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Pelvic nerve input mediates descending modulation of homovisceral processing in the thoracolumbar spinal cord of the rat

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Abstract

Background and aims—Colonic afferents project to the lumbosacral and thoracolumbar spinal cord via the pelvic and hypogastric/lumbar colonic nerves, respectively. Both spinal regions process inflammatory colonic stimuli. The role of thoracolumbar segments in processing acute colorectal pain is questionable, however, since the lumbosacral spinal cord appears sufficient to process reflex responses to acute pain. Here we demonstrate that activity in pelvic nerve colonic afferents actively modulates thoracolumbar dorsal horn neuron processing of the same colonic stimulus via a supraspinal loop: homovisceral descending modulation.

Methods—Dorsal horn neurons were recorded in the rat thoracolumbar spinal cord following acute or chronic pelvic neurectomy and cervical cold block.

Results—Acute pelvic neurectomy or lidocaine inhibition of lumbosacral dorsal roots facilitated the excitatory response of thoracolumbar dorsal horn neurons to colorectal distention (CRD) and decreased the percentage of neurons inhibited by CRD, suggesting colonic input over the pelvic nerve inhibits thoracolumbar processing of the same stimulus. Ectopic activity developed in the proximal pelvic nerve following chronic neurectomy reactivating the inhibitory circuit, inhibiting thoracolumbar neurons. Cervical cold block alleviated the inhibition in intact or chronic neurectomized rats. However, the facilitated response following acute pelvic neurectomy was inhibited by cervical cold block exposing an underlying descending facilitation. Inhibiting pelvic nerve input following cervical cold block had minimal effect.

Conclusion—These data demonstrate that input over the pelvic nerve modulates the response of thoracolumbar spinal neurons to CRD via a supraspinal loop, and that increasing thoracolumbar processing increases visceral hyperalgesia.

Keywords

visceral pain; descending modulation; brainstem; inhibition; facilitation; spinal cord; colorectal distention; electrophysiology

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Introduction

The mechanisms underlying visceral pain lag behind our understanding of somatic pain. Somatic tissue is innervated by nerves that project centrally to a contiguous region of spinal cord¹. In contrast, visceral organs are dually innervated by sensory afferents which project in the same nerves as sympathetic and parasympathetic efferents projecting to two separate regions of the neuraxis. For example, the colorectum is innervated by primary afferents projecting to the thoracolumbar (T13-L2; TL) and lumbosacral (L6-S2; LS) spinal segments through the hypogastric/lumbar colonic nerves and pelvic nerve, respectively^{2, 3}. However, the role of these different pathways in processing visceral pain is unclear. Normal volunteers report pain from colorectal distention (CRD) is referred to sacral dermatomes while there is expansion of the area of referred pain into the TL dermatomes in patients with irritable bowel syndrome or Crohn's disease^{4, 5}. Experimentally, CRD evokes greater Fos expression and neuronal excitability in the LS spinal segments compared to the TL segments, and behavioral responses to acute CRD are mediated by the LS, but not TL spinal segments^{6–9}. However, colonic inflammation increases CRD-induced Fos expression and neuronal excitability in the TL spinal segments^{7, 9, 10}. The interpretation is the LS spinal cord mediates acute and inflammatory colorectal pain; the TL spinal cord contributing to chronic pain conditions.

One hypothesis to explain these observations are differences in the response of colonic afferents originating in the TL and LS DRG. Several *in vitro* studies lend support to this hypothesis^{11–13}, but *in vivo* data suggest differential responses of LS and TL colonic afferents are not sufficient to explain the low level of excitability in the TL spinal segments compared to the LS spinal segments^{14–16}.

Descending inhibitory and facilitatory projections originating in the brainstem modulate the excitability of dorsal horn neurons and reflex responses to noxious somatic and visceral stimuli (see 17 for review). Indeed, neurons in the TL and LS spinal segments responding to visceral stimuli can be biphasically modulated by electrical or chemical stimulation in the rostroventromedial medulla or spinal transection^{18–20}.

In the present study, we demonstrate that colorectal input through the pelvic nerve to the LS spinal cord inhibits processing in the TL spinal cord of that same stimulus and that this inhibition is supraspinally mediated: homovisceral descending modulation. Some of these data have been presented in abstract form^{21, 22}.

Materials and Methods

Animals

Experiments were performed on 146 adult male Sprague-Dawley rats (240–380g). Female rats were not used since it is very difficult to clearly identify and lesion the pelvic nerve. Animals were acclimated to a 12 h/12 h light/dark cycle and housed 2–3 to a cage. Following survival surgery, they were individually housed. Food and water were available *ad libitum*. All experimental procedures were approved by the University of Maryland Dental School Animal Care and Use committee and conform to the guidelines for use of experimental animals published by the International Association for the Study of Pain. Rats were fasted for 18–24 hours prior to the experiment.

Pelvic Neurectomy

A midline laparotomy in Nembutal (50mg/kg) anesthetized rats exposed the abdominal viscera. The major pelvic ganglia were bilaterally exposed, the pelvic nerves dissected free from surrounding tissue and cut. In 4 rats 2% lidocaine was applied to the exposed pelvic nerve for several minutes prior to the nerve cut. In sham animals the nerve was exposed, but not cut. The

wound was closed. The TL spinal cord was exposed by laminectomy for electrophysiological recording immediately after the pelvic neurectomy for the acute experiments and 6–8 days later for chronic experiments. Rats with bilateral pelvic neurectomies cannot micturate so the bladder was expressed manually twice daily.

In a pilot study, there were no labeled cell bodies in the L6-S2 DRG following pelvic neurectomy and tracer injection into to colon, confirming elimination of colonic afferent input to the LS spinal cord.

Electrophysiology

Extracellular signal unit recording was performed in acute and chronic neurectomized, acute and chronic sham surgery, and intact rats (data from intact rats were previously published⁹ and included here for comparison). It was deemed unnecessary to repeat the intact rat recordings since the same person did all the recordings and these experiments were all conducted over a 2 year period. Preparation for extracellular recording was as previously described⁹. Briefly, rats were anaesthetized with Nembutal, catheterized for infusing additional anesthetic (5–10 mg/kg/h) and paralytic (pancuronium bromide (0.2 mg/hr) while artificially ventilating, maintaining an end tidal CO₂ of 3.5–4.5% and a mean blood pressure of 100–130 mmHg.

A laminectomy exposed the T13-L2 spinal segments, the dura matter cut and the spinal cord covered in warm mineral oil. A 5–6 cm balloon attached to Tygon tubing was inserted into the descending colon to provide graded intensities of CRD (20,40,60,80 mmHg; 20s duration).

Single units were recorded extracellularly in the T13-L2 spinal segments (0.2–1.5mm from the cord dorsum) with tungsten microelectrodes (1–2 MΩ, Micro Probe Inc., Potomac, MD). A window discriminator was used to isolate single units, the data stored on computer with Spike 2 software (Cambridge Electronics Design, UK) for online and offline analysis. Units that responded to 80 mmHg colorectal distension (CRD) were used for further study.

There were three general phenotypic responses to CRD: Abrupt (on and off with the stimulus), Sustained (sustained afterdischarge) and Inhibited (inhibited by distention)⁹. Neurons were classified on the basis of their response to 80 mmHg CRD. Abrupt unit activity was quantified as the mean discharge frequency during the 20 s of the CRD stimulus minus the mean background activity determined in the preceding 20 s. Sustained unit activity was measured as the mean discharge frequency during the 40 s after onset of distension minus background activity. Units were classified as Inhibited if the response to 80 mmHg CRD exceeded a 20% decrease in response from the mean spontaneous activity in the 20 s preceding the distention. The data are expressed as mean ± SEM. Data were analyzed in SigmaStat using Chi-Square, t-test, paired t-test, two-way ANOVA as appropriate. Posthoc comparisons (Student Newman-Keuls) were performed if the ANOVA term was significant. A *p* value < 0.05 was considered significant.

In the cold block experiments, the C3-C5 spinal segments were exposed by laminectomy. Crushed frozen saline was placed over the exposed spinal cord to produce cold block.

In the dorsal root lidocaine experiments, a laminectomy of the L1-L5 vertebrae exposed the L6-S2 dorsal roots, which were gently elevated so a strip of saline soaked gauze could be slid between the roots and the spinal cord. Warm agar (2–3%) dissolved in saline was poured around the isolated dorsal roots and spinal cord extending up to the TL spinal cord. A small pool was made over the recording site. A second pool exposed the L6-S2 dorsal roots. This pool was filled with saline until lidocaine application.

Immunocytochemistry

In two experiments Fos expression was used as a measure of activity. In the first experiment the effect of chronic pelvic neurectomy/sham surgery on CRD-induced Fos expression was determined. In the second experiment the exposed T9-T10 spinal segments were bathed in lidocaine and the dorsal lateral funiculi were transected bilaterally or the spinal cord was transected. The incision was closed and rats given 7 days to recover.

On the day of the experiment, rats were sedated with halothane and a distention balloon placed in the colon. Rats were loosely restrained in plastic tubes and distended to 80 mmHg for 2 hrs (30 s on, 90 s off). Animals were transcardially perfused with 120 ml saline, followed by 400 ml of 4% paraformaldehyde in 0.1M phosphate buffer (PB). The T13-L2 and L6-S2 spinal cord segments were removed, postfixed overnight and transferred to 30% sucrose at 4°C for 48 hours. Thirty micron transverse sections were cut in a cryostat. Every fourth section was immunostained for Fos with a standard ABC protocol using rabbit anti c-Fos (1:50,000; Oncogene Science, Cambridge, MA) and biotinylated goat anti-rabbit IgG (1:2000; Jackson ImmunoResearch Lab, PA).

The labeled cells were counted in the whole section by a blinded observer. The sections were initially scanned and the 5–10 sections that appeared the most densely labeled were selected for quantification of the peak average number of Fos labeled cells per section in each animal which was used to determine group means. Data were analyzed by One Way ANOVA, $p < 0.05$ was considered significant.

Results

Neurons were recorded in the TL spinal cord. The mean depth of neurons from the different experimental groups ranged from $1010 \pm 51 \mu\text{m}$ to $1184 \pm 39 \mu\text{m}$ and there was no difference between any of the groups.

Acute pelvic neurectomy increases the response of TL dorsal horn neurons to colorectal distention

Sixty-five CRD-responsive neurons were recorded in the TL spinal cord segments between 4 and 10 hrs following surgery. Acute pelvic neurectomy significantly shifted the phenotypic distribution of TL viscerosensitive neurons compared to intact rats (Figure 1A; Chi-square, $p < 0.001$).

Only 1 Sustained neuron was recorded from the 13 intact rats⁹ removing this group from any statistical analysis. However, a sufficient number of Sustained neurons ($n=8$) were recorded from Sham rats to compare with Sustained neurons ($n=13$) from acutely neurectomized rats. The abdominal surgery likely increased the number of Sustained neurons in the sham group. However, compared to sham surgery, acute neurectomy decreased the threshold for Sustained neurons. In Sham rats 29% of Sustained neurons responded to 20 mmHg CRD, compared to 85% following acute neurectomy (Chi-square, $p < 0.02$). At least 90% of Sustained neurons responded to greater intensities of CRD (40, 60, 80 mmHg) regardless of treatment. There was no change in the threshold of Abrupt neurons.

Acute neurectomy increased the magnitude of response of Abrupt and Sustained neurons to CRD compared with corresponding neurons from Sham animals (two way ANOVA, $p < 0.001$ for Abrupt and Sustained neurons, Figure 1B,C). The response of Abrupt neurons from Sham rats did not differ from intact rats. Following acute neurectomy, the response of Abrupt neurons increased slightly at 60 mmHg CRD and doubled at 80 mmHg CRD. Likewise, the response of Sustained neurons following acute neurectomy was significantly greater than Sustained

neurons in Sham rats at noxious intensities of CRD (Figure 1C). Acute neurectomy had no effect on Inhibited neurons compared to Sham (Figure 1D).

These results suggest that CRD-evoked activity conveyed centrally in the pelvic nerve inhibits the response of TL dorsal horn neurons to noxious CRD. Alternatively, cutting the pelvic nerve could produce an injury discharge evoking excessive afferent input to the LS spinal cord, which in turn, could sensitize TL dorsal horn neurons to CRD. To rule out this possibility, the pelvic nerve was soaked in lidocaine for 5 minutes prior to lesioning in four rats. There were no differences in the response of Abrupt (n=9) or Sustained (n=3) neurons compared to acute neurectomy (Figure 1B,C), demonstrating that the increased response of TL dorsal horn neurons following acute pelvic neurectomy was not caused by an injury discharge.

To further test that removing pelvic nerve input increased the response of TL dorsal horn neurons to CRD, lidocaine or saline was applied to the LS dorsal roots in intact animals while recording from a TL dorsal horn neuron. Saline had no effect on the response of Abrupt neurons to CRD (Figure 2A). In contrast, lidocaine increased the response to CRD of the TL neurons within 5 minutes. Pooled data show saline had no effect in 5 cells, but lidocaine significantly increased the response in those 5 cells (one way RM ANOVA, $p < 0.001$).

Chronic pelvic neurectomy does not increase the response of TL dorsal horn neurons to colorectal distention

The acute pelvic neurectomy data showing an increase in excitatory processing in the TL dorsal horn are inconsistent with behavioral data that shows approximately 1 week following bilateral L5-S3 dorsal rhizotomy⁷ or pelvic neurectomy (unpublished observations), there is a loss of the visceromotor response to CRD. Therefore, the effect of pelvic neurectomy on the response of dorsal horn neurons was examined 6–8 days following surgery (chronic neurectomy).

In contrast to the effect of acute neurectomy, chronic neurectomy did not alter the phenotypic distribution of visceroreceptive TL dorsal horn neurons compared to chronic sham or intact rats (Chi-Square, $p=0.107$; Figure 3A). In addition, there was no change in the response or threshold of Abrupt neurons (Figure 3B). The response of Sustained neurons from chronic neurectomized rats was similar to neurons from acute Sham rats, but significantly less than acute neurectomized rats (Figure 3C). There was no change in the magnitude of response of inhibited neurons (Figure 3D).

Taken together, these data suggest that one week following pelvic neurectomy the response of TL neurons returns towards that in sham surgery or intact rats. The response magnitude decreases and the phenotypic distribution approaches that in intact rats. One explanation for these data is that the cut pelvic nerve develops ectopic activity that nonspecifically restores the inhibitory drive through the LS spinal cord that inhibits the response of TL dorsal horn neurons to CRD.

Does the cut pelvic nerve develop ectopic activity that could inhibit TL dorsal horn neurons?

The most direct approach to address this question is to electrically stimulate the cut pelvic nerve in the chronic neurectomized rats while recording from teased fibers in the LS dorsal roots. The electrical stimulus would identify pelvic nerve afferents and their spontaneous activity could be quantified. This is not practical since the pelvic nerve is extremely small and it is impossible to find the central end of the cut nerve one week later. Therefore, two experiments were done to indirectly address this question. First, normal rats without any noxious stimulation express little Fos protein in the spinal cord. We reasoned that ongoing ectopic activity in the cut pelvic nerve might be sufficient to induce Fos expression in the LS dorsal horn. While surgery and manipulating the incision (to express the bladder) resulted in

some Fos expression in the LS spinal cord of chronic sham rats (Figure 4), presumptive ectopic activity in the cut pelvic nerve significantly increased Fos expression in the LS spinal cord ($p < 0.005$), which was not affected by CRD.

In a second experiment, lidocaine applied to the LS dorsal roots of chronic neurectomized rats to block the centripetal conduction of ectopic activity decreased the nonspecific inhibition of TL dorsal horn neurons. Lidocaine increased the response by $28 \pm 9\%$ (Figure 5; the response to CRD increased in 8 neurons, decreased in 2 neurons and did not change in 3 neurons). Saline applied to the LS dorsal roots had no effect ($0.2 \pm 4.1\%$ increase; t-test, $p < 0.05$). These data further support the hypothesis that ectopic discharges induced by chronic pelvic neurectomy inhibits the response of TL dorsal horn neurons to CRD.

Pelvic nerve inhibition of TL dorsal horn neuron processing of CRD is supraspinally mediated

The next experiments addressed the hypothesis that the pelvic nerve modulation of TL dorsal horn neuronal processing is supraspinally mediated. First, Fos expression was used as a measure of CRD-evoked activity. Repetitive CRD induced 40 ± 8 Fos cells per section in intact rats. Four days following bilateral lesions of the dorsal lateral funiculi or spinal transection at T10, CRD-induced Fos expression increased to 89 ± 12 and 153 ± 22 cells per section, respectively (One way ANOVA, $p < 0.001$; Figure 6). These data demonstrate descending modulation of TL dorsal horn neurons to CRD.

In the second experiment, cold block was applied to the cervical spinal cord while recording from TL dorsal horn neurons in intact, acute and chronic neurectomized rats. Cervical cold block significantly increased the mean response to CRD in intact (8 cells increased, 5 cells decreased; Two way ANOVA, $p < 0.05$; Figure 7A) and chronic neurectomized (6 cells increased; $p < 0.05$; Figure 7B) rats, suggesting most neurons received tonic descending inhibition.

In contrast, cervical cold block decreased the facilitated response in 11/12 neurons from acute neurectomized rats (two way ANOVA, $p < 0.005$; Figure 7C). The interpretation of these data is that acute neurectomy decreases descending inhibition of TL dorsal horn neurons facilitating their response to CRD (see Figure 1). The subsequent decrease in response following cervical cold block suggests that acute neurectomy also unmasks a tonic descending facilitation that is attenuated by the cold block.

In addition to the change in the magnitude of response, cold block changed the phenotype of TL dorsal horn neurons. In intact and chronic neurectomized rats, there were few neurons with Sustained responses to CRD (Figures 1 and 3). In contrast, almost 40% of neurons in acute neurectomized rats were Sustained. Acute colonic inflammation with mustard oil, while changing the response magnitude of dorsal horn neurons does not change the phenotype of the neuron^{9, 23}. However, following cold block 6/13 TL neurons from intact rats transitioned from Abrupt to Sustained responses (Figure 7 A1). In contrast, 4/12 Sustained neurons from acute neurectomized rats transitioned to Abrupt responses during cold block (Figure 7 C1). When the cold block was removed and the spinal cord warmed, the neurons reverted back to their original phenotype. These data suggest that descending activity shapes the response of dorsal horn neurons to CRD and invites speculation that dorsal horn neurons intrinsically have Abrupt responses that matches the afferent input and that Sustained responses are the product of spinal and supraspinal modulation.

In order to differentiate between descending modulation and direct intraspinal modulation of TL viscerosensitive neurons by pelvic nerve input, 11 neurons were tested first for the effects of cold block at the cervical spinal level and then lidocaine was applied to the LS dorsal roots.

Cervical cold block increased the response of TL neurons by 26%. Subsequent lidocaine application to the LS dorsal roots reduced the cold block response by less than 5%, suggesting there was minimal direct modulation of the TL neurons by LS afferents/dorsal horn neurons.

Discussion

In the present study, we examined the effect of acute and chronic neurectomy on the response to CRD of thoracolumbar viscerosensitive dorsal horn neurons (Figure 8). Acute neurectomy increased activity in TL neurons that was manifest as a decrease in the percentage of Inhibited neurons with an increase in the percentage of Sustained neurons and an increase in the magnitude of response of Abrupt and Sustained neurons. Recording from individual neurons before and after lidocaine block of the LS dorsal roots in intact rats confirmed the acute neurectomy data. In contrast, neuronal responses following chronic neurectomy were similar to intact rats suggesting ectopic activity in the axotomized pelvic nerve nonspecifically reactivated the inhibition. Our results lead to the conclusion that colonic afferents in the pelvic nerve inhibit TL dorsal horn neuronal processing of the homovisceral stimulus conveyed by colonic afferents in the hypogastric/lumbar colonic nerves. This inhibition is supraspinally mediated since cervical cold block in intact and chronic neurectomized rats reduced the inhibition, facilitating TL responses to CRD. In addition, the inhibition of responses following cervical cold block in the acute neurectomized rats revealed an underlying tonically active descending facilitation. This is the first example of homovisceral descending modulation of viscerosensitive processing in the spinal cord.

Pelvic neurectomy and ectopic activity

Six to eight days after pelvic neurectomy, the response of TL dorsal horn neurons was similar to intact rats, both in terms of the response magnitude and phenotypic distribution. We hypothesized this was due to ectopic activity generated in the cut pelvic nerve reactivating the inhibitory circuit. Low frequency ectopic activity begins within several hours of nerve injury, gradually increasing to peak within the first few weeks and maintaining for several months^{24, 25}. Lidocaine applied to the L6 to S2 dorsal roots in chronic neurectomized rats blocked any presumptive ectopic activity in the pelvic nerve from accessing the spinal cord. This removed the inhibition of TL dorsal horn neurons, increasing their response to CRD. The interpretation that the cut pelvic nerve generates ectopic activity is supported by the increase in nonevoked Fos expression in LS spinal cord segments following chronic neurectomy. These data indicate that activation of viscerosensitive neurons in the LS spinal cord drives an activity-dependent inhibition of homovisceral processing in the TL spinal cord and that ectopic activity in the cut pelvic nerve is sufficient to maintain this inhibition.

“Sympathetic” vs. “parasympathetic” modulation

The present results draw some comparison to vagal nerve inhibition of spinal responses to somatic and visceral stimuli. Stimulation of the upper GI tract induces Fos in the nucleus of the solitary tract, but not the appropriate segments of the thoracic spinal cord^{26, 27} paralleling CRD-induced Fos in the LS spinal cord. Electrical stimulation of the cervical or cardiopulmonary branch of the vagus nerve inhibits the response of thoracic neurons to somatic and visceral stimuli^{28, 29}. Low intensity vagal stimulation is pronociceptive, higher stimulus intensities becoming increasingly antinociceptive in both animal and clinical studies^{30–32}. The vagus and pelvic nerves supply the parasympathetic efferent innervation of the viscera inviting speculation that stimulation of primary afferents in parasympathetic nerves drives descending modulation of TL spinal processing of afferent activity in sympathetic nerves. Indeed, local anesthetics in the brainstem reduced the inhibitory effect of vagal nerve stimulation³³. Our data are consistent with this interpretation. Whether this homovisceral

modulation is specific to the colon or a general principle of organization of the visceral system is unknown.

Descending modulation of viscerο-nociceptive transmission in the spinal cord

It is now generally accepted that nociceptive processing in the spinal cord is subject to bidirectional descending modulation^{17, 34, 35}. In the absence of tissue injury, descending inhibition is predominant and masks descending facilitation. Spinal transection generally increases the response of dorsal horn neurons to somatic and visceral stimuli, and reflex responses to noxious somatic stimuli become more robust, suggesting hyperexcitability within the flexion reflex circuit in the spinal cord. However, low intensity electrical stimulation in the RVM facilitates the response of LS dorsal horn neurons to somatic and visceral stimuli, and only at higher intensity stimulation does facilitation switch to increasingly potent inhibition^{18, 19, 36}.

Following injury or inflammation, descending facilitation becomes predominant^{37–40} (but see⁴¹). Spinalization decreases inflammation-induced allodynia and hyperalgesia^{42, 43}, and lidocaine administration to the rostroventromedial medulla (RVM) following spinal nerve ligation reverses or prevents allodynia and hyperalgesia⁴⁴.

In the present study the pelvic neurectomy and dorsal root lidocaine experiments documented that the inhibition of TL processing of CRD was driven by pelvic nerve input to the LS dorsal horn, while the cold block/lidocaine experiments demonstrated it was supraspinally mediated. What our experiments revealed was a tonic descending facilitation that is normally masked by the descending inhibition. Acute neurectomy facilitated the response to distention. It was assumed acute neurectomy removes the descending inhibition so TL dorsal horn neurons would respond 'normally' to colonic input over the TL dorsal roots. However, the cervical cold block following acute neurectomy inhibited the response of most neurons, suggesting that in addition to descending inhibition, there was an underlying tonic descending facilitation. Therefore, these data provide supporting evidence for coincident descending inhibitory and facilitatory modulation of colonic input to the TL spinal cord.

Clinical significance

Colonic inflammation increases excitability of the TL dorsal horn to CRD. There is an increase in the discharge of TL dorsal horn neurons, an increase in CRD-induced Fos expression and the TL dorsal horn contributes to behavioral responses to CRD^{7, 9, 10}. These data are similar to the present observations following acute neurectomy or spinal cold block/transection although the experimental manipulations are in opposite directions (increasing or decreasing colonic input to the LS dorsal horn). Since the RVM modulates responses to CRD following inflammation^{45, 46} or pelvic neurectomy, these data suggest that a shift in the balance of descending inhibition towards facilitation, irrespective of cause, increases excitatory processing of colorectal input in the TL spinal cord resulting in visceral hyperalgesia. Clinically, patients with abdominal pain originating in the lower bowel report an expansion of the area of referred pain into the thoracic dermatomes during experimental gut distention^{4, 5}. Experimentally increasing abdominal pain increases the area of referral in patients⁴⁷. Treatment to reduce abdominal pain also reduces the area of referred pain, suggesting increasing excitability in the TL spinal cord contributes to clinically relevant visceral pain and hyperalgesia.

Acknowledgements

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Abbreviations

CRD	colorectal distention
LS	lumbosacral
TL	thoracolumbar
aPNx	acute pelvic neurectomy
cPNx	chronic pelvic neurectomy

Figure 1.

Acute neurectomy (aPNx). A: the phenotypic distribution of TL dorsal horn neurons as a percent of neurons recorded. The number of neurons is noted above each bar. The phenotypic distribution between intact, aSham and neurectomy is significantly different (Chi-square, $p < 0.001$). B,C,D: The magnitude of response of Abrupt (B), Sustained (C) and Inhibited (D) neurons. Data from intact rats (previously published) are shown for comparison, but were not used in the statistical analysis. Symbols may be horizontally offset for clarity. * $p < 0.05$, # $p < 0.001$ vs. acute Sham.

Figure 2.

A: Peristimulus time histograms showing the increase in response of a TL Abrupt neuron following application of lidocaine, but not saline, to the L6-S2 dorsal roots in an intact rat. Examples of the spike shape recorded under each condition are shown on the right (scale bars: 1 ms and 50 μ V). B: Pooled data from 5 neurons showing the increase in response to lidocaine. * $p < 0.001$ vs. saline.

Figure 3.

Chronic neurectomy (cPNx). A: The phenotypic distribution of TL dorsal horn neurons as a percentage of neurons recorded. The number of neurons is noted above each bar. There was no change in the distribution (Chi-square, $p = 0.107$). B,C,D: The magnitude of response of Abrupt (B), Sustained (C) and Inhibited (D) neurons. * two-way ANOVA, $p < 0.05$ vs. aPNx. Data from intact rats (previously published) are shown for comparison but were not used in the statistical analysis.

Figure 4.

Fos expression in the LS spinal cord following cPNx or cSham ± CRD. Examples are shown in the photomicrographs. The graph shows the mean number of Fos positive nuclei per section in each treatment group. * $p < 0.005$ vs. Sham.

Figure 5.

A: Peristimulus time histograms showing the increase in response of a TL Abrupt neuron following application of lidocaine, but not saline, to the L6-S2 dorsal roots of a chronic neurectomized rat. Examples of the spike shape recorded under each condition are shown on the right (scale bars: 1 ms and 200 μ V). B: Pooled data showing the increase in response. * $p < 0.05$ vs. saline.

Figure 6. The effect of bilateral DLF lesions (DLFx) or spinal transection (SCx) on CRD-induced Fos expression in the TL spinal cord. * $p < 0.05$, ** $p < 0.001$ vs. intact; # $p < 0.01$ vs. SCx.

Figure 7.

The effect of cervical cold block on the response of TL dorsal horn neurons from intact (A), chronic (B) and acute (C) neurectomized rats. A1, B1, C1: peristimulus time histograms showing examples of the increase in response and change in neuronal phenotype under the different experimental conditions. The time and scale bars in C1 are the same for A1 and B1. Examples of the spike shape recorded under each condition are shown on the right (scale bars: 1 ms and 50 μ V). A2, B2, C2: the pooled data showing the effects of cold block. * $p < 0.05$, *** $p < 0.005$ vs. cold block; # $p < 0.01$ vs. same pressure during cold block.

Proposed Model for Spinal Processing of Colonic Nociception

Figure 8.

Summary diagram of the proposed model for TL spinal processing of noninflammatory colorectal stimuli highlighting homovisceral modulation. The line thickness denotes the strength of the projection. Lines with perpendicular endings are inhibitory synapses. Lines with Y shaped endings are excitatory synapses. The supraspinal site has both inhibitory (black area) and facilitatory (white area) descending projections. A: Descending input to the TL spinal cord is dependent on TL and LS input to the source of descending modulation. This feeds back to decrease TL activity (modulation of LS activity was not studied). B: Acute pelvic neurectomy removes colonic input (dotted line) to the LS spinal cord resulting in less excitatory drive of the descending modulatory circuit. The net result is a decrease in inhibition or an increase in

facilitation. C: After a few days ectopic activity in the pelvic nerve (dashed line) reactivates the 'normal' descending modulation to the TL spinal cord.