted by inspecting the colonic tissue to determine if any inflammation was still present at this time point.[17] Macroscopic scoring was based on wall thickness and the presence and extent of adhesions, ulceration, hyperaemia and the presence or absence of diarrhoea.

### Gastrointestinal motility assay

The distal colons of control, 56-day and 56-day/DFU animals, identified as the part of the colon between the hypogastric flexure and the pelvic brim, was removed and placed in iced cold Kreb's (mmol L$^{-1}$: NaCl, 121; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; $NaH_2PO_4$, 1.2; and glucose 8; aerated with 95%$O_2$/5%$CO_2$; Sigma, St Louis, MO, USA). A 7 cm segment of distal colon was pinned on either end in a 50 mL organ bath lined with Sylgard and continuously perfused with prewarmed oxygenated Kreb's at 10 mL min$^{-1}$ so that the temperature in the bath was brought up to 37 °C. Gastrointestinal motility was monitored using the Gastrointestinal Motility Monitoring system (GIMM; Med-Associates Inc., Saint Albans, VT, USA). Briefly, the distal colon was illuminated from beneath and a digital video camera was used to film the fecal pellet being propelled in the anal direction. The digital images were transferred to a PC and analysed at a later time using software designed for

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

the GIMM. Five individual runs from the number of animals specified from each group were filmed with 5 min allowed to elapse between runs. The rate of motility of fecal pellets was analysed by monitoring the time it took for a fecal pellet to traverse 5 cm of the colon. The rates of propulsion were compared between all groups.

Spatiotemporal maps were constructed from the digital videos that were acquired from individual runs. Briefly, changes in colonic diameter were plotted over time (vertical-axis). The image of the colon in each video frame was converted to a silhouette and the diameter along the entire length was calculated and converted into a grey-scale. The smallest diameter, or fully contracted, is white, and the largest diameter, or dilated, is black. Each frame of video produced a single row of pixels corresponding to the diameter of each segment along the entire length of imaged colon. Each computed image has a calibration scale that indicates the change in diameter in grey-scale (mm), time (s) and length (mm) for each spatio-temporal map produced for each experiment (GIMM software, Med-Associates Inc.).

## Electrophysiological recordings

Experiments were performed on the distal colons from control and 56-day animals that were removed and placed in iced Kreb's solution. The tissue was then placed in a Sylgard coated dissecting dish with ice cold Kreb's containing Nifedipine (5 $\mu$mol $L^{-1}$) and atropine (200 nmol $L^{-1}$) (Sigma) to eliminate smooth muscle contraction. The tissue was cut open along the mesenteric border and the mucosa, submucosa, and circular muscle of the colon were subsequently removed with forceps exposing the myenteric plexus on the longitudinal smooth muscle. The preparation was then moved to a 2.5 mL recording chamber.

Preparations were continuously perfused at 10 mL $min^{-1}$ with Kreb's solution containing nifedipine and atropine maintained at 37 °C. Glass microelectrodes used for recording were filled to the shoulder with 1.0 mol $L^{-1}$ KCl, and the remainder filled with 2.0 mol $L^{-1}$ KCl and had resistances in the range of 50–150 MΩ. Myenteric ganglia were visualized at ×200 with Hoffman modulation contrast optics through an inverted microscope (Nikon Diaphot, Melville, NY, USA) and individual myenteric neurons were randomly impaled. Transmembrane potential was measured with an Axoclamp-2A amplifier (Axon Instruments, Union City, CA, USA) and electrical signals were acquired and analysed using PowerLab Chart (version 5.01; AD Instruments, Castle Hills, Australia). Input resistance and resting membrane potential were determined for each neuron and compared with control animals. APs were elicited by a 500 ms depolarizing current pulse. Synaptic activation of neurons was elicited by direct stimuli applied to fibre tracts in interganglionic connectives with monopolar extracellular electrodes made from Teflon-insulated platinum wire (a single pulse of 0.5 ms). Neurons were deemed unhealthy if they had an input resistance below 50 MΩ or had an AP that peaked at a level <0 mV, and were excluded from the study.

Using criteria described previously for classifying neurons in the guinea-pig small intestine.[19,20] S neurons were identified by the existence of fEPSP and the lack of a shoulder on the repolarizing phase of the AP. AH neurons were identified by the presence of a shoulder on the repolarization portion of the AP and an AHP of >1 s. Amplitude of the maximal fEPSP were acquired while injecting hyperpolarizing current to hold the membrane potential of the neurons to approximately −90 mV to avoid APs. Excitability of neurons was evaluated by injecting 500 ms depolarizing current pulses with increasing current between each pulse until the maximum number of APs elicited were obtained. The AHP of AH neurons were measured by eliciting an AP that peaked only after the offset of a single depolarizing current injection and then integrating the voltage more negative then resting membrane potential over time until membrane potential returned to resting levels. Only antidromically evoked APs were analysed to compare the shapes between control and 56 days. The magnitude was determined by integrating the voltage greater than resting membrane potential over the time until the voltage returned to resting membrane potential. The duration of the AP was determined by measuring the time at half the repolarization the AP. The amplitude was measured by taking the difference in voltage between the peak of the AP and resting membrane potential.

## Measurement of prostaglandin $E_2$ content

The distal colon from control, 56-day and 56-day/DFU animals were cut along the mesenteric border and a 0.5 cm full thickness segment of distal colon from all animal groups was homogenized in 5 mL $g^{-1}$ tissue in 0.1 mol $L^{-1}$ phosphate buffer (0.1 mol $L^{-1}$ phosphate, pH 7.4, 1 mmol $L^{-1}$ EDTA) containing 10 $\mu$mol $L^{-1}$ indomethacin (Sigma). Samples were assayed in triplicate at concentrations of 1 : 10, 1 : 100, 1 : 1000 in an immunoassay buffer and prostaglandin $E_2$ ($PGE_2$) levels were determined by enzyme immunoassay according to manufacturers instructions ($PGE_2$ EIA kit; Cayman Chemical Company, Ann Arbor, MI, USA).


© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

### Measurement of myeloperoxidase activity

MPO is an enzyme found in the intracellular granules of neutrophils and can be used as a marker of neutrophil infiltration. Tissues from control, colitis, 14, 28, 56 days and 56 days/DFU were collected and assayed. The distal colon was cut open along the mesenteric border and 0.5 cm length of full thickness distal colon weighing 100–200 mg was homogenized in 50 mg $mL^{-1}$ of potassium phosphate buffer (50 mmol $L^{-1}$ potassium phosphate, pH 6.0) and spun at 20 000 ***g*** for 20 min. The supernatant was removed and HTAB buffer (Hexadecyltrimethylammonium Bromide: 5 g 1 $L^{-1}$ in 50 mmol $L^{-1}$ potassium phosphate buffer; Sigma) was added, and the pellet was homogenized again and spun at 10 000 ***g***. The samples were then with three cycles of sonication/freezing and spun at 10 000 ***g*** for 10 min. The level of myeloperoxidase (MPO) activity was determined from the supernatent by adding 200 $\mu$L of *O*-dianisidine buffer solution (16.7 mg *O*-dianisidine dihydrochloride; Pfaltz&Bauer, Inc., Waterbury, CT, USA) in 5 mmol $L^{-1}$ phosphate buffer containing %0.005 $H_2O_2$ (Merck). The change in absorbance at 450 nm was determined over a 3-min period by a Maxline Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA, USA). Values are expressed as units of MPO activity per gram of tissue sample, where one unit of MPO is defined as that which degrades 1 $\mu$mol of hydrogen peroxide per minute.

### Statistical analyses

Statistical analyses were performed using GraphPad Prism software (version 4.0a for Macintosh, GraphPad Software, San Diego, CA, USA). Continuous data differences between groups were determined by performaing a Student's unpaired *t*-test or a one-way ANOVA with a Newman–Keuls multiple comparison post-test. Proportional data were analysed by Fisher's exact test. *P*-values < 0.05 were determined to be significant. *n* values represent individual neurons or colons for each experiment and are indicated where appropriate.

## RESULTS

### Inflammation is not detectable 56 days after TNBS administration

During active colitis in the guinea pig, indicators of inflammation include elevated MPO activity, a marker of neurotrophil infiltration, and elevated $PGE_2$ levels.[18,21] Furthermore, the gross macroscopic damage scores are significantly higher in these animals than in control animals, and animals exhibit transient weight loss during the first 48 h after TNBS administration.[8,17] We, therefore, used these indicators to test when the inflammation subsided after TNBS treatment and whether it was still present 8 weeks after TNBS administration.

Animals from control, 56-day and 56-day/DFU groups had comparable weights. Animals treated with TNBS lost weight (8.8 ± 2.0 g) during the first 48 h post-TNBS administration, which is comparable to previous studies of TNBS-induced colitis in guinea pigs[9,17] indicating that colitis was induced by the TNBS treatment. Gross damage scores in 14-day, 28-day, 56-day and 56-day/DFU animals were comparable to control levels (Fig. 1A), which provides an indication of the timeline of the recovery from inflammation.

Previously, we reported that TNBS-induced colitis is associated with an elevation of the COX product, $PGE_2$, and that the COX-2 inhibitor, DFU alleviates TNBS-induced changes in motility and AH neuron excitability.[18]. In the current study, we tested whether COX-2 activation persists 8 weeks after TNBS administration. We measured the levels of $PGE_2$ in tissue from control, 6-, 56-day and 56-day/DFU animals that were administered the COX-2 blocker, DFU. We found that the $PGE_2$ levels were comparable in the control, 56-day and 56-day/DFU groups (Fig. 1B), and were significantly elevated during inflammation at the 6-day time point. The $PGE_2$ levels at the 6-day time point were comparable to the levels that we previously reported for the 6-day animals that were injected only with an ethanol vehicle associated with the DFU injections[18] (one-way ANOVA with a Neuman–Keuls *post-hoc* test).

MPO activity levels were evaluated at the various time points after TNBS administration as another determinant of the timeline for recovery from TNBS. Increased MPO activity was detected during the first 14 days after TNBS administration, whereas MPO levels were comparable to control values at the 28- and 56-day post-TNBS time points (Fig. 1C). Collectively, these data indicate that inflammation resulting from TNBS administration is resolved by day 28, and therefore, the colon is non-inflamed for at least 4 weeks at the 56-day time point.

### Colonic motility is still altered 56 days after TNBS exposure

The myenteric plexus is primarily involved in regulating intestinal motility. Additionally, inflammation-

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

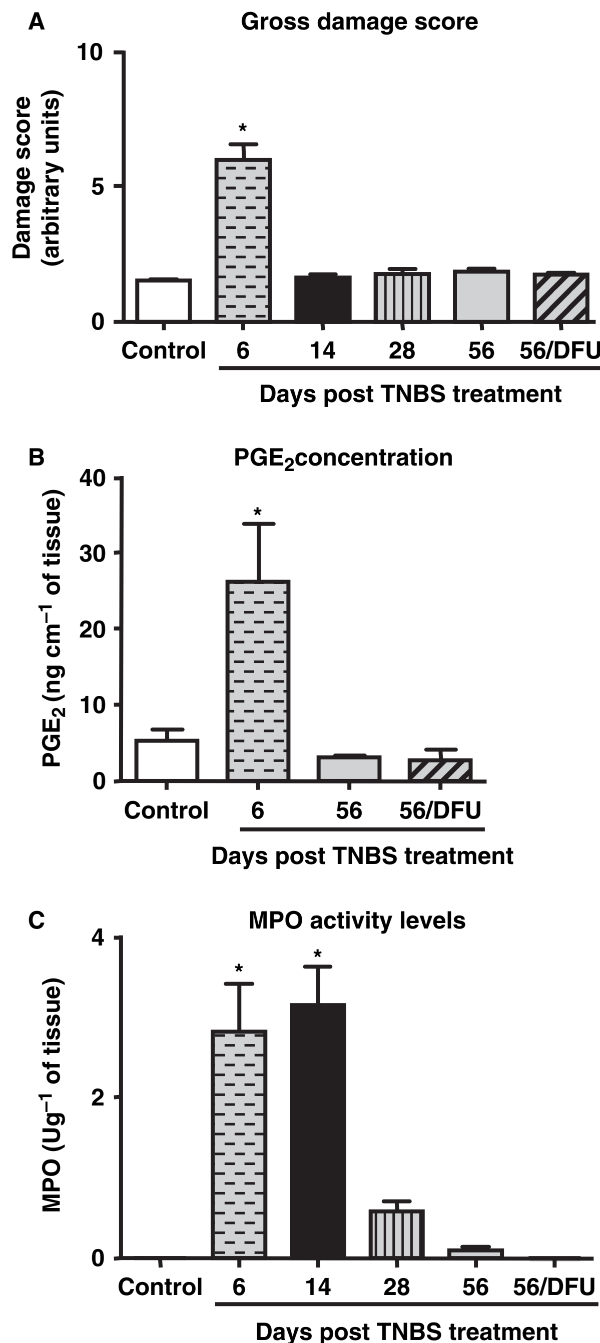


**Figure 1** Presence of inflammation is not detected 56 days after trinitrobenzene sulfonic acid (TNBS) administration. (A) Graph representing average damage scores ± SEM indicating that the elevated damage scores in animals with colitis returns to normal by 14 days after TNBS administration ($n$ = 15 for control, $n$ = 13 for 6 days, $n$ = 10 for 14 days, $n$ = 5 for 28 days, $n$ = 15 for 56 days, $n$ = 6 for 56 days/DFU; $^{*}P < 0.05$ when compared to control, 14, 28, 56 days and 56 days/DFU; ANOVA with Newman–Keuls multiple comparison post-test). (B) Graph representing average levels of prostaglandin $E_2$ ($PGE_2$) ± SEM in tissue from control, 6-, 56-day and 56-day/DFU animals. ($n$ = 5 for control, $n$ = 4 for 6 days, $n$ = 8 for 56 days, $n$ = 3 for 56 days/DFU; $^{*}P < 0.05$ when compared to control, 56 days and 56 days/DFU; ANOVA with Newman–Keuls multiple comparison post-test). (C) Graph representing the average myeloperoxidase activity ± SEM indicating that the activity is elevated 6 and 14 days after TNBS administration and then return to activity levels comparable to control ($n$ = 5 for control, $n$ = 13 for 6 days, $n$ = 10 for 14 days, $n$ = 5 for 28 days, $n$ = 11 for 56 days, $n$ = 3 for 56 days/DFU; $^{*}P < 0.01$ when compared to control, 28, 56 days and 56 days/DFU; ANOVA with Newman–Keuls multiple comparison post-test).

induced changes in colonic motility have been reported in the guinea pig[17] and mice.[22] This dysmotility in the guinea pig is associated with the activation of COX-2.[18] We, therefore, investigated if changes in colonic motility persist after the resolution of inflammation, and if so, whether persistent alterations in motility can be reversed by the inhibition of COX-2. The rate of propulsive motility was measured in colons from weight-matched controls, 56-day post-TNBS animals, and 56-day TNBS animals treated with DFU for 4 days immediately prior to euthanasia.

The rates of propulsion of fecal pellets in colons from 56-day and 56-day/DFU animals were comparable to each other, but were both significantly reduced as compared to values from control animals (Fig. 2A). Furthermore, spatio-temporal pattern of the propulsion was altered in the 56-day and 56-day/DFU groups. In the control animals, the pattern of motility was linear as the fecal pellet moved through the lumen of the colon. However, in the 56-day and the 56-day/DFU groups a step-wise pattern was detected (Fig. 5B). These data suggest that changes in gut function persist 56 days after TNBS administration, and that COX-2 products are not involved with maintaining this dysmotility.

### Altered electrophysiological properties of myenteric neurons persist after resolution of TNBS colitis

During active inflammation, marked changes in the electrophysiological properties of myenteric neurons are detected.[7,16] In the guinea pig TNBS colitis model there is an increase in the excitability of the AH neurons and in S neurons a facilitation of fEPSP amplitudes.[7] We, therefore, tested whether the changes in electrophysiological properties of myenteric neurons persist after the recovery from prior exposure to TNBS.

Data from a total of 11 AH neurons and 19 S neurons were obtained from eight distal colons of animals

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

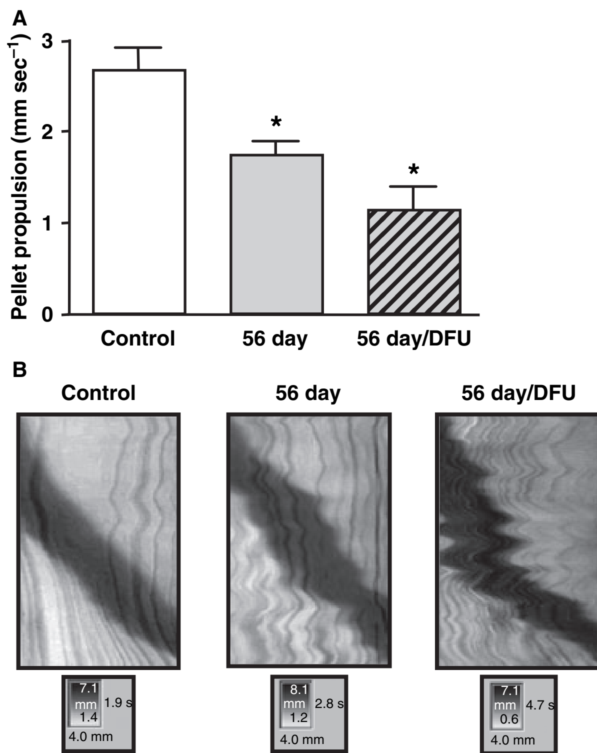


**Figure 2** Dysmotility persists 8 weeks after trinitrobenzene sulfonic acid (TNBS)-induced inflammation, even if the animals are administered DFU. (A) Graph representing the average rate of propulsion ± SEM of a fecal pellet to traverse a 5 cm length of colon from control, 56-day, 56-day/DFU animals ($n$ = 5 for control, $n$ = 9 for 56 days, $n$ = 3 for 56 days/DFU; $*P < 0.05$ as compared to control, ANOVA with Newman–Keul's multiple comparison post-test). (B) Representative spatio-temporal maps constructed from a pellet being propelled in the anal direction in control, 56-day, 56-day/DFU colons. Note how the spatio-temporal pattern of motility is different in the 56-day and 56-day/DFU colons as compared to the linear pattern in the control colons. Scales are indicated for each group.

previously exposed to TNBS (56-day animals). The properties of these neurons were compared to eight AH neurons and 19 S neurons from nine distal colons of weight-matched control animals. Neurons that had APs with no shoulder present on the repolarizing portion and no late AHP were identified as S neurons. Neurons that had APs that had a shoulder present on the repolarizing portion of the AP and having a long AHP that lasted 1 to 20 s were classified as AH neurons. The electrophysiological properties of the neurons from control animals were comparable to those reported in previous studies.[7,23–28] Furthermore, many of the properties of AH neurons and most of the properties of S neurons from the 56-day animals were comparable to control animals (Tables 1 and 2). However, marked changes were detected in several AH neuron properties and in fast synaptic potentials in S neurons. These differences were similar to the changes observed during active TNBS colitis.[7]

*AH neurons* We have previously reported that myenteric AH neurons from colons of animals during active TNBS colitis have slower accommodation that was attributed to a reduction in the magnitude of the AHP that accompanies each AP.[7] In the present study, these alterations persisted 56 days following TNBS administration. The AH neurons in tissue from 56-day animals had a slower accommodation to a 500 ms significant decrease in the magnitude of the AHP in the AH neurons from 56-day animals as compared to AH neurons from control tissue was identified (Fig. 3D). The number of APs fired per 500 ms. depolarizing current pulse and AHP magnitude were comparable to our previous reports during active TNBS colitis (unpaired $t$-test).[7] Furthermore, the slower accommodation and decrease in AHP magnitude was accompanied with a higher percentage of AH neurons that exhibited anodal break APs [weight-match control, 0/8 (0%); 56 days, 5/11 (45.5%); $P < 0.05$, Fisher's exact test]. This number of neurons exhibiting anodal break APs in the 56-day animals and during active TNBS-induced colitis as we previously reported were comparable (Fisher's exact test).[7] Fast synaptic input

**Table 1** AH neuron electrical and synaptic properties that were unaltered by prior inflammation

| | Control ($n$ = 8) | 56 days ($n$ = 11) |
|---|---|---|
| Resting membrane potential (mV) | −63 ± 1 | −61 ± 3 |
| Input resistance (mΩ) | 65 ± 2 | 87 ± 11 |
| Number of neurons with sEPSPs | 5/5 | 10/10 |
| Number of neurons with sIPSPs | 0/5 | 0/10 |
| Number of neurons with spontaneous activity | 0/8 | 1/11 |

Values are average ± SEM for continuous data and analysed with unpaired $t$-test. Proportional data are the number of neurons exhibiting characteristic out of total number of neurons that were tested for that parameter and analysed with Fisher's exact test ($P$-values > 0.5 for all parameters). sEPSPs, slow excitatory postsynaptic potentials; sIPSPs, synchronized inhibitory postsynaptic potentials.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

**Table 2** S Neuron electrical and synaptic properties that were unaltered by prior inflammation

| | Control ($n$ = 19) | 56 days ($n$ = 19) |
|---|---|---|
| Resting membrane potential (mV) | −48 ± 1 | −44 ± 1 |
| Input resistance (mΩ) | 86 ± 10 | 121 ± 16 |
| Max. number of APs/500 ms | 8 ± 3 | 9 ± 3 |
| Number of neurons with fEPSPs | 19/19 | 19/19 |
| Number of neurons with sEPSPs | 14/16 | 14/16 |
| Number of neurons with sIPSPs | 0/16 | 0/16 |
| Number of neurons with spontaneous activity | 0/19 | 2/20 |
| Number of neurons with anodal break APs | 2/17 | 7/19 |

Values are average ± SEM for continuous data and analysed with unpaired $t$-test. Proportional data are the number of neurons exhibiting characteristic out of total number of neurons that were tested for that parameter and analysed with Fisher's exact test ($P$-values > 0.1 for all parameters). fEPSP, fast excitatory postsynaptic potentials; sEPSPs, slow EPSPs; sIPSPs, synchronized inhibitory postsynaptic potentials.

was not detected in any of the 7 AH neurons from control animals, whereas fEPSPs were detected in four out of the 11 AH neurons from the 56-day animals. These data indicate that the hyperexcitability of myenteric AH neurons detected during the active phase of TNBS colitis persists after the resolution of colitis.

During TNBS colitis no change in the AP shape was detected in myenteric AH neurons suggesting that the hyperexcitability was due to changes in the underlying currents of the AHP.[7] In the present study, no difference was detected in the temporal properties of antidromically evoked APs in AH neurons of tissue from weight-matched control and 56-day animals (Table 3). These data suggest that the mechanism for the increase in excitability is due to a change in the currents of the AHP and not due to the currents that underlie the APs as was detected in the submucosal plexus of animals that were previously exposed to TNBS.[8]

*S neurons* The amplitudes of the evoked fast synaptic potentials are facilitated in S neurons from actively inflamed colons.[7,8] In the current study, significant facilitation of fEPSP amplitudes in myenteric S neurons was detected 56 days after administration of TNBS (Fig. 4). The EPSP amplitudes in the 56 days were comparable to those detected during active colitis (unpaired $t$-test).[7] Collectively, these data

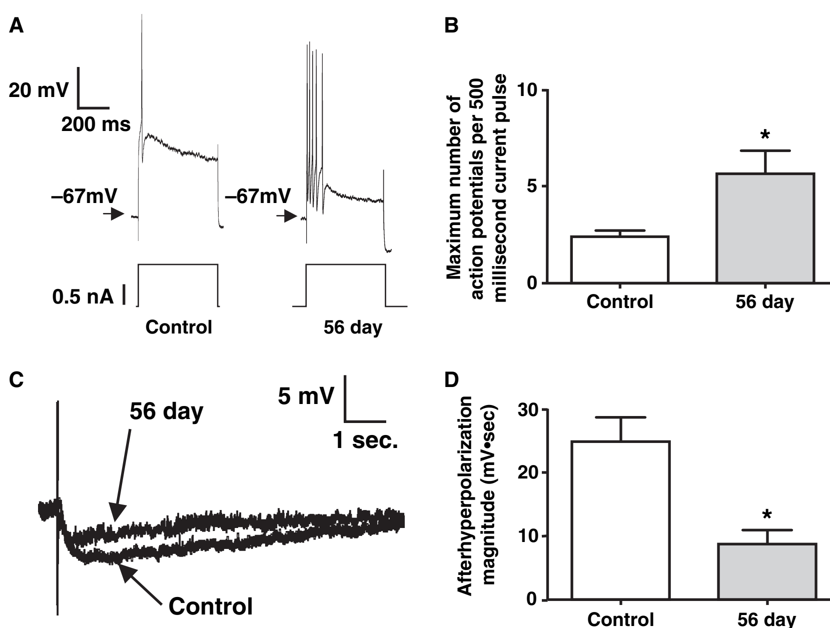


**Figure 3** Hyperexcitability of AH neurons persists 8 weeks after trinitrobenzene sulfonic acid (TNBS) administration. (A) Representative traces from neurons in control and 56 days tissue. Traces are on the same voltage, current, and time scale. (B) Graph of the average of the maximum number ± SEM of action potentials fired during a 500 ms depolarizing current pulse in AH neurons from control ($n$ = 8) and 56 days ($n$ = 11) tissue (*$P$ < 0.05, unpaired $t$-test). (C) Representative traces from control and 56-day animals depicting the decrease in afterhyperpolarization magnitude in postcolitis animals. (D) Graph of the average afterhyperpolarization magnitudes ± SEM in AH neurons from control ($n$ = 7) and 56 days ($n$ = 9) tissue (*$P$ < 0.05, unpaired $t$-test).

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

**Table 3** AH neuron action potential shape is not affected by prior inflammation

| | Control ($n$ = 4) | 56 days ($n$ = 3) |
|---|---|---|
| Action potential amplitude (mV) | 72 ± 2 | 72 ± 2 |
| Action potential width at half repolarization (ms) | 1.8 ± 0.1 | 1.9 ± 0.2 |
| Action potential magnitude (mV•ms) | 128 ± 8 | 135 ± 8 |

All values are the average ± SEM. Each characteristic was analysed with an unpaired $t$-test ($P$-values > 0.5).

suggest that changes in the electrophysiological properties of myenteric neurons could contribute to the dysmotility that is detected at this time point.

## DISCUSSION

The purpose of this investigation was to determine if changes in motility and the properties of myenteric neurons persist after the resolution of experimental colitis. We have previously demonstrated that during TNBS-induced colitis the rate of propulsive motility in the distal colon is decreased, the AH neurons are hyperexcitable, and the fEPSPs of S neurons are facilitated.[7] In the present study, altered colonic motility and many of the properties of the neuroplasticity detected during colitis were present weeks following recovery from inflammation. Our data are indicative that TNBS-induced inflammation is self limited, which is consistent with previous reports from postcolitis models in rat and guinea-pig.[9,29] However, changes in gut physiology persist at least 4 weeks following resolution of inflammation, indicating that the changes are long-lasting and may contribute to the clinical observations in patients in remission from IBD.[1]

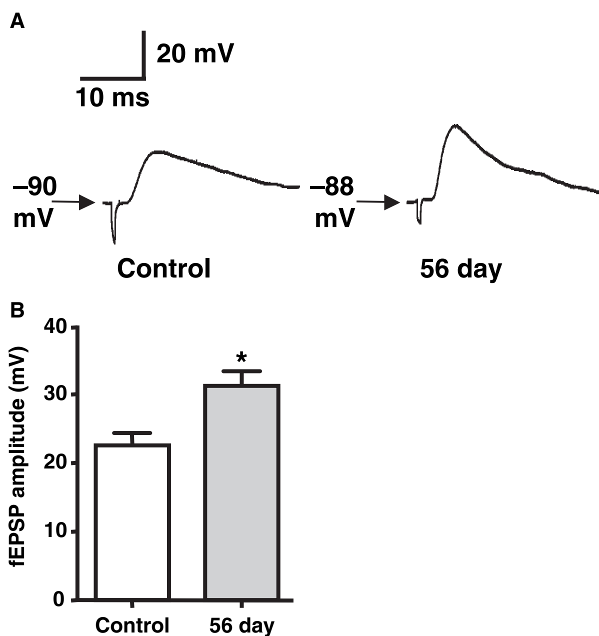


**Figure 4** Facilitated fast excitatory postsynaptic potential (fEPSP) amplitudes persist in S neurons after the resolution from inflammation. (A) Representative traces of fEPSPs recorded from neurons in control and 56 days tissues. Traces are on the same voltage and time scales. (B) Graph representing the average ± SEM fEPSP amplitudes in S neurons from control ($n$ = 19) and 56 days ($n$ = 19) tissue (*$P$ < 0.005, unpaired $t$-test).

The myenteric plexus is primarily responsible for coordinating proper motility throughout the intestine. Our data indicate that disrupted peristalsis persists in postcolitis animals as the rate of propulsion of a fecal pellet was decreased as compared to control. It was determined that this dysmotility is due to disruption of coordinated motor activity, as reflected by a step-wise spatio-temporal pattern that was detected. The peristaltic reflex is thought to involve release of serotonin from enterochromaffin cells activating AH neurons.[30,31] We have determined that serotonin signalling is altered by TNBS colitis, which could affect the peristaltic reflex.[17,32] However, recently, we have indicated that the elements of serotonin signalling have returned to normal levels at the 56-day time point,[9] which suggests that the altered motility pattern at this time could involve changes in the properties of the myenteric neurons. Alternatively, the dysmotility could be due to changes in smooth muscle activity. For instance, in a rat model of post-inflammatory colitis, inflammation affects the ICC and contractility of smooth muscle, however only alterations of myogenic contractions of smooth muscle persisted after the resolution of inflammation.[33,34] It is likely that both the neural and myogenic activity contribute to the persistent dysmotility after the resolution of inflammation. Investigating the roles played by both components is important for a full understanding of the pathophysiology of intestinal dysmotility and the identification of potential therapeutic targets.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

Neurophysiological properties of myenteric AH neurons were still altered at the 56-day time point when compared to control animals. When data from 56-day animals obtained in the current study were compared to values from our previous study of AH neurons in actively inflamed tissue,[7] we found that the changes still detected at 56 days were not statistically different from the inflamed condition. In other words, no recovery of these properties was detected at this time point. During active TNBS colitis the majority of these changes are resolved when the $PGE_2$ levels from full thickness tissue were reduced by COX-2 inhibition. In the current study, the changes persist when the $PGE_2$ levels from full thickness tissue are comparable to control tissue. In a rat study of persistent postinflammatory changes in mucosal secretion, COX-2 inhibition restored secretion to a normal level.[35] However, in the current study colonic motility was not restored by postinflammatory administration of the COX-2 inhibitor, DFU. Furthermore, the AH neurons, which are sensitive to $PGE_2$,[36,37] were still hyperexcitable even though $PGE_2$ levels are not elevated at this time point but is at the 6-day time point.[18] Collectively, these data suggest that the changes occur during the active state of inflammation potentially through a COX-2 sensitive mechanism, but once initiated do not require sustained elevations in COX-2 derived prostaglandins to maintain them. It is worth considering that since blocking COX-2 during active inflammation partially restores the intestinal motility, that the administration of DFU in the present study is too late to restore gut function. However, we reasoned that inhibition of COX-2 for 4 days prior to the electrophysiological studies would be long enough to restore function as this treatment protocol is sufficient during active inflammation. Future investigations are warranted to elucidate if administration during active TNBS colitis would prevent the persistent dysmotility and AH neuron hyperexcitability that are detected at the 56-day time point.

In the current study, when examining AH neurons 56 days following TNBS administration, we detected a decrease in the AHP magnitude with no changes in the resting membrane potential or input resistance. As described in our previous evaluation of AH neuron properties during active TNBS-induced colitis,[7] no changes in the temporal properties of the AP were detected at the 56-day time point suggesting that a change in the channels that contribute to the AHP may be responsible. The two channels that contribute to the magnitude of the AHP are the intermediate conductance calcium-activated potassium channel (IK),[38–40] and the hyperpolarization-activated cation channel (HCN).[41] With the decrease in AHP magnitude persisting at least 4 weeks after the resolution of inflammation and being comparable to the AHP magnitude 6 days after TNBS treatment, it is possible that there are persistent changes in the relative expression of these channels. For example, there are multiple isoforms of the HCN channel, each with different kinetics,[42] and in the rat hippocampus persistent changes in network activity can affect gene transcription leading to expression of different HCN isoforms.[43] With inflammatory-induced changes in the electrophysiology of myenteric neurons, which could alter the enteric neural network activity, then perhaps there are changes in the relative expression of the HCN channel to isoforms with faster kinetics leading to a decrease in the AHP magnitude. Further studies into whether there are changes in the expression levels or changes to pre-existing channels due to inflammation are warranted.

Facilitation of fEPSP amplitudes in myenteric S neurons persisted 56 days after TNBS administration as compared to weight-matched control animals. The fEPSP amplitudes in post-colitis tissue were comparable to the amplitudes of fEPSPs in S neurons from actively inflamed tissue.[7] As inflammation was not detected at this time, it is feasible that this change initially occurs during chronic-colitis. During chronic-colitis increased activity of protein kinase A (PKA), independent of $5\text{-}HT_4$ receptors, contributes to the augmentation of the fEPSP amplitude, which potentially leads to phosphorylation of ion channels involved with the regulation of transmitter release.[44] If the upregulation of PKA continues for a prolonged time period, this could lead to a downstream effect on transcription and change the expression of proteins or ion channels that regulate neurotransmission, which could explain the persistence of the augmented fEPSPs. Further investigation of whether the upregulation of PKA persists at this time point is warranted to determine if this is the case.

In conclusion, changes in the neurophysiological properties of myenteric neurons persist after the resolution of inflammation and likely contribute to the dysmotility that is detected. Furthermore, we determined that by 4 weeks after TNBS administration inflammation was not present. It is likely that these changes in neurophysiology and motility are a persistent reflection of those that occur during active colitis. Nonetheless, these changes coupled with the persistent changes in the submucosal plexus, which regulates secretion,[9] indicate that prior inflammation can lead to continuous changes in gut function and could


© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

contribute to colonic dysfunction that is reported during remission from IBD.[1]

## ACKNOWLEDGMENTS

This work was supported by NIH grants DK62267, NS26995 (GMM), and Ruth L. Kirschstein National Research Service Award F31 NS055512–01 from NINDS (EMK), and a grant from the Crohn's and Colitis Foundation of Canada (CCFC; KAS, GMM). KAS is an Alberta Heritage Foundation for Medical Research Medical Scientist and the CCFC Chair in IBD Research at the University of Calgary.

## REFERENCES

1 Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. *Gastroenterology* 1996; **111**: 1683–99.
2 Vasina V, Barbara G, Talamonti L *et al.* Enteric neuroplasticity evoked by inflammation. *Auton Neurosci* 2006; **127**: 264–72.
3 Spiller RC. Postinfectious irritable bowel syndrome. *Gastroenterology* 2003; **124**: 1662–71.
4 Chadwick VS, Chen W, Shu D *et al.* Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002; **122**: 1778–83.
5 Bercik P, Wang L, Verdu EF *et al.* Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. *Gastroenterology* 2004; **127**: 179–87.
6 Coldwell JR, Phillis BD, Sutherland K, Howarth GS, Blackshaw LA. Increased responsiveness of rat colonic splanchnic afferents to 5-HT after inflammation and recovery. *J Physiol* 2007; **579**: 203–13.
7 Linden DR, Sharkey KA, Mawe GM. Enhanced excitability of myenteric AH neurones in the inflamed guinea-pig distal colon. *J Physiol* 2003; **547**: 589–601.
8 Lomax AE, Mawe GM, Sharkey KA. Synaptic facilitation and enhanced neuronal excitability in the submucosal plexus during experimental colitis in guinea-pig. *J Physiol* 2005; **564**: 863–75.
9 Lomax AE, O'Hara JR, Hyland NP, Mawe GM, Sharkey KA. Persistent alterations to enteric neural signaling in the guinea pig colon following the resolution of colitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G482–91.
10 Brookes SJ. Classes of enteric nerve cells in the guinea-pig small intestine. *Anat Rec* 2001; **262**: 58–70.
11 Spencer NJ, Smith TK. Mechanosensory S-neurons rather than AH-neurons appear to generate a rhythmic motor pattern in guinea-pig distal colon. *J Physiol* 2004; **558**: 577–96.
12 Bertrand PP, Kunze WA, Bornstein JC, Furness JB, Smith ML. Analysis of the responses of myenteric neurons in the small intestine to chemical stimulation of the mucosa. *Am J Physiol* 1997; **273**: G422–35.
13 Furness JB, Kunze WAA, Bertrand PP, Bornstein JC. Intracellular recording from myenteric neurons of the guinea-pig ileum that respond to stretch. *J Physiol* 1998; **506**: 827–42.
14 Kunze WA, Furness JB. The enteric nervous system and regulation of intestinal motility. *Annu Rev Physiol* 1999; **61**: 117–42.
15 Wood JD. Cellular neurophysiology of enteric neurons. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*. Burlington, MA: Academic Press, 2006: 629–83.
16 Palmer JM, Wong-Riley M, Sharkey KA. Functional alterations in jejunal myenteric neurons during inflammation in nematode-infected guinea pigs. *Am J Physiol* 1998; **275**: G922–35.
17 Linden DR, Chen JX, Gershon MD, Sharkey KA, Mawe GM. Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G207–16.
18 Linden DR, Sharkey KA, Ho W, Mawe GM. Cyclooxygenase-2 contributes to dysmotility and enhanced excitability of myenteric AH neurones in the inflamed guinea pig distal colon. *J Physiol* 2004; **557**: 191–205.
19 Bornstein JC, Furness JB, Kunze WA. Electrophysiological characterization of myenteric neurons: how do classification schemes relate? *J Auton Nerv Syst* 1994; **48**: 1–15.
20 Wood JD. Physiology of the enteric nervous system. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*. New York: Raven Press, 1994: 423–82.
21 Fretland DJ, Widomski D, Tsai BS *et al.* Effect of the leukotriene B4 receptor antagonist SC-41930 on colonic inflammation in rat, guinea pig and rabbit. *J Pharmacol Exp Ther* 1990; **255**: 572–6.
22 Boyer L, Ghoreishi M, Templeman V *et al.* Myenteric plexus injury and apoptosis in experimental colitis. *Auton Neurosci* 2005; **117**: 41–53.
23 Wade PR, Wood JD. Electrical behavior of myenteric neurons in guinea pig distal colon. *Am J Physiol* 1988; **254**: G522–30.
24 Wade PR, Wood JD. Synaptic behavior of myenteric neurons in guinea pig distal colon. *Am J Physiol* 1988; **255**: G184–90.
25 Lomax AE, Sharkey KA, Bertrand PP, Low AM, Bornstein JC, Furness JB. Correlation of morphology, electrophysiology and chemistry of neurons in the myenteric plexus of the guinea-pig distal colon. *J Auton Nerv Syst* 1999; **76**: 45–61.
26 Wada-Takahashi S, Tamura K. Actions of reactive oxygen species on AH/type 2 myenteric neurons in guinea pig distal colon. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G893–902.
27 Tamura K, Ito H, Wade PR. Morphology, electrophysiology, and calbindin immunoreactivity of myenteric neurons in the guinea pig distal colon. *J Comp Neurol* 2001; **437**: 423–37.
28 Nurgali K, Furness JB, Stebbing MJ. Correlation of electrophysiology, shape and synaptic properties of myenteric AH neurons of the guinea pig distal colon. *Auton Neurosci* 2003; **103**: 50–64.
29 Asfaha S, Bell CJ, Wallace JL, MacNaughton WK. Prolonged colonic epithelial hyporesponsiveness after colitis: role of inducible nitric oxide synthase. *Am J Physiol* 1999; **276**: G703–10.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd 

30 Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine4 receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. *Gastroenterology* 1998; **115**: 370–80.
31 Grider JR, Kuemmerle JF, Jin JG. 5-HT released by mucosal stimuli initiates peristalsis by activating 5- HT4/5-HT1p receptors on sensory CGRP neurons. *Am J Physiol* 1996; **270**: G778–82.
32 Linden DR, Foley KF, McQuoid C, Simpson J, Sharkey KA, Mawe GM. Serotonin transporter function and expression are reduced in mice with TNBS-induced colitis. *Neurogastroenterol Motil* 2005; **17**: 565–74.
33 Ozaki H, Hori M, Kinoshita K, Ohama T. Intestinal dysmotility in inflammatory bowel disease: mechanisms of the reduced activity of smooth muscle contraction. *Inflammopharmacology* 2005; **13**: 103–11.
34 Wells RW, Blennerhassett MG. Persistent and selective effects of inflammation on smooth muscle cell contractility in rat colitis. *Pflugers Arch* 2004; **448**: 515–24.
35 Zamuner SR, Warrier N, Buret AG, MacNaughton WK, Wallace JL. Cyclooxygenase 2 mediates post-inflammatory colonic secretory and barrier dysfunction. *Gut* 2003; **52**: 1714–20.
36 Kelles A, Janssens J, Tack J. Electrical behaviour of interleukin-1 beta (IL-1 beta) and prostaglandin-E2 (PGE2) on colonic myenteric neurones. *Neurogastroenterol Motil* 2002; **14**: 321–30.
37 Manning BP, Sharkey KA, Mawe GM. Effects of PGE2 in guinea pig colonic myenteric ganglia. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G1388–97.
38 Vogalis F, Harvey JR, Furness JB. TEA- and apamin-resistant K(Ca) channels in guinea-pig myenteric neurons: slow AHP channels. *J Physiol* 2002; **538**: 421–33.
39 Vogalis F, Harvey JR, Neylon CB, Furness JB. Regulation of K+ channels underlying the slow afterhyperpolarization in enteric afterhyperpolarization-generating myenteric neurons: role of calcium and phosphorylation. *Clin Exp Pharmacol Physiol* 2002; **29**: 935–43.
40 Furness JB, Robbins HL, Selmer IS *et al.* Expression of intermediate conductance potassium channel immunoreactivity in neurons and epithelial cells of the rat gastrointestinal tract. *Cell Tissue Res* 2003; **314**: 179–89.
41 Xiao J, Nguyen TV, Ngui K *et al.* Molecular and functional analysis of hyperpolarisation-activated nucleotide-gated (HCN) channels in the enteric nervous system. *Neuroscience* 2004; **129**: 603–14.
42 Surges R, Brewster AL, Bender RA, Beck H, Feuerstein TJ, Baram TZ. Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. *Eur J Neurosci* 2006; **24**: 94–104.
43 Brewster A, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J Neurosci* 2002; **22**: 4591–9.
44 Krauter EM, Linden DR, Sharkey KA, Mawe GM. Synaptic plasticity in myenteric neurons of the guinea pig distal colon: Presynaptic mechanisms of inflammation-induced synaptic facilitation. *J Physiol* 2007; **581**: 787–800.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd