Video Article

In Vitro Characterization of the Electrophysiological Properties of Colonic Afferent Fibers in Rats

Youqiang Meng[1], Li Dong[1], Biying Sun[1], Ping Luo[1], Guohua Zhang[1], Weifang Rong[1]

[1]Hongqiao International Institute of Medical Research, Tongren Hospital and Department of Physiology, Faculty of Basic Medical Sciences, Shanghai Jiaotong University School of Medicine

Correspondence to: Weifang Rong at weifangrong@shsmu.edu.cn

URL: https://www.jove.com/video/56090
DOI: doi:10.3791/56090

Keywords: Neuroscience, Issue 127, Colonic afferents, pelvic ganglion, thoracolumbar hypogastric nerve, lumbosacral pelvic afferents, extracellular recording, colon

Date Published: 9/27/2017

Citation: Meng, Y., Dong, L., Sun, B., Luo, P., Zhang, G., Rong, W. *In Vitro* Characterization of the Electrophysiological Properties of Colonic Afferent Fibers in Rats. *J. Vis. Exp.* (127), e56090, doi:10.3791/56090 (2017).

Abstract

Dysfunction of the colonic sensory nerves has been implicated in the pathophysiology of several common conditions, including functional and inflammatory bowel diseases and diabetes. Here, we describe a protocol for the *in vitro* characterization of the electrophysiological properties of colonic afferents in rats. The colorectum, with the intact pelvic ganglion (PG) attached, is removed from the rat; superfused with carbogenated Krebs solution in the recording chamber; and cannulated at the oral and anal ends to allow for distension. A fine nerve bundle emanating from the PG is identified, and the multiunit afferent nerve activity is recorded using a suction electrode. Distension of the colonic segment elicits gradual increases in multiunit discharge. A principal component analysis is conducted to differentiate the low-threshold, the high-threshold, and the wide-dynamic range afferent fibers. Chemical sensitivity of colonic afferents can be studied through the bath or intraluminal administration of test compounds. This protocol can be modified for application to other species, such as mice and guinea pigs, and to study the differences in the electrophysiological properties of thoracolumbar/hypogastric and lumbosacral/pelvic afferents of the descending colon in normal and pathological conditions.

Video Link

The video component of this article can be found at https://www.jove.com/video/56090/

Introduction

The gastrointestinal tract (GIT) is richly innervated with extrinsic afferent nerves that convey sensory signals from the gut to the central nervous system and that contribute to the gut-brain interaction. Altered excitability of these extrinsic afferents, as well as altered central processing of the afferent inputs, underlies visceral pain and other symptoms of GI conditions, including functional and inflammatory bowel diseases[1]. Sensory information from the colorectum is conveyed primarily through the thoracolumbar/hypogastric and the lumbosacral/pelvic nerves (PN)[2]. There has been an increased interest in studying the electrophysiological properties of these primary afferent fibers in rodent disease models. However, *in vivo* electrophysiological recordings of the colonic afferents in rodents is a technical challenge and requires considerable surgical skills. In addition, hemodynamic changes, tissue movement, and anesthetics may also impact nerve activity and sensitivity to test stimuli *in vivo*. Therefore, in recent years, an increasing number of studies have employed *in vitro* (*ex vivo*) preparations of different species, including mice, rats, guinea pigs, and humans, to examine the mechanisms of sensory transduction in colonic afferents and the altered excitability in disease conditions.[3,4,5,6,7,8]

Two types of *ex vivo* colonic preparation have primarily been reported: the "flat-sheet" preparation[5,9,10] and the "tube" preparation[3,4]. A video protocol for the "flat-sheet" murine colorectum preparation has been previously published[11]. In this protocol, the mouse colorectum, with the PN) or lumbar splanchnic nerves (LSN) attached, is harvested and superfused in a tissue chamber. The colorectum is cut open longitudinally, and the nerve bundle is extended into a recording compartment filled with paraffin oil. Nerve activity is recorded using a monopolar platinum-iridium electrode. The protocol allows for the identification of the receptive fields of individual afferent fibers by using unbiased electrical stimulation. It localizes the application of chemical stimuli, as well as the application of different mechanical stimulation paradigms (*e.g.,* focal mucosal probing and circumferential stretch), to the afferent nerve endings. Because the nerve must be extended to a separate chamber from the tissue chamber, it is critical to keep the attached nerve relatively long; the successful dissection of the nerves poses a challenge to those new to this methodology. More recently, Nullens *et al.* published a video protocol for the *in vitro* recording of the mesenteric afferents in murine jejunal and colonic segments[12]. In this "tube" preparation, the gut segment with the mesentery attached is kept intact, thus allowing for graded distension and the intra- and extra-luminal administration of different chemicals. Since the mesentery nerve is recorded using a suction electrode, which can be positioned close to the tissue, afferent activity can be recorded even though the mesentery nerve is relatively short. However, the mesentery nerve consists of mixed populations of vagal and spinal afferent fibers that innervate the jejunum or thoracolumbar hypogastric. Lumbosacral pelvic afferents innervate the colorectum, which cannot be discriminated in this protocol. Here, we present a detailed protocol for

the electrophysiological recording of rat colonic afferents using the "tube" colorectum preparation with an intact PG. This method may allow for the characterization of the functional properties of lumbar splanchnic (hypogastric) and lumbosacral pelvic afferents.

Protocol

The experimental protocol reported here has been approved by the Animal Ethical Committee of Shanghai Jiaotong University School of Medicine (# SYXK2013-0050). The dissection of the colorectum with intact ganglion and nerve trunk takes a minimum of 15 minutes for a person quite experienced in this technique. It is therefore necessary to keep the animal alive but under deep anesthesia whilst performing the dissections, to ensure viability of the tissue for subsequent electrophysiological recording.

1. Preparation of Perfusion Solution and Test Compounds

1. Prepare 5 L of Krebs solution: 113 mM NaCl, 5.9 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM $CaCl_2$, and 11.5 mM glucose. Saturate the solution with a 95% O_2 + 5% CO_2 gas mixture. Pre-cool ~500 mL of oxygenated Krebs in the fridge.
2. Prepare aliquots of stock test compounds (1 mM capsaicin in ethanol and 10 mM 5-hydroxytryptamine (5-HT) in saline) as needed. Dilute the stock in Krebs (for bath application) and in saline (for intraluminal administration) to the final concentration just prior to use.

2. Preparation of the Recording Electrode

1. Pull the recording electrode from standard glass tubes without inner filaments (1.5 mm outer diameter) using a conventional electrode puller. Adjust the puller settings for heat and pull so that the shank of the pulled electrodes is between 20 and 25 mm.

3. Tissue Collection

1. Anaesthetize the rat deeply using sodium pentobarbital (80 mg/kg, i.p.).
2. While ensuring sterility, expose the abdominal cavity by performing a midline incision on the abdominal wall using a scalpel. Pull the mesentery and other tissues aside to expose the colorectum.
3. Place the animal under the dissecting microscope. Through careful dissection, locate the left PG and identify the PN and the LSN joining it. Cut these nerves a few millimeters away from the PG.
   NOTE: The PG lies close to the colorectal junction. Typically, 3 - 4 PN from the lumbosacral spinal cord and an LSN project to the PG (**Figure 1**).
4. Cut the symphysis bone to expose the rectum. Remove the tissues (*i.e.,* urinary bladder, *etc.*) above the colorectum; be careful to leave the PG intact.
5. Sacrifice the rat by the intravenous injection of an overdose of pentobarbital. Transect the colon about 3 cm above the PG and remove the colorectum from the animal using forceps.
6. Transfer the colorectum to a Petri dish filled with pre-cooled Krebs solution. Remove the feces by gently flushing the colon. Remove the remnant urinary bladder and other tissues carefully, without compromising the PG.

4. Dissection of the Colonic Afferent Nerves

1. Place the colon into a recording chamber (20 mL) and perfuse the tissue continuously with carbogenated Krebs solution. Set the perfusion rate at 15 mL/min.
2. Cannulate the colorectum at both the oral and anal ends. Start the intraluminal infusion of the colon with saline at a rate of 10 mL/h in the oral to anal direction.
3. Locate the major PG under the dissecting microscope. Use insect pins to expose the ganglion. With careful dissection, find a fine branch of nerve emanating from the ganglion and running towards the colon (**Figure 1**). Cut the nerve close to the ganglion.
   NOTE: **Figure 1** illustrates a recording from a nerve branch distal to the PG. The nerve presumably contains a mixture of pelvic and lumbar splanchnic afferent fibers. Alternatively, a recording can be made from the PN and/or the LSN proximal to the PG.
4. Turn on the heating bath and warm the Krebs solution to keep the chamber temperature at 34 ± 0.5 °C.

5. Preparation of the Suction Electrode

1. Take a pre-pulled glass pipette (step 2) and inspect it under the dissecting microscope. Break the tip of the electrode with a pair of forceps so that it is of a size compatible with the diameter of the nerve to be recorded.
2. Bevel the tip by placing it close to a lighter flare.

6. Electrophysiological Recording

1. Connect the beveled electrode to the electrode holder. Connect a 10-mL syringe to the side port on the holder to apply negative or positive pressure to the electrode.
2. Connect the holder to the headstage of the bioamplifier and mount the headstage onto a manipulator.
3. Move the electrode to the tissue bath and fill the electrode with the Krebs solution by applying gentle suction until it contacts the silver wire of the holder. Place the electrode tip close to the cut end of the nerve and apply negative pressure to suck the nerve into the electrode. Apply more negative pressure so that ~1 mm of the nerve is pulled into the electrode and forms a tight seal.

4. Turn on the bioamplifier and set the filter to 300 - 3,000 Hz. Monitor the signal on the oscilloscope and record the nerve signal (20-kHz sampling rate) and the intraluminal pressure signal (100-Hz sampling rate) using a computer with a spike data processing software.

7. Testing the Colonic Afferent Sensitivity

1. Apply ramp distension of the colon by closing the three-way tap on the outlet cannula while continuously infusing intraluminally. Monitor the intraluminal pressure until it reaches 60 mmHg, at which time open the three-way tap on the draining cannula.
2. Repeat this procedure at regular intervals of 15 min. Apply drugs extra- or intraluminally to test the chemical sensitivity of the afferent nerves.

Representative Results

Figure 1 is the schematic illustration of the experimental setup for the *ex vivo* "tube" colorectum preparation, with a representative recording from a nerve distal to the PG. The nerve presumably contained a mixture of pelvic and lumbar splanchnic afferents. In preparations from normal rats, the colonic afferent nerves typically have a low level of irregular spontaneous activity. Ramp distension of the colon induces a gradual increase in the firing rate (**Figure 1B**). The mechanosensory property of each nerve is represented by plotting the intraluminal pressure-afferent nerve response curve (**Figure 1C**). Averaged pressure-response curves can be compared between groups (*i.e.,* control versus treated) to reveal whether a treatment alters the mechanosensory property of the colonic afferent nerves (see Rong *et al.*[13] for details on the pressure-afferent response curve).

Alternatively, recordings can be made from the PN or the LSN proximal to the PG. **Figure 2** is a representative recording from a PN bundle that exhibited a mechanosensory response qualitatively similar to that of the bundle distal to the PG, shown in **Figure 1B**. Principal component analysis of the neural signal allows for the identification of single-unit activity. The discriminated single units are classified as low-threshold (LT) fibers, wide-dynamic range (WDR) fibers, and high-threshold (HT) fibers, according to the profiles of the responses to distension. LT units are activated at a low distension pressure, and the firing rate reaches a maximum at about 20 mmHg. WDR units are also activated at a low distension pressure, and the firing rate continues to increase as the intraluminal pressure increases (**Figure 2A** and **C**). HT units are activated at a distension pressure >20 mmHg (see Dong *et al.*)[4].

The chemical sensitivity of the colonic afferents can be tested by superfusion (bath application) or intraluminal infusion. For example, the bath application of 10 μM 5-HT caused a modest increase, whereas capsaicin at 0.3 μM evoked robust increases in pelvic afferent discharge that were followed by a period of decreased activity due to desensitization (**Figure 3**).

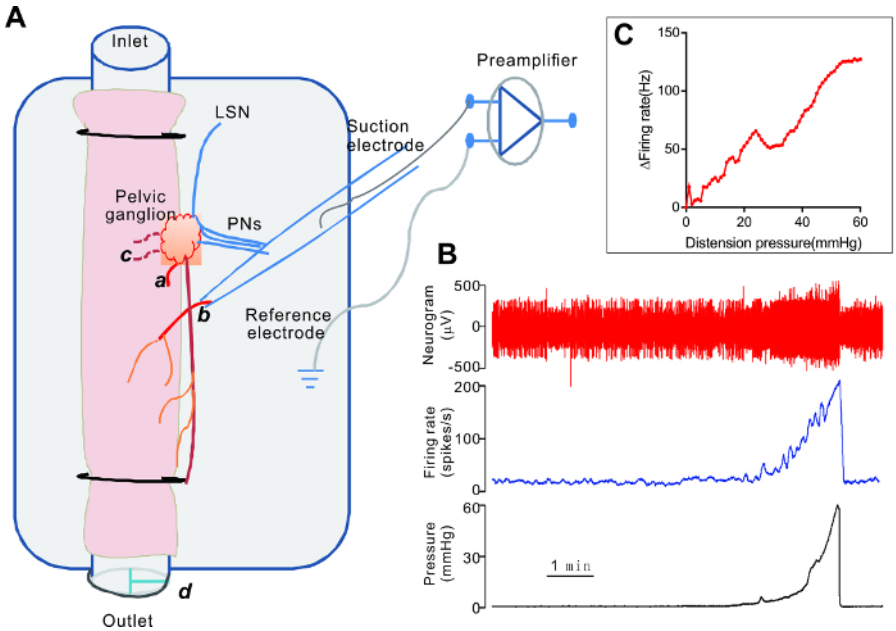

Figure 1. Electrophysiological Recording of Rat Colonic Afferent Nerves *In Vitro*. A. A schematic illustration of the *ex vivo* "tube" colon preparation of the rat. The colorectum, with the attached PG, is superfused in the recording chamber and is cannulated at both ends. Intraluminal infusion is achieved using a syringe pump and moves in the oral to anal direction. Distension of the colon is effected by closing the three-way tap (*d*) on the outlet cannula. A fine nerve branch from the PG is cut and recorded using a suction electrode. A branch of the LSN and three branches of the PN joining the PG are also shown. *a*. The proximal cut end of a fine nerve projecting to the colon. *b*. The distal cut end of the nerve sucked into the electrode. *c*. The cut end of two fine nerves projecting to the urinary bladder. LSN: lumbar splanchnic nerves, PN: pelvic nerves. **B.** The representative traces of the colonic afferent nerve signal and intraluminal pressure. **C.** A plot of the pressure-response curve of this nerve. Please click here to view a larger version of this figure.

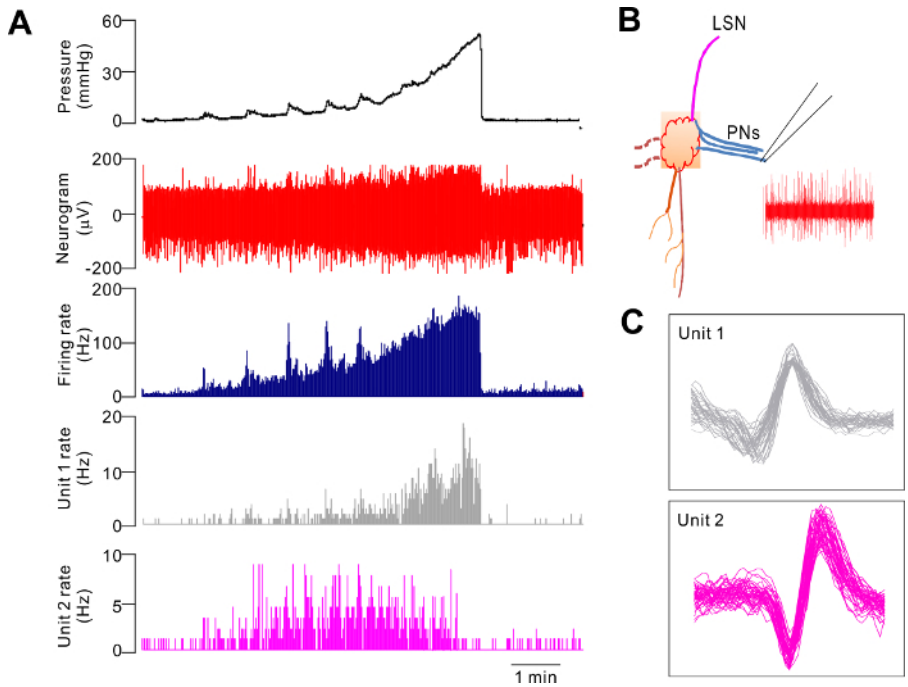

Figure 2. Representative Electrophysiological Recording from a Branch of the Pelvic Nerves during Ramp Distension of the *Ex Vivo* Colorectum. A. The response pattern of two single units (WDR and LT units), together with the multiunit mechanosensory response during ramp distension. **B.** A schematic illustration of the nerve bundle being recorded. LSN: lumbar splanchnic nerves, PN: pelvic nerves. **C.** Superimposed waveform of the two single units. Please click here to view a larger version of this figure.

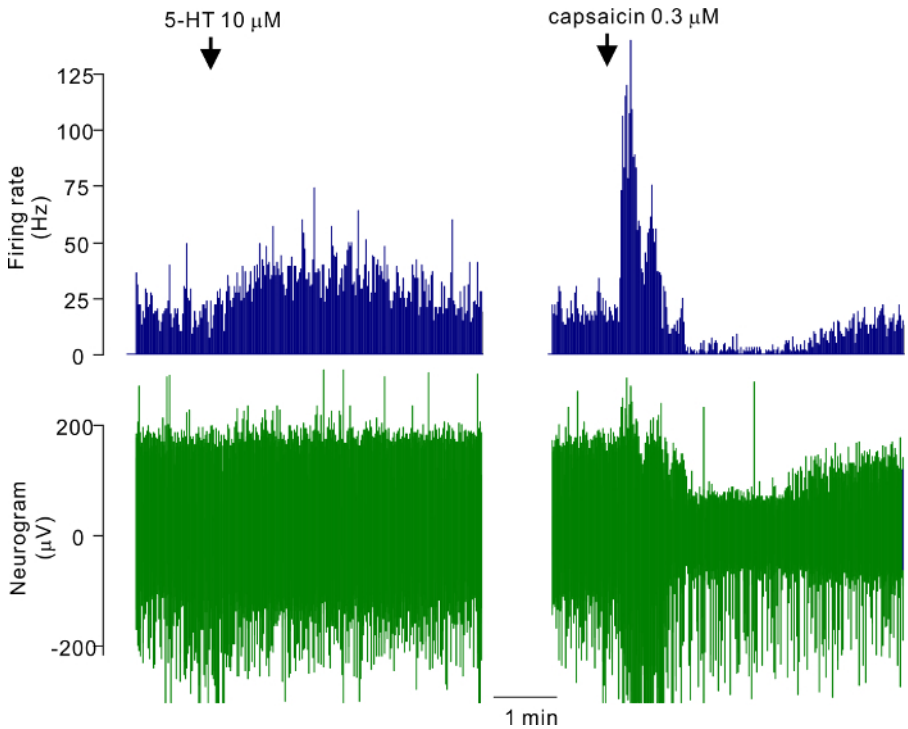

Figure 3. Representative Electrophysiological Recording from a Branch of the Pelvic Nerves During the Bath Application of 5-HT and Capsaicin. (Left) Application of 10 μM 5-HT. **(Right)** Application of 0.3 μM capsaicin. Please click here to view a larger version of this figure.

Discussion

The protocol presented here is a relatively straightforward experimental method to assess the electrophysiological properties of the colonic afferents of rats. The protocol (from tissue dissection to setting up the nerve recording) usually takes about 2 h to complete. Tissue collection (step 3) and preparation of the suction electrode (step 5) are the critical steps. It is crucial to be able to locate the PG, the LSN, and the PN and

to take care not to damage the ganglion and nerves during tissue dissection. The tip of the glass pipette must be broken and beveled to a size compatible with the nerve bundle.

The preparation is usually viable for several hours, allowing for the study of the mechanosensitivity, the chemosensitivity, and the pharmacological profiles of the colorectal afferent nerves. Compared with the "flat-sheet" preparation[11], in which mechanical stimulation is applied through mucosal stroking or circumferential stretching, this "tube" preparation allows for ramp or graded distension in a manner similar to the application of colorectal distension (CRD) in anesthetized or awake animals to assess visceral pain. Using a similar protocol, Wynn *et al.*[3] studied the contribution of purinergic receptors to the mechanosensory transduction of rat colonic afferents and found that colonic afferents were activated by the bath application of ATP (P2X and P2Y agonist) and α,β-meATP (P2X agonist) and that distention-induced pelvic afferent discharge was attenuated by P2 receptor antagonists. More recently, we compared the mechanosensory responses of colonic afferents between normal and streptozotocin (STZ)-induced diabetic rats. We found a significant decrease in multiunit afferent nerve responses to ramp distension of the *ex vivo* colon in diabetic rats (3-6 weeks after STZ injection) compared to the control rats. The decreased mechanosensory response in colonic afferents observed *in vitro* was consistent with an attenuated visceromotor response (VMR) to *in vivo* CRD in the diabetic group. Single-unit analysis indicated that impaired mechanosensitivity of LT and WDR fibers may underlie the afferent hyposensitivity in the diabetic colon[4]. Using a similar protocol, we also successfully recorded from murine colonic afferents and detected significantly increased colonic afferent excitability in DSS-induced IBD mice (unpublished observations).

The colorectum is innervated by the thoracolumbar/hypogastric and the lumbosacral/pelvic afferent fibers[1,2]. It emerges that these two sets of primary afferents may differ significantly in their functional properties and may signal different qualities of colonic stimuli[14,15,16,17]. An important advantage of the current protocol is its potential to comparatively study the hypogastric and pelvic afferents of the colorectum. As illustrated in **Figure 1**, a lumbar splanchnic (hypogastric) nerve and 3 - 4 branches of the PN pass through the major PG and then project to the descending colon and the urinary bladder. We noted that the mechanosensory response of the PN bundle was qualitatively similar to that of the nerve distal to the PG, which presumably contained mixed lumbar splanchnic and pelvic afferents. Technically, it is highly feasible to place two suction electrodes near the PG to simultaneously record the LSN and the PN and to assess if they respond differently to mechanical and chemical stimulation.

Disclosures

The authors declare no conflict of interest.

Acknowledgements

This protocol was supported by research grants from the National Natural Science Foundation of China (#31171066, #81270464) and the Sino-German Science Center (GZ919).

References

1. Al-Chaer, E.D., Traub, R.J. Biological basis of visceral pain: recent developments. *Pain.* **96** (3), 221-225 (2002).
2. Christianson, J.A., Traub, R.J., Davis, B.M. Differences in spinal distribution and neurochemical phenotype of colonic afferents in mouse and rat. *J Comp Neurol.* **494** (2), 246-259 (2006).
3. Wynn, G., Rong, W., Xiang, Z., Burnstock, G. Purinergic mechanisms contribute to mechanosensory transduction in the rat colorectum. *Gastroenterology.* **125** (5), 1398-1409 (2003).
4. Dong, L. *et al.* Impairments of the Primary Afferent Nerves in a Rat Model of Diabetic Visceral Hyposensitivity. *Mol Pain.* **11**, (2016).
5. Lynn, P.A, Blackshaw, L.A. In vitro recordings of afferent fibres with receptive fields in the serosa, muscle and mucosa of rat colon. *J Physiol.* **518** (Pt 1), 271-282 (1999).
6. Page, A.J. *et al.* Different contributions of ASIC channels 1a, 2, and 3 in gastrointestinal mechanosensory function. *Gut.* **54** (10), 1408-1415 (2005).
7. Hockley, J.R. *et al.* P2Y Receptors Sensitize Mouse and Human Colonic Nociceptors. *J Neurosci.* **36** (8), 2364-2376 (2016).
8. Peiris, M. *et al.* Human visceral afferent recordings: preliminary report. *Gut.* **60** (2), 204-208 (2011).
9. Feng, B., Gebhart, G.F. Characterization of silent afferents in the pelvic and splanchnic innervations of the mouse colorectum. *Am J Physiol Gastrointest Liver Physiol.* **300** (1), G170-G180 (2011).
10. Feng, B. *et al.* Activation of guanylate cyclase-C attenuates stretch responses and sensitization of mouse colorectal afferents. *J Neurosci.* **33** (23), 9831-9839 (2013).
11. Feng, B., Gebhart, G. F. *In vitro* Functional Characterization of Mouse Colorectal Afferent Endings. *J Vis Exp.* (95), e52310 (2015).
12. Nullens, S. *et al.* In Vitro Recording of Mesenteric Afferent Nerve Activity in Mouse Jejunal and Colonic Segments. *J Vis Exp.* (116) (2016).
13. Rong, W., Hillsley, K., Davis, J.B., Hicks, G., Winchester, W.J., Grundy, D. Jejunalafferent nerve sensitivity in wild-type and TRPV1 knockout mice. *J Physiol.* **560** (Pt 3), 867-881 (2004).
14. Brierley, S.M. *et al.* Differential chemosensory function and receptor expression of splanchnic and pelvic colonic afferents in mice. *J Physiol.* **567** (Pt 1), 267-281 (2005).
15. Brierley, S.M., Jones, R.C. 3rd, Gebhart, G.F., Blackshaw, L.A. Splanchnic and pelvic mechanosensory afferents signal different qualities of colonic stimuli in mice. *Gastroenterology.* **127** (1), 166-178 (2004).
16. La, J.H., Schwartz, E.S., Gebhart, G.F. Differences in the expression of transient receptor potential channel V1, transient receptor potential channel A1 and mechanosensitive two pore-domain K+ channels between the lumbar splanchnic and pelvic nerve innervations of mouse urinary bladder and colon. *Neuroscience.* **186**, 179-187 (2001).
17. Wang, G., Tang, B., Traub, R.J. Differential processing of noxious colonic input by thoracolumbar and lumbosacral dorsal horn neurons in the rat. *J Neurophysiol.* **94** (6), 3788-3794 (2005).

