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TITLE:

***In vitro* recording of mesenteric afferent nerve activity in mouse jejunal and colonic segments**

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SHORT ABSTRACT: *(10 words minimum, 50 words maximum)*

Mesenteric afferent nerves convey information from the gastrointestinal tract towards the brain regarding normal homeostasis as well as pathophysiology. Gastrointestinal afferent nerve activity can be assessed by mounting isolated intestinal segments with attached afferent nerves into an organ bath, isolating the nerve, and assessing basal as well as stimulated activity.

LONG ABSTRACT: *(150 words minimum, 300 words maximum)*

Afferent nerves not only convey information concerning normal physiology, but also signal disturbed homeostasis and pathophysiological processes of the different organ systems from the periphery towards the central nervous system. As such, the increased activity or 'sensitization' of mesenteric afferent nerves has been allocated an important role in the pathophysiology of visceral hypersensitivity and abdominal pain syndromes.
Mesenteric afferent nerve activity can be measured *in vitro* in an isolated intestinal segment that is mounted in a purpose-built organ bath and from which the afferent nerve is isolated, allowing researchers to directly assess nerve activity adjacent to the gastrointestinal segment. Activity can be recorded at baseline in standardized conditions, during distension of the segment or following the addition of pharmacological compounds delivered intraluminally or serosally. This technique allows the researcher to easily study the effect of drugs targeting the peripheral nervous system in control specimens; besides, it provides crucial information on how neuronal activity is altered in disease. It should be noted however that measuring afferent neuronal firing activity only constitutes one relay station in the complex neuronal signaling cascade, and researchers should bear in mind not to overlook neuronal activity at other levels (e.g. dorsal root ganglia, spinal cord or central nervous system) in order to fully elucidate the complex neuronal physiology in health and disease.
Commonly used applications include the study of neuronal activity in response to the administration of lipopolysaccharide, and the study of afferent nerve activity in animal models of irritable bowel syndrome. In a more translational approach, the isolated mouse intestinal segment can be exposed to colonic supernatants from IBS patients. Furthermore, a modification of this technique has been recently shown to be applicable in human colonic specimens.

INTRODUCTION: *(150 words minimum, 1500 words maximum)*

Sensory signaling and pain perception is a complex process that results from an intricate interplay between afferent nerves, spinal neurons, ascending and descending facilitatory and inhibitory pathways and several different brain regions. As such, changes at one or more of these levels may result in altered sensory signaling and visceral pain in disease states. To study all these different aspects of sensory signaling multiple techniques have been developed ranging from single cell experiments (e.g. calcium imaging on neurons) to whole animal models (e.g. behavioral responses such as the visceromotor response). The technique described in this paper allows researchers to specifically assess afferent nerve activity *in vitro* from an isolated segment of small bowel or colon in rodents. In short, an isolated gastrointestinal segment (usually jejunum or colon) is mounted in a purpose-built recording chamber perfused with a physiological Krebs solution. The afferent nerve is dissected free and connected to an electrode allowing registration of afferent neuronal activity in splanchnic or pelvic afferent nerves. Nerve activity can be recorded basally or in response to increasing intraluminal pressures and/or pharmacological compounds that can be applied either directly into the recording chamber (serosally), or via the intraluminal perfusate (mucosally) to assess their effect on afferent discharge (1-6).
Afferent neuronal activity in response to other stimuli, such as mucosal stroking, probing using von Frey hairs or stretching of the segment can be studied in a modified experimental setup in which the intestinal tissue is pinned down and opened longitudinally (which in contrast to our setup using an intact segment), as was described in a previous issue of *Jove* (7;8). In addition, only recently, a technique was described to study colonic afferent nerve activation in the colonic wall itself via calcium imaging, again using a pinned down, longitudinally opened segment (9).

An alternative version of this *in vivo* technique consists out of measuring neuronal activation near the afferent's entry into the spinal cord. In short, the sedated animal is placed in the prone position, exposing the lumbosacral spinal cord to which the afferent nerve of interest projects by means of laminectomy, constructing a similar paraffin-filled well using the skin of the incision and draping the dorsal rootlet over a platinum bipolar electrode (10;11). This technique furthermore allows researchers to characterize fibers based upon their conduction velocity, and distinguish unmyelinated C-fibers from thinly myelinated Aδ-fibers.

Recording afferent nerve discharge *in vitro* from isolated gut segments can also been done using human specimens, as two research groups independently published *first-in-man* manuscripts recording colonic afferent nerve activity in human resection specimens (12;13). The implementation of this technique could result in a more readily translation of murine data to the humane state, and could allow researchers to easily identify drugs targeting the sensitized sensory nerve. The clinical importance of characterizing the afferent nerve activity, as well as the discovery of new therapeutic reagents that target exorbitant afferent nerve activity, has been elaborately discussed by many experts in the field (14-16). Several targets have already been identified and verified, such as the transient receptor potential (TRP) channels, opioid and cannabinoid receptors among others. As such, increased TRPV1-expression has been demonstrated in patients with irritable bowel syndrome, and correlates well with visceral pain

scores (17); furthermore, TRPV1 antagonists decreased visceral hypersensitivity in an animal model of acute colitis and visceral pain (18;19).

The aforementioned *in vitro* technique complements the more commonly known *in vivo* measurement of afferent nerve activity. During *in vivo* neuronal activity measurement, nerve activity can be measured directly in the sedated animal during which the segment of interest in identified and subsequently intubated, and a liquid paraffin-filled well is constructed using the abdominal wall and skin of the rodent (20). The afferent nerve of interest is then identified, sectioned and placed on a bipolar platinum electrode, allowing neuronal activity measurement. This technique allows the researcher to modulate afferent nerve activity in living albeit sedated animals; as such, one can study neuronal activity is response to e.g. luminal distension or the intravenous administration of a compound.

Translational research nowadays mainly focuses on the application of human-derived supernatants (*e.g*. from colonic biopsies, cultivated peripheral blood mononuclear cells,…) on jejunal and/or colonic mouse afferents (21;22). As such, it was shown that that colonic mucosal biopsy supernatans from patients with irritable bowel syndrome can cause hypersensitivity in mouse colonic afferents, guinea pig submucous neurons and mouse dorsal root ganglion neurons (21;23;24).

Finally, recording neuronal activity is not restricted to the mesenteric and/or pelvic afferents, as afferent nerve activity has been characterized in an animal model of rheumatoid arthritis measuring afferents supplying the knee joint (25). Others have characterized bladder afferent nerve activity as well (26-28), and demonstrated that pelvic afferents from the bladder as well the gastrointestinal tract converge, possibly resulting in neuronal crosstalk (29).

PROTOCOL *(min 2 max 10 pages)*

All animal experiments described below were approved by the Committee for Medical Ethics and the use of Experimental Animals at the University of Antwerp (file number 2012-42).

1. Tissue preparation of jejunal and colonic afferent nerves

1.1) Preparation of the jejunal afferent nerve

1.1.1) Humanely kill the adolescent or adult rodent of interest by means of the desired euthanasia method that has been approved prior to the experiment by the local Ethical Committee (*e.g*. terminal sedation followed by cardiac puncture, cervical dislocation,…).

1.1.2) Place the sacrificed laboratory animal in the supine position and perform an abdominal midline incision through the skin and abdominal muscle layer using a scalpel, extending from the xyphoid process until the pubic bone.

1.1.3) Bath the abdominal cavity with cold Krebs solution in order to prevent the intra-abdominal tissues from drying out (Krebs composition: 120.03 mM NaCl, 6.22 mM KCl, 1.57 mM NaH_2PO_4, 15.43 mM $NaHCO_3$, 1.21 mM $MgSO_4$, 11.52 mM D-glucose and 1.52 mM $CaCl_2$).

1.1.4) Rapidly excise the entire jejunum, taking care not to damage surrounding structures, keeping the bowel's mesentery, which contains jejunal blood vessels and afferent nerves, intact.

1.1.5) Place the excised jejunum in ice-cold Krebs solution and keep on ice, while oxygenating the Krebs solution continuously with carbogen (95% O_2, 5% CO_2).

1.1.6) Cut the jejunum with sharp scissors in approximately 3-cm long loops; the mesenteric bundle containing the vessels and nerve should originate somewhere near the center of the respective loop.

1.1.7) Flush each segment with Krebs solution using a blunt catheter to remove luminal contents and chyme as these contain digestive enzymes that will accelerate the deterioration of the tissue sample *in vitro*. During flushing, take care not to damage the lumen of the loop, as the destruction of the *villi* will result in the release of mediators that can alter the afferent nerve activity

1.1.8) Identify consistently the segment you wish to measure afferent nerve activity from (e.g. the first jejunal segment distally of the ligament of Treitz), and place this in a purpose-built recording chamber that is constantly perfused with warm, oxygenated Krebs solution at a rate of 10 mL/min; the Krebs' temperature in the recording chamber should be kept constant at 34 °C. The bottom of the recording chamber should be covered with a thin Sylgard® silicone elastomer layer.

1.1.9) Mount the jejunal segment in the organ bath so that the oral end is connected to the syringe driver providing luminal flow and the aboral end connects to the outflow. The segment can be slightly stretched but take care not to exert excessive tension. Attach both ends firmly using 4/0 silk ligatures to the in- and outflow ports.

1.1.10) Attach the syringe driver to the oral end, and perfuse the jejunal segment intraluminally with Krebs solution (non-oxygenated, ambient temperature) at a rate of 10 mL/h.

1.1.11) Pin the mesentery of the mounted intestinal segment flat against the Sylgard bottom layer, using insect pins. The mesentery should be stretched out in order to optimize

visualization of the mesenteric bundle; no strain should be exerted on the bundle or the jejunum.

1.1.12) Perform a test ramp distension (*vide infra)* by closing the output port until the intraluminal pressure of the intestinal segment reaches 60 mmHg, in order to verify that no intraluminal Krebs solution is leaking from the mounted segment. A smooth rise in intraluminal pressure without interruptions should be observed. Of note, small contractions of the segment (peristaltic waves) can be observed during the initial distension phase; if required, peristaltic activity can be blocked by adding 1 µM of the L-type calcium channel blocker nifedipine to the Krebs solution.

1.1.13) Under a stereomicroscope, gently start to peel off the fat tissue from the mesentery, starting at a remote distance from the jejunum, and expose both blood vessels in the mesenteric bundle. The afferent jejunal nerve can be localized in between both vessels as a thin, white thread encapsulated in adipose tissue. Only work your way up towards the jejunum when you cannot adequately identify both mesenteric vessels and/or the afferent nerve.

1.1.14) Dissect the jejunal mesenteric nerve of the segment free over a distance of several millimeters.

1.1.15) Transect the nerve using sharp tissue scissors. If required, the myelin layer can be carefully peeled off.

1.1.16) Using a micromanipulator, lower the tip of the suction electrode, connected to a syringe with plunger, into the organ bath; then, by manipulating the plunger, gently aspirate some Krebs solution from the organ bath so that the tip of the electrode is submerged in the Krebs solution (figure 1).

1.1.17) Position the tip of the suction electrode immediately next to the transected afferent nerve strand and draw the transected nerve strand into the capillary over its entire length.

1.1.18) Maneuver the tip of the electrode towards some adipose tissue and aspirate this into the glass capillary while firmly aspirating with your plunger, thereby mechanically 'sealing' the nerve in the capillary from the contents of the organ bath.

1.1.19) Verify the recording of afferent nerve activity with your data acquisition system, e.g. by performing a ramp distension-induced increase in afferent firing (*vide infra*). Following the isolation of the nerve into the suction electrode, a 15 min break should be respected in the experimental protocol in order to obtain a steady state spontaneous afferent nerve activity before performing your actual experiments.

1.1.20) To perform ramp distension, distend the intestinal segment by closing the output port, leading to gradual rise in pressure in the intestinal segment (up to 60 mmHg). The desired experimental protocol should only be performed when three consecutive ramp distensions (with a 15 min interval) yield a reproducible multi-unit discharge (figure 2).

1.2) Preparation of the lumbar splanchnic afferent (colonic afferent nerve)

The dissection of colonic afferent nerves requires a more detailed dissection. Deviations from the former 'jejunal' protocol are listed below:

1.2.1) Humanely cull the animal, place it in the supine position, perform a midline laparotomy

and excessively pour ice-cold Krebs solution in the abdominal cavity.
(Krebs composition: 118 mM NaCl, 4.75 mM KCl, 1 mM NaH_2PO_4, 22 $NaHCO_3$, 1.2 mM $MgSO_4$, 11 mM D-glucose and 2.5 mM $CaCl_2$, 3 µM indomethacin). Note the altered composition of the Krebs solution; indomethacin is added to the solution in order to prevent alterations of afferent nerve activity by prostaglandins.
1.2.2) Discard the adipose tissue, bladder and internal genitalia, and gently shift the small bowel to one side in the abdominal cavity. Perform an extended prelevation of the distal part of the colon with intact mesenteric nerves from the abdomen. Important landmarks that may be included in this dissection to aid further preparation of the tissue include the abdominal aorta and vena cava, the left kidney and the pelvic floor musculature. Take care not to exert traction on the connecting tissue between colon and the abdominal aorta, as this region contains the lumbar splanchnic nerves.
1.2.3) Transfer the tissue segment into the Sylgard lined organ chamber. Use the left renal artery, which originates from the abdominal aorta, as a starting point. Follow the abdominal in the aboral direction, after which you will encounter the superior mesenteric artery together with the celiac and superior mesenteric ganglion. Finally, the connecting tissue between the distal part of the colon and the aorta will be reached, in which the nerve of interest is located.
1.2.4) Identify the inferior mesenteric artery originating from the abdominal aorta. The lumbar splanchnic afferent nerve can be identified at the base of the inferior mesenteric artery; it runs parallel to the artery (figure 3).
1.2.5) Following the transection of the nerve, gently peel of the myelin sheath and tease the nerve into several fine threads. Make sure to keep a safe distance from the colon.
1.2.6) Draw one of these single threads into the suction electrode, 'seal' the capillary with surrounding adipose tissue as previously described and perform the desired experimental protocol.
1.2.7) Discard any superfluous tissue that is present in the organ bath (e.g. kidneys, abdominal vessels, muscle tissue), as these may disturb the afferent signal.
1.2.8) Nifedipine (1µM) can be added to the Krebs solution should the afferent nerve signal be disturbed by spontaneous intestinal movement due to smooth muscle cell contractions.
1.2.9) Drugs can be administered serosally by dissolving the desired compound into the Krebs that perfuses the organ chamber, or temporarily halt the perfusion and directly administer the compound into the organ bath. A third option consists out of dissolving the compound of interest into the Krebs solution in the syringe drive, that way the drug shall be administered intraluminally. Desensitization of the afferent nerve can occur when cumulative dosages of a compound are administered too fast consecutively.

[Place figure 1 here]
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2. Data acquisition

Nerve activity is recorded via a suction electrode connected to a Neurolog Headstage (NL100AK, Digitimer), amplified (gain 10k, NL104, Digitimer) and filtered (NL125, Digitimer, bandpass 500-5000 Hz). The signal is digitized by a CED1401 analog-digital converter (Cambridge Electronic

Design) and sampled at 20 kHz for analysis using the Spike2 software (Cambridge Electronic Design).

3. Analysis

Afferent nerve discharge can be reported as the overall number of impulses/second for the entire nerve or can be further analyzed using specialized software as multi-unit recordings of overall nerve activity contain action potentials of different shape, amplitude and width, corresponding to different nerve units in each afferent fiber (figure 2). These individual wavemarks are matched to predefined templates using the computerized Spike2 Cambridge Electronic Design software (UK), allowing discrimination between single-units. Before allocation of a spike to a certain wavemark, a signal-to-noise ratio of >2:1 should be enforced. The action potential responses are calculated by subtracting the spontaneous activity at baseline (intraluminal pressure of 0 mm Hg) from the response during distension at fixed time points (5 mm Hg of increment from 0 to 20 mmHg intraluminal pressure, increments of 10 mm Hg from 20 mm Hg onwards). Baseline afferent discharge was measured using a bin size of 10 seconds.

The single-unit analysis can be used to classify fibers based upon their discharge profile during ramp distension (figure 4). In addition, single-unit analysis can be utilized to study the chemosensitive profile of different types of units, as not all types of units will display the same altered firing activity in response to a drug or compound.

Low threshold fibers typically exert increased discharge at the lower distension pressures, high threshold fibers (HT) at the highest distension pressures, whereas wide dynamic range fibers (WDR) demonstrate increased firing during the entire ramp distension as graphically shown in figure 4. Mechanically insensitive afferent (MIA) nerve fibers typically do not respond to ramp distensions (Blackshaw, Curr Opin Pharmacol 2002; Booth, NGM 2008; Deiteren, NGM 2015). The nerve firing response at 20 mmHg is expressed as the percentage of the maximum firing response during distension (LT%) and reflects the extent of firing at low distension pressure. Therefore, LT fibers are characterizes by an LT% >55%, whereas HT are defined by a value of <15%. Values for WDR units range between 15 and 55% (Booth, NGM 2008). A MIA displays spontaneous afferent firing that is unaffected by distensions.

[Place figure 4 here]

REPRESENTATIVE RESULTS:

1. Multi unit jejunal afferent nerve activity

Jejunal afferent nerve activity was measured at baseline and in response to ramp distension in 9 eight-week old male OF-1 mice. Animals were housed in groups in standardized conditions (6 animals per cage, 20-22 °C, humidity 40-50%, 12h light-dark cycle) with unlimited access to tap water and regular chow. Jejunal segments of mice displayed irregular spontaneous afferent nerve discharge at baseline at an intraluminal pressure of 0 mmHg (mean spontaneous activity 11.47 $\pm$ 3.31 imp/s).

The jejunal afferent nerve activity increased upon performing ramp distensions up until 60 mmHg. Typically, the increase in afferent nerve activity following the rise in intraluminal pressure is characterized by a biphasic response (figure 5), consisting of an initial rapid increase in firing activity up until the intraluminal pressure reaches 20 mmHg, which can mainly be attributed to the increased firing rate of *low threshold* fibers. This is then followed by a plateau phase, after which a second increase in firing activity can be observed from 40 mmHg onwards, representing the activation of predominantly *high threshold* fibers.

[Place figure 5 here]

2. Single unit jejunal afferent nerve activity

Based upon their waveforms, single-units can be discriminated in each multi-unit recording and classified in one of the aforementioned categories. In 9 mice, we discriminated 40 different units (4.44 $\pm$ 1.01 units/jejunal afferent nerve), with the LT units being the most prevalent ones, followed by WDR and HT fibers (*figure 6*).

The firing activity of the different units in response to ramp distension can be observed in figure 7.

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Figure Legends:

Figure 1: Schematical overview of the purpose-built organ chamber and suction electrode
Detailed overview of the technical setup with the suction electrode and the organ chamber in place.

Figure 2: Representative tracing of the *in* vitro recording of jejunal afferent nerve activity.
Typical recording of jejunal multi-unit afferent nerve activity (imp.s^{-1})(upper panel) at baseline and in response to 2 ramp distensions up until 60 mmHg (lower panel), and the subsequent identification (wavemark analysis) of different single-units in the nerve signal (third panel).

Figure 3: Neuroanatomy of the colon
A) Sensory information from the colon is conveyed via the lumbar colonic nerves (LCN) towards the central nervous system, with the LCN running in close proximity to the inferior mesenteric artery (IMA). A portion of the fibers from this lumbar colonic nerve will course along the intermesenteric nerve (IMN) to form the lumbar splanchnic nerves (LSN). **B)** A schematic overview of the experimental set-up. Afferent recording of the LCN is performed in an organ both by means of a suction electrode connected to the data acquisition system. Ramp distension can be performed upon closure of the outlet port while continuing the inflow of Krebs solution.

Figure 4: Schematic representation of the different afferent fiber units based on their mechanosensitive profile. Units are classified based upon the percentage (LT%) of their firing rate at 20 mmHg distension pressure compared to the maximum firing response during distension. Low threshold fibers (upper left panel) predominantly display an increased nerve activity at low distension pressures, resulting in an LT% of over 55%. High threshold units (upper right panel) on the contrary only display an increase in firing rate at noxious pressures (%LT < 15). Wide dynamic range fibers (lower left panel) display a gradual increase in nerve activity during the entire distension (%LT ranging between 15 and 55), whereas mechanically insensitive fibers (lower right panel) do not respond to increasing distension pressures.
LT%: (afferent firing at 20 mmHg / maximal afferent firing)

Figure 5: Mesenteric afferent nerve discharge (imp.s^{-1}) in wild-type mice during ramp distension. Mesenteric multi-unit afferent nerve discharge (imp/s^{-1}) in wild-type mice during ramp distension for the whole nerve.

Figure 6: Single unit distribution of 40 units identified in jejunal afferent nerves from 9 wild-type mice. HT: high threshold fiber, LT: low threshold fiber, MIA: mechanically insensitive fiber, WDR: wide dynamic range fiber.

Figure 7: Pressure-response curves for the different types of subunits in wild-type mice. HT: high threshold, LT: low threshold, MIA: mechanically insensitive afferent, WDR: wide dynamic range.

DISCUSSION: *(3-6 paragraphs)*

The protocol in this paper describes a reproducible laboratory technique to study mesenteric afferent nerve activity in rodents as used by our group as well as others (3;4;7;8;12;20;21;30).
By recording afferent nerve activity in close proximity to the jejunum or colon, one can isolate the first part of the afferent signal transduction chain and easily study the contribution and alterations that occur at the sole afferent level without interference from the central nervous system. The disadvantage then off course is the fact that *in vitro* observations cannot always be effortless extrapolated to the *in vivo* setting, as the *in vitro* setup only incorporates one relay station in the complex nerve signaling cascade. As such, a broader picture must be made incorporating all other stations, such as the dorsal root ganglia, central nervous system (e.g. functional brain imaging) and descending (inhibitory) efferent pathways.
Another advantage of this method constitutes the rather simple technical procedure, as one no longer has to monitor the wellbeing of the lab animal that provides the gastrointestinal specimen. On the other hand is the *in vitro* measurement of neuronal activity not suitable for elucidating the effect of a systemically administered drug on afferent nerve discharge, however researchers can overcome this obstacle by systemically administering the drug of interest to the animal, then followed by the *ex vivo in vitro* recording of afferent nerve activity.
Researchers attempting to implement this technique must also bear in mind that the identification and isolation of the mesenteric afferent and pelvic afferents obviously requires knowledge of basic anatomy and technical training, and researchers ought to be acquainted with the basic principles of neuronal electrophysiology.
The *in vitro* setting furthermore allows to easily identify possible pharmacological targets, and provides insight on the physiological role of neuronal activity as well as altered sensory signaling in several disease processes.
In case of jejunal afferent measurements, several tissue segments of a single animal can be studied simultaneously, a feature that is rather difficult using an *in vivo* setup. Researchers however should cautiously interpret results obtained from different segments, as regional differences could bias results. Therefore we would recommend to consistently measure afferent nerve activity from the same site (e.g first segment distally from the ligament of Treitz or the duodenojejunal flexure).

Critical steps in the protocol include the rapid isolation of the tissue, the aspiration of the nerve strand into the suction electrode and the adequate 'sealing' of the glass capillary from the organ bath by aspirating surrounding adipose tissue into the capillary. The aperture of the glass capillary should be precisely determined: an aperture that is too small will complicate the aspiration of the nerve strand into the electrode, whereas a too wide aperture will hinder the 'sealing' of the capillary with adipose tissue, resulting in redundant background noise that will hamper the analysis (low signal-to-noise recordings). Also to allow for reliable single-unit classification, splanchnic afferents should be divided in different strands in order to reduce the number of units in the recording. Typically, we would suggest aiming to have a maximum of 4-5 units in each recording. Researchers therefore ought to adjust the aperture based upon the fiber of interest, and the lab animal that is applied.
Another critical point encompasses the sufficient grounding of the experimental setup. The

suction electrode and recording chamber should be adequately grounded and covered by a Faraday cage in order to minimize interfering electrical fields that impede the analysis of neuronal activity, whereas all other equipment including the recording apparatuses, syringe driver et cetera should be installed outside the cage.

Typical applications of this technique comprise the screening of pharmacological compounds that can alter sensitization of mesenteric afferents during pathologies that are characterized by visceral hypersensitivity and pain. As already mentioned before, these target of these compounds can be encountered somewhere along the intricate nervous system ranging from the enteric intrinsic nervous system to the brain; as such, characterizing and modulating afferent nerve activity contributes to the broader picture that also encompasses calcium imaging of the intrinsic enteric nerves and dorsal root ganglia, measurement of the visceromotor response as an indicator of visceral hypersensitivity *in vivo*, and functional brain imaging, among others.

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SN performed the experiments described above, performed the data analysis and drafted the manuscript. AD and JDM implemented the technique at our research facilities and aided in the data analysis. HC aided in performing the experiments. SF, JDM and BDW designed the study. All authors critically read and approved the final manuscript. SN is an aspirant of the Fund for Scientific Research (FWO), Flanders (11G7415N). This work was supported financially by the FWO (G028615N and G034113N).

DISCLOSURES

The authors have nothing to disclose.

TABLE OF SPECIFIC REAGENTS/EQUIPMENT – *supplementary Excel file*

REFERENCES *(10 minimum— use* JoVE*'s style files to interface with reference software)*

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Figure 1

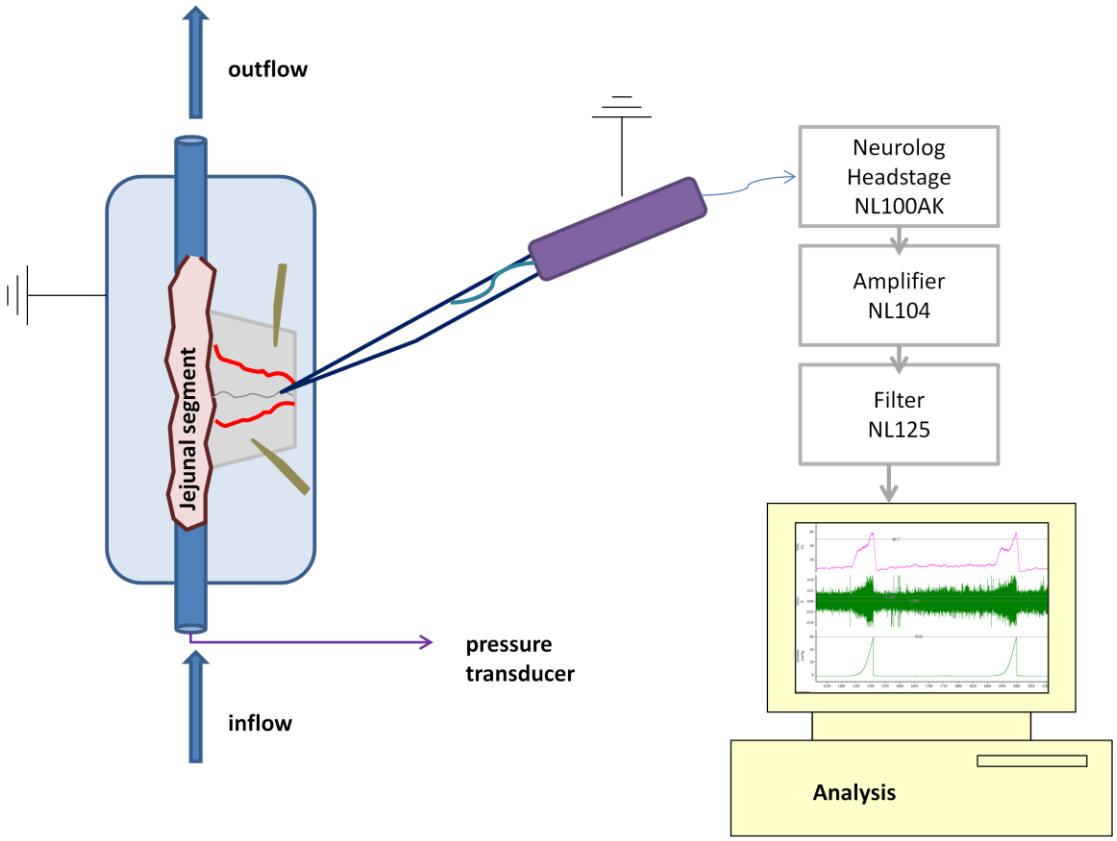

outflow
Jejunal segment
pressure
transducer
inflow
Neurolog
Headstage
NL100AK
Amplifier
NL104
Filter
NL125
Analysis

Figure 2

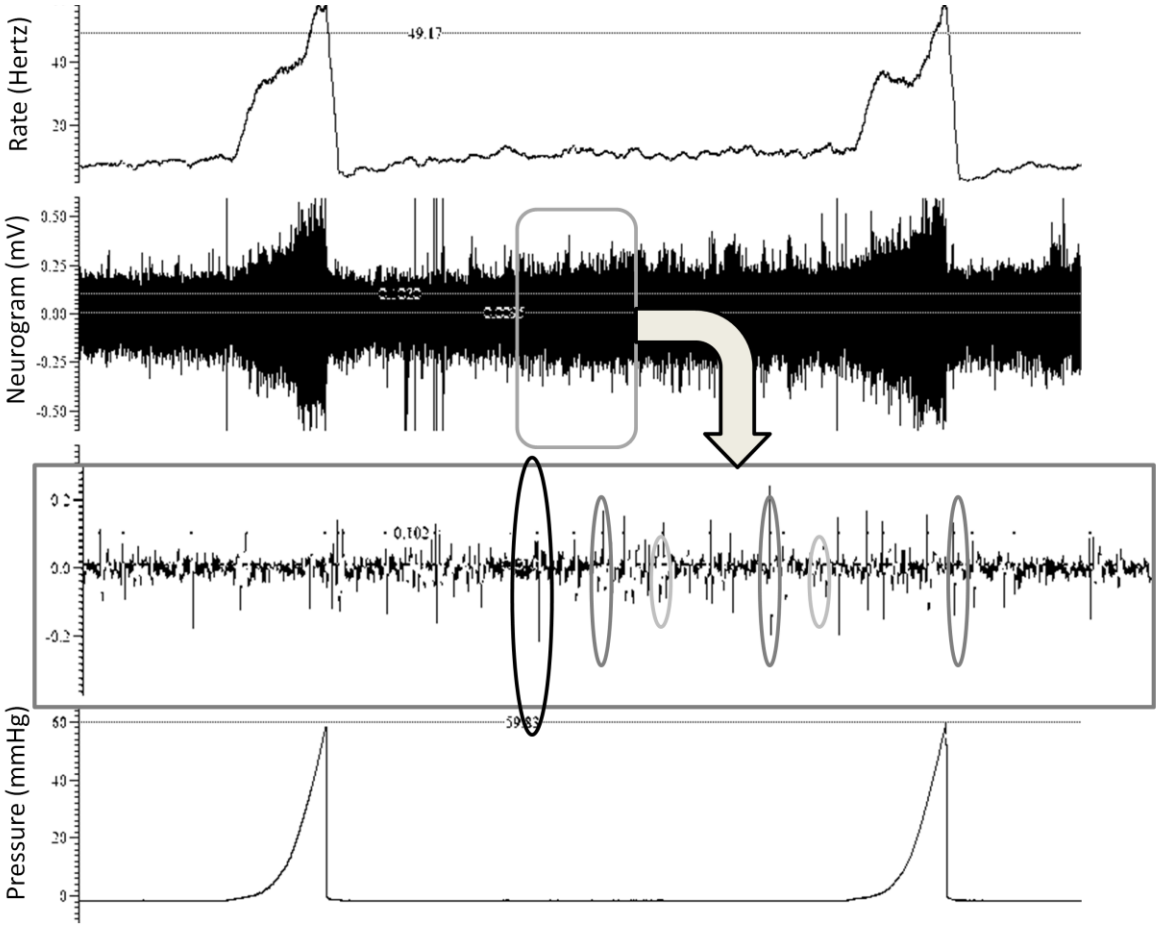

Rate (Hertz)
49.17
40
20
Neurogram (mV)
0.50
0.25
0.00
-0.25
-0.50
0.102
0.2
0.0
-0.2
Pressure (mmHg)
60
40
20
0

Figure 3

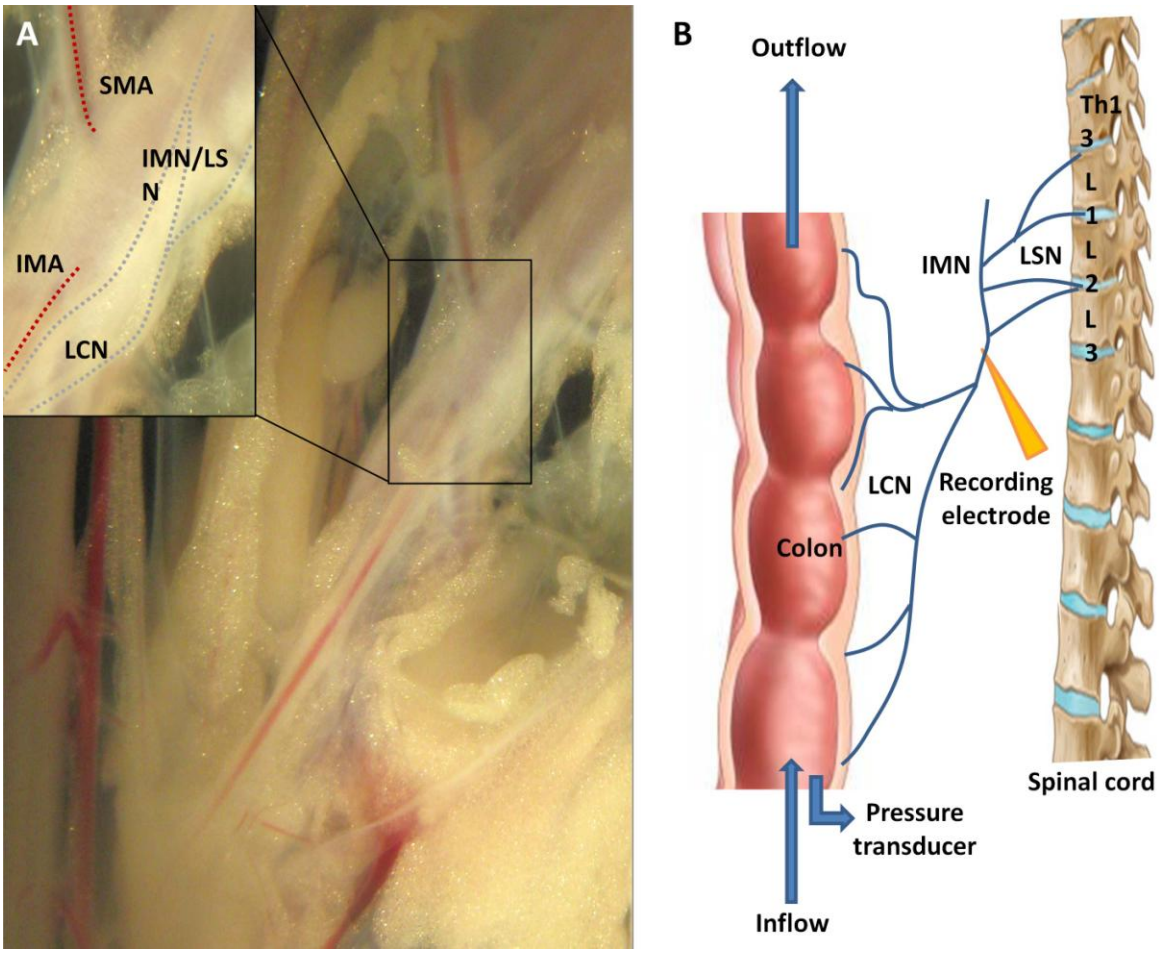
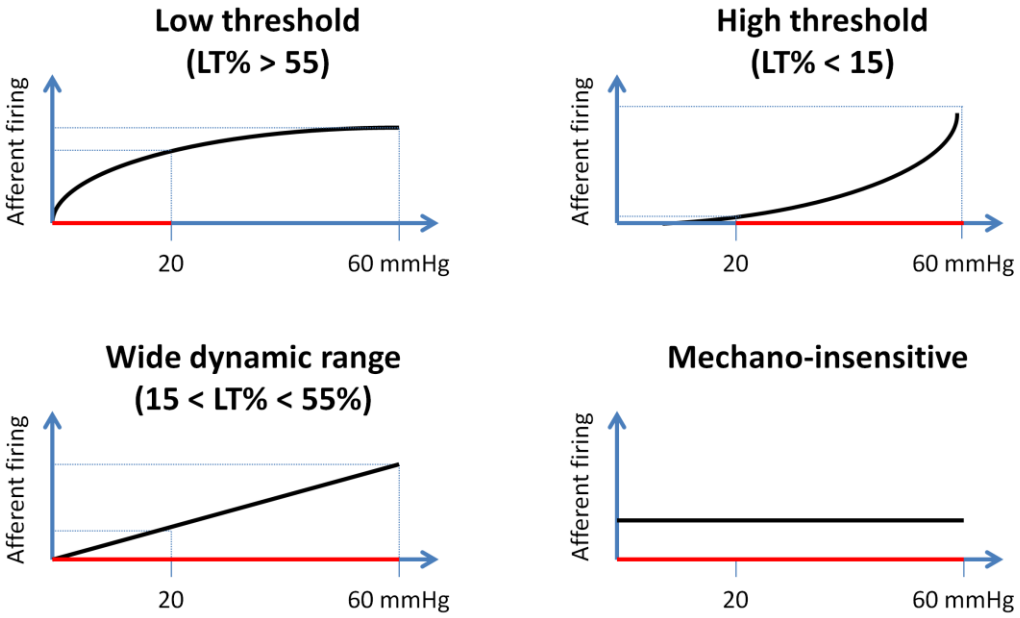

A
SMA
IMN/LSN
IMA
LCN
B
Outflow
Th1
3
L
1
L
2
L
3
IMN
LSN
LCN
Recording electrode
Colon
Spinal cord
Pressure transducer
Inflow

Figure 4



LT% = (afferent firing at 20 mmHg)/ (max afferent firing)

Figure 5

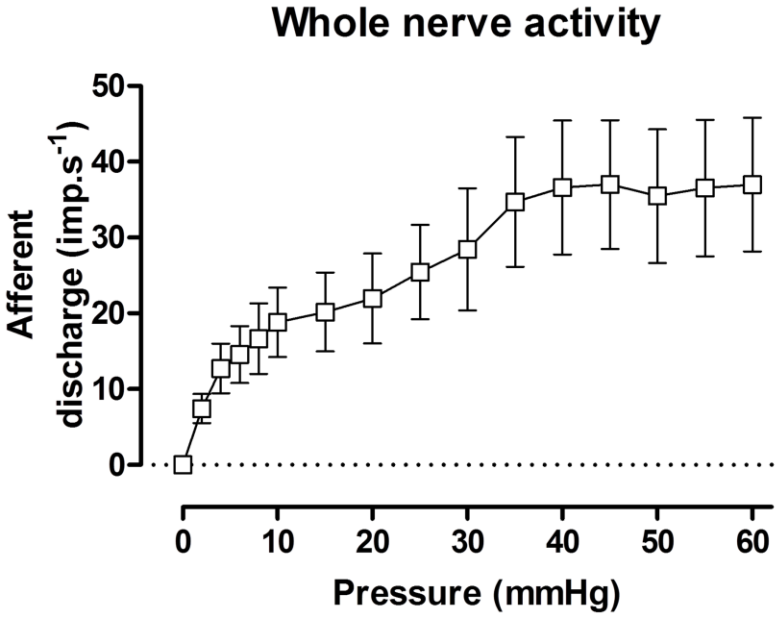

Whole nerve activity
Afferent discharge (imp.s-1)
50
40
30
20
10
0
0
10
20
30
40
50
60
Pressure (mmHg)

Figure 6

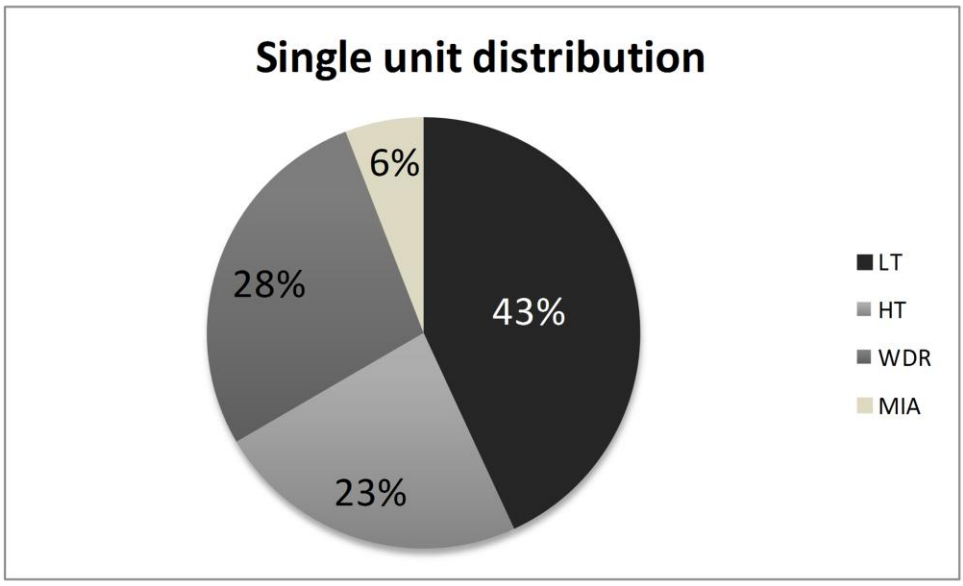
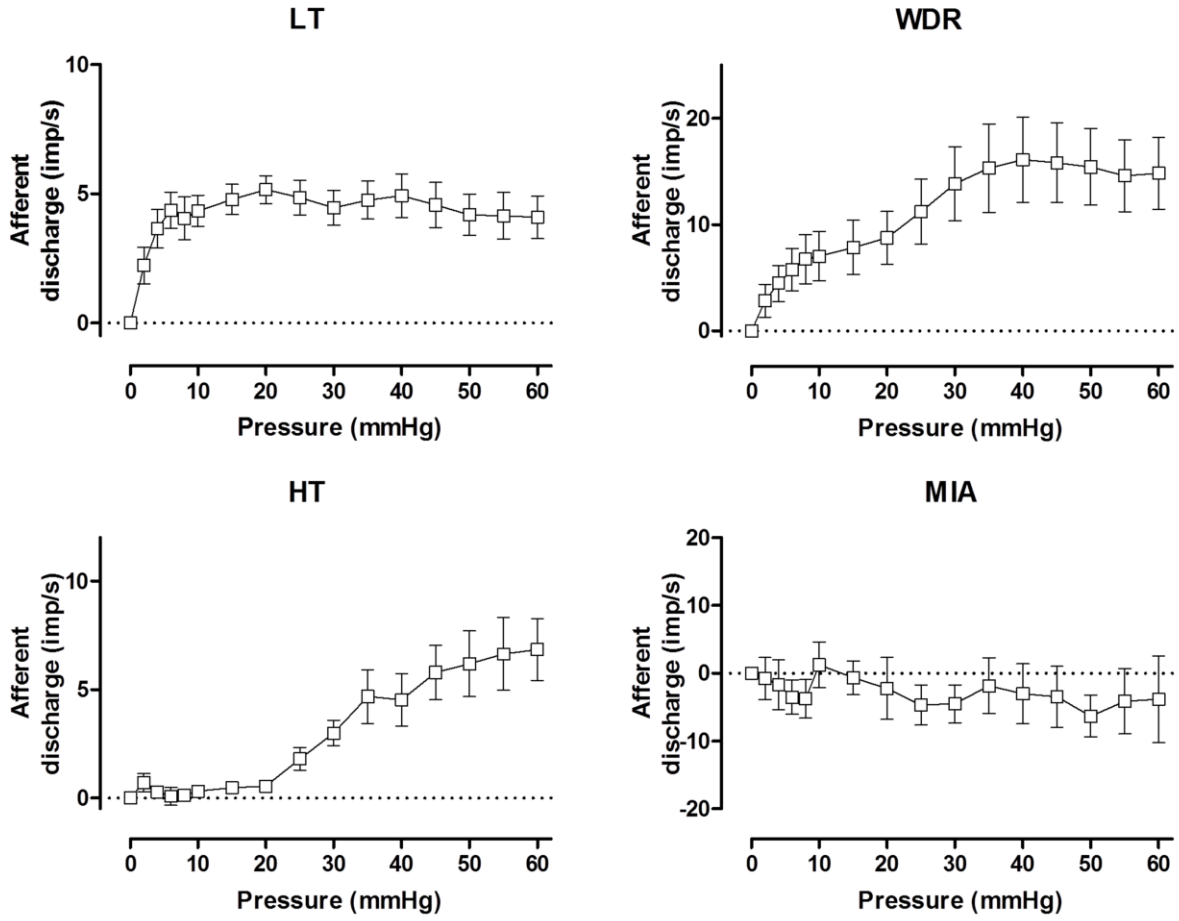


Figure 7


LT
WDR
HT
MIA
Afferent discharge (imp/s)
Pressure (mmHg)
0
10
20
30
40
50
60
-20
-10
5