2021

Molecular Basis of Cross-Sensitization in Colonic Inflammation-Induced Somatic Hypersensitivity

Parshva K. Mehta
Virginia Commonwealth University

Acknowledgment

I am incredibly grateful to Dr. Liya Qiao for welcoming me into her lab, and for her support and guidance as my advisor. None of my accomplishments over the past year would be possible without her input.

I would like to thank Dr. Jack Grider and Dr. Zhao Lin for their advice and encouragement as my committee members.

I am appreciative of Dr. Jack Grider for serving as the Chair of the Committee.

I would also like to offer a special thanks to Ben Hernandez, Namrata Tiwari, and Shanwei Shen who have been wonderful lab members and have been very helpful.

Finally, I am beyond grateful to all of my friends and family who have supported me.

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Abstract

MOLECULAR BASIS OF CROSS-SENSITIZATION IN COLONIC INFLAMMATION-INDUCED SOMATIC HYPERSENSITIVITY

By: Parshva Mehta

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Physiology and Biophysics at Virginia Commonwealth University

Virginia Commonwealth University, 2021

Professor: Dr. Liya Qiao, PhD
VCU School of Medicine
Physiology and Biophysics

A major portion of pain experienced by patients with an Irritable Bowel Disease (IBD) or Irritable Bowel Syndrome (IBS) can be attributed to visceral hypersensitivity. Visceral stimuli transmitted through primary afferent neurons in the dorsal root ganglia (DRG) induce a nociceptive response. Notably, a subset of patients have also experienced the development of somatic pain, such as leg pain, after diagnosis of a bowel disorder. The aim of this investigation is to ascertain which biochemical mediators are involved in the development of such

viscerosomatic cross-sensitization. Initially, the Von Frey Test was used to find behavioral evidence of somatic referred pain; TNBS-induced mice exhibited a greater withdrawal response to hindpaw stimulation than control mice. Then, fast blue retrograde tracing was conducted and hindpaw innervation was identified to occur via primary afferent neurons located in the DRG at L4 spinal level. Immunohistochemical studies were conducted on L4 DRG tissue to evaluate levels of cellular mediators known to be involved in pain signaling, such as phosphor(p)-Akt. L4 DRG from TNBS-treated mice exhibited significantly higher levels of phospho-Akt than control animals. The increased p-Akt was located in Piezo2-expressing L4 DRG neurons and was suppressed in mice lacking TrkB.T1. These findings suggest that the PI3K/Akt pathway regulated by TrkB.T1-mediated cellular events participates in viscerosomatic cross-organ sensitization.

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by

PARSHVA MEHTA
B.A in Biology, University of Virginia 2018

Advisor: DR. LIYA QIAO, Ph.D.
PROFESSOR
VCU SCHOOL OF MEDICINE
PHYSIOLOGY AND BIOPHYSICS

Virginia Commonwealth University
Richmond, Virginia
May 2021

Introduction

1.1 Inflammatory Bowel Disease

Inflammatory Bowel Diseases are characterized by inflammation and disruption within the gastrointestinal tract. The prevalence of IBD in North America and Europe has been a significant problem and it continues to grow due to a young age of onset and low mortality (1). Cases are rapidly increasing in newly industrialized countries around the world, particularly in Asia, Africa, and South America. Studies reveal that there is no significant difference in IBD cases amongst men and women, and there also does not seem to be significant variation by race (2). Early onset of this disease is fairly common; previous findings by McDowell et al. estimate that up to a fourth of patients may develop IBD by adolescence. A 2019 study approximated that 2 million people have been affected by IBD in North America, and that this number is projected to double by 2030 (3). In terms of financial cost, this disease was expected to cost the healthcare systems of the United States and Canada around 10 billion US dollars as of 2018 (3). Given the increasing prevalence around the world and the projected population affected, this disease can become a significant global burden. When considering the future, the early onset of IBD may also present complex cases/comorbidities due to longer disease duration (1).

IBDs can be differentiated into two types: Crohn's Disease (CD) or Ulcerative Colitis (UC); if either of these cannot be confidently diagnosed, then the condition is considered IBD-undifferentiated (IBD-U) (4). Common symptoms of IBDs in affected patients include diarrhea, abdominal pain, weight loss, and/or bloody stools (5).

Crohn's Disease (CD) can involve any aspect of the gastrointestinal tract ranging from the mouth to the anus. Most commonly, CD affects the ileum and/or the large intestine, generally in

a discontinuous pattern. The symptoms vary based on the location and severity of disease, resulting in varying clinical presentation from one patient to another. Common symptoms include abdominal pain, watery diarrhea, and weight loss. This pain is acute and severe, generally localizing to the right lower quadrant (5). The abdominal pain and the diarrhea tend to be episodic and can occur intermittently for years prior to diagnosis. A key feature of Crohn's is that it affects all layers of the bowel (1), potentially leading to strictures or fistulas. CD can cause serious problems for the skin and also lead to biliary stones over time (2). Systemic symptoms can also be seen due to CD, with fatigue being the most common (due to persistent inflammation and malabsorption) (5).

Ulcerative Colitis (UC) generally involves inflammation confined to the colon, and therefore symptoms are not as diverse as those in CD (4, 8). The inflammation tends to start in the rectum and spreads in a continuous pattern towards the proximal colon. While the large intestine gets inflamed, the small intestine continues to function normally (7). In contrast with CD, UC only affects the innermost layer of the colon or other affected areas. UC frequently presents as bloody diarrhea, and patients often describe tenesmus, a sensation of incomplete emptying. The extent of blood present in the stool can be indicative of the severity of disease (8). Physical exams often reveal left lower or upper quadrant abdominal pain. If UC lasts for 8-10 years, it can lead to osteoporosis or even colon cancer. With longer disease courses, patients may also develop colonic strictures which cause obstruction/pain (8).

The cause of IBDs continues to be a mystery; various factors seem to be involved, but there has not been a universal aspect which is consistent among all patients. Genetic and environmental factors are certainly involved, and patients with compromised or inappropriate immune responses are particularly susceptible (6, 7). Recent Genome wide association studies

(GWAS) have revealed 240 nonoverlapping genetic loci, of which 30 are common to both UC and CD. Analysis of these loci indicate their role in important pathways for intestinal homeostasis (5). Environmentally, the intestinal microbiota is the major driver of IBD; the gut bacteria affect the immune system, host metabolism, and GI development in major ways.
The goal of treatment is to induce remission of IBD, and it varies based on the severity of the disease. Nonbiological therapies such as aminosalicylates, thiopurines, and steroids provide symptomatic relief but do not change the long-term course of disease progression. Biological therapies involving immunomodulators, such as tumor necrosis factor-α (TNF-α) antibodies, have induced or maintained remission in certain patients, but a significant portion of IBD patients were or became non-responders to this treatment; new methods of treatment targeting specific pathways have become increasingly necessary (9).

1.2 Visceral Hypersensitivity

While the inflammation in IBD is considered to be more severe than that in IBS, a recent study noted that there can be a significant amount of overlap in symptoms; up to 40% of IBS symptoms were present in patients with IBS and IBD (10). One of the primary symptoms, abdominal pain, is present in a plethora of gastrointestinal disorders. This pain may be due to visceral hypersensitivity; visceral hypersensitivity is defined as enhanced perception of mechanical triggers applied to the bowels (14). Such improper signaling of the viscera through a signaling reflex pathway is known to occur in both IBD and IBS, and may be affecting up to 20% of the global population (11). There are two types of hypersensitivity: hyperalgesia and allodynia. Hyperalgesia is when there is an intensified pain sensation in response to stimuli

which usually provoke pain. Allodynia is when there is nociceptive sensation in response to normal stimuli (12).

The sensory pathway through which visceral hypersensitivity occurs is known as the "gut-brain axis," with signals going from the periphery to the central nervous system (13). Perceivable sensations, such as pain, in the colon and rectum are generated by extrinsic afferent neurons (13, 15). The conscious perception of pain/discomfort in the viscera occurs is signaled to the brain via these neurons, which respond to noxious mechanical stimuli. These neurons can be subdivided by the location of soma; splanchnic nerve cell bodies are located within the thoracolumbar dorsal root ganglion (DRG), and pelvic nerve cell bodies are within the lumbosacral DRG. DRGs are groups of cells bodies located all along the human body and convey sensory information from the Peripheral Nervous System (PNS) to the Central Nervous System (CNS). Each DRG is composed of a cluster of pseudounipolar neurons whose axons project onto the spinal cord transmitting the sensory signal towards the brain (16). In particular, these neurons are known to control our sensation of pain and temperature; for this reason, they have immense potential as targets in the treatment of chronic pain. Colonic innervation occurs primarily through the thoracolumbar spinal cord with the signal relaying through the splanchnic nerves. Rectal innervation is mainly thought to transmit signals through the lumbosacral spinal cord via the pelvic nerves. Peripheral portions of the afferents in the DRG

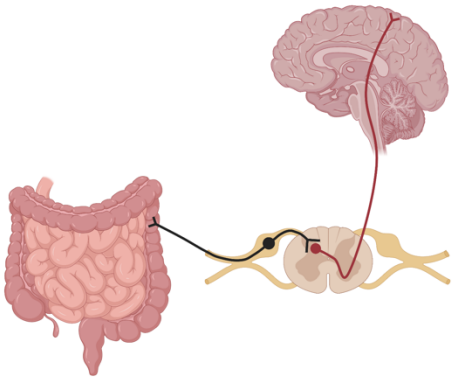


Figure 1: Visceral Organ Sensation. Visceral organs are innervated by pseudounipolar neurons, or primary afferent neurons, which transmit onto second order neurons in the spinal cord. These second order neurons then convey sensory information to the brain.

innervating the colorectum then terminate onto second order neurons in the dorsal horns of vertebrae in the spinal cord which transmit the signal to the brain (13, 17).

In the mouse colon, eight different types of afferent fibers have been identified and five of these have also been recorded in humans: mucosal afferents, muscular afferents, muscular/mucosal afferents, vascular endings, and silent afferents (13, 18). Mucosal afferents have low thresholds for activation, responding mainly to distortion of the colonic mucosal epithelium. Muscular afferents have low distention thresholds in response to the colorectum, and likely contribute to nociception when there is high stimulus intensity. Muscular/mucosal afferents respond to mucosal distortion and circular stretch and aren't found in splanchnic innervation of the distal colon. Vascular endings wrap around blood vessels in the mesentery and submucosa and have the greatest similarity to cutaneous nociceptors. These afferents respond to high-threshold stimuli, inflammatory/immune mediators, and also noxious distention. The last group of afferents discussed are mechanically insensitive or "silent" in normal conditions. Three different types of silent afferents have been identified: those that respond to chemical stimuli (inflammatory markers) and do not develop mechanical sensitivity, those who develop mechanical but not chemical sensitivity, and finally those stimulated chemically and mechanically. (18)

Ultimately, both low threshold and high threshold mechanosensitive afferents are affected during sensitization and contribute to pain/discomfort; this response can be further amplified by the recruitment of silent afferents capable of developing mechanosensitivity (13). Inflammation leads to increased signal intensity and frequency, along with lower thresholds of activation for these afferent neurons. Experiments comparing control and viscerally inflamed mice showed an increase in mechanosensitive afferents with a near equivalent decrease in silent

afferents. In mice exposed to conditions of hypersensitivity for 3 consecutive days, the proportions of mechanosensitive afferents remained higher for weeks after the inflammatory agent was last administered (19). The exact mechanism by which inflammation induces such changes in the extrinsic afferent fibers is still controversial; many chemical mediators may play a role, including but not limited to amines, peptides, cytokines, and neurotrophins.

Many scientists have attempted to better understand the mechanism of sensitization by inducing visceral pain in animal models. Although it is difficult for models to serve as a true representation of this condition in humans, previous studies with mice and rats show behavioral similarity to that of post-infectious IBS patients, who still perceive pain after inflammation has abated (20, 22). Inflammation is induced in these animals through the use of certain haptens, substances which cannot induce inflammation on their own, but cause an inflammatory response once attached to specific proteins. 2,4,6-trinitrobenzene sulfonic acid (TNBS) is a common hapten which has been shown to cause inflammation, peaking after 4-5 days and persisting around a month post-injection. Hypersensitivity in the treated persisted for up to 16 weeks after inflammation has ceased (21).

1.3 Neurotrophin Signaling

Neurotrophins are a group of proteins that are known for their role in embryonic development of the PNS and CNS (23). These proteins are essential for neuronal survival, proliferation, and regulation. Outside of their importance during the embryonic stages, neurotrophins, particularly nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), play an important role in nociceptive signaling as well. Expression of four

neurotrophins has been identified in mammals: NGF, BDNF, neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) (24).

These neurotrophins mediate cellular processes by binding to two types of receptors, Trk tyrosine kinase receptors and the $p75^{NTR}$ receptor (24). The Trk receptors each have high selectivity for specific neurotrophins; NGF binds to TrkA, BDNF and NT-4 bind to TrkB, and NT-3 binds to TrkC. The $p75^{NTR}$ receptor can bind to all of the mammalian neurotrophins with low affinity (24, 25). The locations of both types of receptors are variable throughout the body and neurotrophin binding can affect cellular function in diverse ways. After binding, the neurotrophin/receptor complex is internalized by the cell and growth factor receptors maintain the signal from endosomes (26). Trk receptor signaling is known to promote cell proliferation through signaling including but not limited to the PI3K/Akt, MEK/MAPK pathways, and PLCγ pathways (25). Meanwhile, the $p75^{NTR}$ receptor is capable of supporting or inhibiting cell proliferation. In neurons with both Trk and $p75^{NTR}$ receptors, their physical interaction can increase ligand affinity and delay degradation of the Trk receptor, prolonging the signal (24). The immature form of the neurotrophins, pro-neurotrophins, are also capable of binding to the $p75^{NTR}$ receptor when sortilin is present, leading to transmission of an apoptotic signal via the Jun kinase pathway (27).

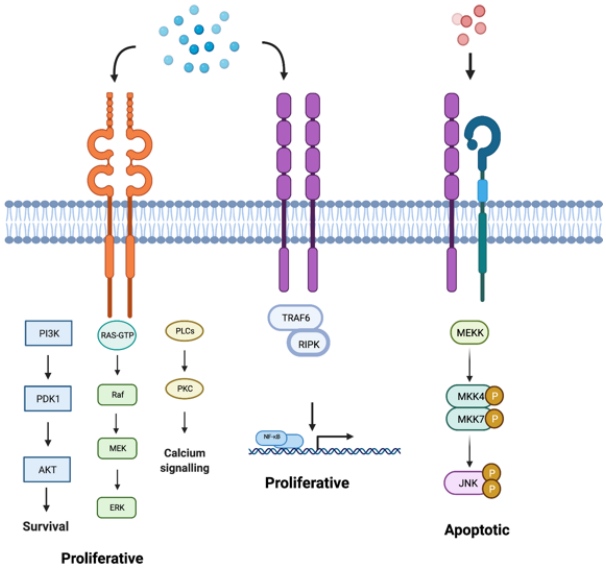


Figure 2: **Neurotrophin Signaling Pathways.** Neurotrophin binding to Trk receptors (orange) or $p75^{NTR}$ receptors (purple) set off signaling cascades promoting neuronal survival, synaptic plasticity, and/or cell growth. However, binding of pro-neurotrophins to $p75^{NTR}$ receptor in the presence of sortillin (blue) leads to initiation of apoptotic signals.

1.4 BDNF and NGF

As previously mentioned, BDNF and NGF play an important role in pain signaling. NGF has been found to increase activity of primary afferents through various mechanisms, while BDNF is more involved in central sensitization (25). In adulthood, NGF was originally thought to be a means of treating peripheral neuropathy due to its neurotrophic effects, but administration in clinical trials exacerbated the pain (28). Further experimentation supported this, as levels of NGF mRNA were elevated during inflammatory conditions (29). In regard to visceral pain, levels of colonic NGF expression in patients with IBS were positively associated with disease severity and gut barrier dysfunction (30). The augmented pain sensation in response to NGF may occur through various mechanisms: lower thresholds, increased recruitment, and/or increased transcription of the nociceptors (25). NGF signaling is also believed to play a role in the development of mechanosensitivity in silent afferents (31). Additionally, BDNF was found to be localized primarily in NGF-sensitive neurons expressing TrkA (32,33). BDNF protein is found to increase in response to nociceptor activity and can rapidly increase when there are high levels of NGF during inflammation. BDNF can also be released in response to mucosal NMDA receptor activation, which is known to be involved in visceral hypersensitivity; this increase in BDNF was found to occur via an Erk dependent pathway.

Ultimately, there are various mechanisms through which neurotrophins can lead to the sensitization of DRG neurons. In TNBS-induced colitis, increased expression of the TRPV1 receptor was noted in L1 and S1 level DRGs in conjunction with increased NGF expression; this increased expression also occurred with NGF treatment and was attributed to activation of the PI3K/Akt pathway (34). CGRP, an important nociceptive marker, also had elevated levels after

NGF treatment. CGRP is colocalized with TrkB in lumbar and sacral (L1 and S1 DRG), and increased CGRP expression correlated with BDNF activation (35). When given BDNF treatment, increased amount and density of CGRP fibers were also noted. Therefore, NGF may upregulate BDNF expression, which goes on to upregulate CGRP expression. Further analysis revealed that this upregulation occurs through the PLC_{γ} pathway downstream of BDNF-TrkB, and the Mek/Erk and PI3K/Akt pathways were not involved (25). Another important molecule, cAMP-responsive element binding (CREB) protein, was also activated in the neurons which showed upregulation of CGRP. The involvement of this molecule was confirmed by BDNF antibody treatment, which attenuated the CREB activation (36). Our growing understanding of these signaling cascades sheds light on the complexity of visceral hypersensitivity, and also on which molecules may be worthy targets in medicine.

1.5 Cross-Sensitization

Pain from visceral organs tends to be diffuse and poorly localized, and also can be associated with somatic pain elsewhere. This "referred" pain has been recognized in numerous conditions affecting different organs. Such cross-sensitization has been seen in patients between the stomach and heart, esophagus and heart, colon and urinary bladder, and many other organ pairings (37). Patients with gastrointestinal conditions often concurrently have comorbidities like bladder disorders, neck pain, foot pain, etc. The inflammation or damage originating in the GI system from a certain organ led to sensitization elsewhere in response to certain stimuli. One clinical survey revealed that patients with chronic bladder pain are up to 100 times more likely to have IBD relative to the general population (38, 39).

The development of cross-sensitization is not yet fully understood, and further studies exploring the pathogenesis of cross-sensitization require an animal model which develops similar symptoms as those in humans. The rodent TNBS-induced colitis model has shown promise, as rodents in these conditions have shown bladder hypersensitivity and somatic pain in response to mechanical stimulation (40). Additionally, rodents are known to have similar neuron-cell interactions to humans in terms of pain sensation. When considering the mechanism by which cross-sensitization occurs, interactions among primary afferent neurons and non-neuronal cells are considered as major players (37). One afferent neuron is generally surrounded by two or three satellite glial cells (SGCs), and colitis has led to increased coupling between SGCs around colonic afferents (41). The afferent neuron may then release cellular mediators which transmit the signal to SGCs. The SGCs then may communicate with SGCs surrounding another afferent neuron via gap junctions, allowing for transmission of nociceptive signals among neurons within DRG that innervate different organs. For cross-sensitization between organs which are not innervated by DRG neurons in the same spinal segment, it can likely be attributed to communication between DRG through the spinal cord. A recent study showed that TNBS-induced mice developed somatic heterosegmental and homogesegmental hypersensitivity to the

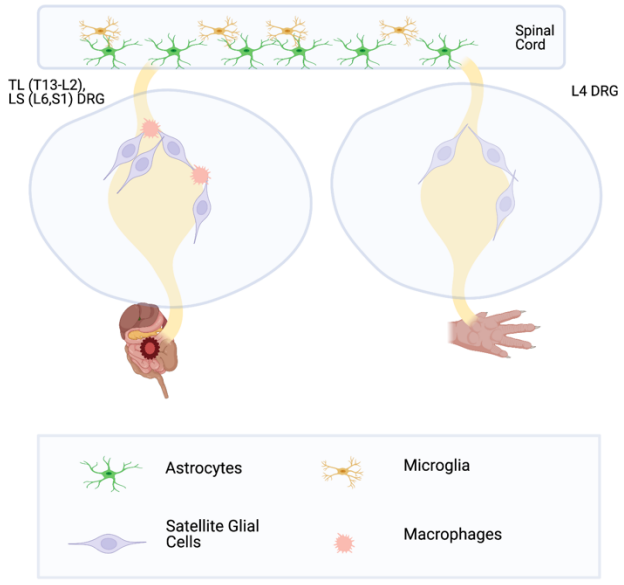


Figure 3: Mouse Model of Cross-Sensitization. Colonic inflammation leads to increased coupling of SGC and accumulation of macrophages due to nociceptive signaling from the neuron. This nociceptive signaling can be transmitted to heterosegmental afferents via microglia-astrocyte networks in the spinal cord.

hindpaw and low back, respectively (42). Colororectal innervation was traced to L6, low-back innervation was also in L6, and hindpaw innervation was traced to L4. TNBS treatment led to hypersensitivity to somatic regions innervated by neurons within the same DRG and to those innervated by a distinct spinal region in L4 (42). The central sensitization likely triggered by the release of mediators into the spinal cord (43). The central response to these mediators is bolstered by activation of microglia and astrocytes in the spinal cord, which are upregulated following colitis (37,44). Astrocyte networks then may convey this signal from DRG at one level to another (Figure 3). Understanding the interactions of the sensory neuron and glial cells in the primary afferent pathway, and also the biochemical mediators involved in the signaling will be very important in the path towards mitigating such referred pain.

1.6 TrkB.T1

TrkB.T1 is a truncated isoform of the TrkB receptor. This isoform is known to be upregulated during fetal growth and becomes one of the common Trk receptor isoforms present in adults (48). TrkB.T1 deficient mice were noted to be able to develop normally, but exhibited anxious behaviors more often than control animals and had neuronal abnormalities in the amygdala. Recent studies indicate that the TrkB.T1 receptor may actually have a maladaptive role in response to disease or injury (49). Specifically, TrkB.T1 receptors are increased in SGCs after peripheral inflammation, maintaining BDNF signaling. This receptor isoform has also been found to induce astrocytic morphogenesis in response to BDNF, and astrocyte networks play a key role in central sensitization post-inflammation (37). TrkB.T1 therefore carries potential as a therapeutic target to treat neuropathic pain.

1.7 Akt

Akt is a serine/threonine protein kinase involved in various cellular processes, primarily to support cell survival/proliferation. Activity of Akt is regulated by post-translational modifications, such as phosphorylation or ubiquitination. Phosphorylation of Akt by a kinase leads to its activation, while dephosphorylation via a phosphatase can be a means of regulation. Additionally, Akt is known to promote survival through inhibition of apoptotic processes and is therefore implicated in various types of cancer (45). Akt can be activated by multiple factors such as cytokines, growth factors and neurotrophins. Growing evidence shows that Akt also participates in the processes of visceral hypersensitivity and somatic pain. For example, an Akt inhibitor was able to block the NGF upregulation which occurred during gastric hypersensitivity (46). TRPV1 upregulation, which was noted to occur with TNBS-induced colitis, involved the PI3K/Akt pathway (34). TNBS-induced colitis models also have shown evidence of CGRP triggering an increase in Akt phosphorylation, which leads to central sensitization and allows for crosstalk between segments along the spinal cord (47).

1.8 Piezo2

The Piezo channel family has been evolutionarily conserved and play a vital role in the mechanotransducive processes of different cell types (50). Piezo2, in particular, is known to be involved in touch sensation, respiration and proprioception. Piezo2 deficient mice have been noted as unable to sense discriminative touch (51). Piezo2 plays a role in sensory pathways and has specifically been localized in sensory neurons of DRG and marks neurons that respond to mechanical stimulation (52). It's role in pain has become clearer with time, and the expression of this channel has notably increased in nerve-injury induced mechanical pain (51). When

considering visceral hypersensitivity, a former study showed that Piezo2 knockdown in the DRG resulted in attenuation of visceral sensation to innocuous or noxious stimuli (53).

1.9 Summary

Previous studies indicate that TNBS-induced inflammation can lead to referred somatic pain innervated by a DRG heterosegmental to the DRG innervating the colorectum. However, the cellular mediators and mechanism(s) by which this can occur are understudied. The goal of this study is to characterize the expression of p-Akt in L4 DRG following colitis induction and the regulation of p-Akt by TrkB.T1-mediated cellular events in order to understand the molecular pathways in viscerosomatic cross-sensitization.

Objectives And Aims

Objectives

The objective of this study is to ascertain which signaling pathway may be involved in the development of referred somatic pain resulting from viscerosomatic cross-sensitization.

Hypothesis

TNBS-induced inflammation leads to somatic hypersensitivity which is associated with changes in neurochemical coding in L4 DRG and is regulated by BDNF/TrkB.T1.

Aims:

Aim 1: Establish behavior evidence of somatic hypersensitivity developing after induction of visceral inflammation

Aim 2: Determine which group of afferent neurons (DRG) innervate the affected somatic region

Aim 3: Investigate the cellular mediator involved in the development of viscerosomatic cross-sensitivity.

Methods

Experimental Animals

Adult Black 6 male mice weighing 25-30 grams were used for all studies All experimental protocols involving animal use were approved by the Institutional animal Care and Use Committee at the Virginia Commonwealth University (IACUC# AM10315). Animal Care was in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and National Institutes of health guidelines. All efforts were made to minimize the potential for animal pain, stress, or distress as well as to reduce the number of animals used.

Introduction of Colonic Inflammation in Mice with TNBS

Under anesthesia (2.5 % isoflurane), a single dose of TNBS (75 µL of 12.5 µg/µL TNBS in 30 % EtOH) was administered into the mouse colon via a polyethylene (PE)-50 catheter through the anus. The proximal tip of the catheter was 2.5 cm inside from the anus. The mouse tail was lifted for 1 min after TNBS installation to avoid drug leakage from the anus. The same amount of 30 % EtOH as vehicle was used in control animals. TNBS-treated mice developed peak colonic inflammation on day 3-4 and persistent visceral hypersensitivity after inflammation resolution (> 7 days) [9,12,19].

Von Frey Test

The mice were placed individually into cages with a mesh floor underneath. They were then allowed to acclimatize to their environment for 30 minutes. After this period, a

microfilament was applied perpendicularly to the plantar surface of the hindpaw when the animal has all four paws resting on the floor. The responses were recorded in a treatment blind manner, with a response considered as positive if a withdrawal behavior (paw licking, shaking, or withdrawal) was noted during or immediately after application. The mice were each consecutively tested for a filament size, and this was repeated five times before moving on to a filament size of higher force. These tests were conducted 1 week, 2 weeks and 3 weeks post-injection. More than one animal was used for each experimental group to show variability.

Perfusion and tissue dissection

Animals were put under anesthesia (2.5% isoflurane) in an induction chamber. The mouse was then kept under anesthesia, and a lateral incision was made through the integument and abdominal wall. A small incision was then made in the diaphragm along the entire length of the rib cage to expose the pleural cavity. Tissue surrounding the heart was trimmed away, and another incision was made to the animal's right atrium. A needle was then secured into the posterior end of the left ventricle, at which point the mouse was perfused with Krebs Buffer to flush out blood. After the fluid became clear, the buffer valve was switched to administer the fixative buffer, 4% paraformaldehyde (PFA). The valve was then closed once the fixative was nearly finished and the mouse became stiff. Next, an incision was made to the back to expose the spinal column, which was then isolated from the animal. Muscle, fat, and soft tissues were then trimmed away from the column, and DRG were collected and stored in 4% PFA for 2 hours. After 2 hours in 4% PFA, the tissues were transferred to sucrose for overnight.

Fast Blue administration

Under anesthesia (2.5% isoflurane), mice were administered Fast Blue in the plantar surface of their hindpaw to retrogradely label the primary afferents responsible for hindpaw sensation. DRG tissues from T13, L1, L2, L3, L4, L5, L6, and S1 were collected 1 week post-Fast Blue administration, after which the mice went through whole-body perfusion as previously mentioned. After dissection, the DRG were kept in 4% paraformaldehyde for 2 hours and then stored in 20% sucrose overnight. Each DRG was then embedded in OCT cryostat medium (Tissue Tek), sectioned at 10 μm thickness, and placed on gelatin coated slides for examination in a microscope.

Immunohistochemistry

The DRG were taken out from 20% sucrose and embedded in OCT cryostat medium (Tissue Tek). Each ganglion was then sectioned at 10 μm thickness and placed on gelatin-coated slides. Slides were then heated at 35° for 15-30 minutes or until the sections were no longer frozen. A hydrophobic border was then placed around the slide using a Pap Pen prior to application of the primary antibody solution. Slides were then incubated overnight with specific primary antibody at room temperature in 2% Normal Donkey Serum and 0.3% Triton Buffer. Following incubation, slides were washed with a 0.1M sodium phosphate buffer at a pH of 7.4 for ten minutes three times. Slides were then incubated with a fluorescent species-specific secondary antibody for two hours. The slides were then washed again with 0.1M sodium phosphate buffer three times for ten minutes each. Citiflour anti-fadent mounting medium (Electron microscopy Science) was then added onto the slides before placement of the cover

slips. Slides were viewed with an Axiocam Carl Zeiss microscope and neurons exhibiting immunoreactivity greater than the background level were considered positively stained.

Transgenic mice

Piezo2 reporter mice were generated by crossing Piezo2EGFP-IRES-Cre mice (Jax Stock No. 027719) with R26-LSL-Gi-DREADD (Jax Stock No. 026219) so that Piezo2-expressing cells can be visualized by mCitrine yellow fluorescent protein (YFP). TrkB.T1 global knockout mice were obtained from NIH/NCI and a gift from Dr. Lino Tessarollo. All these transgenic mice received TNBS treatment in parallel to wildtype mice.

Immunohistochemical Analysis

DRG cells exhibiting immunoreactivity greater than the observed background level were considered positively stained. The number of positive cells in an area of the DRG were then counted. The overall area was also measure excluding areas with no cells and/or with nerve fibers. The area was converted from units of (μm^2) to units of (mm^2), and the cells per area (mm^2) were then calculated.

Statistical Analysis

Results of each study were shown as the mean ± SEM. Multiple groups were compared by ANOVA with differences between means considered significant at $p \leq 0.05$. When two groups were compared, a t test was used.

Figure 4: Von Frey Test. TNBS-induced and control animals were placed in cages with a mesh floor underneath a designated time post-injection. After a 30 minute acclimatization period, their somatic sensitivity was tested by mechanical stimulation of a hindpaw with Von Frey filaments of increasing size.

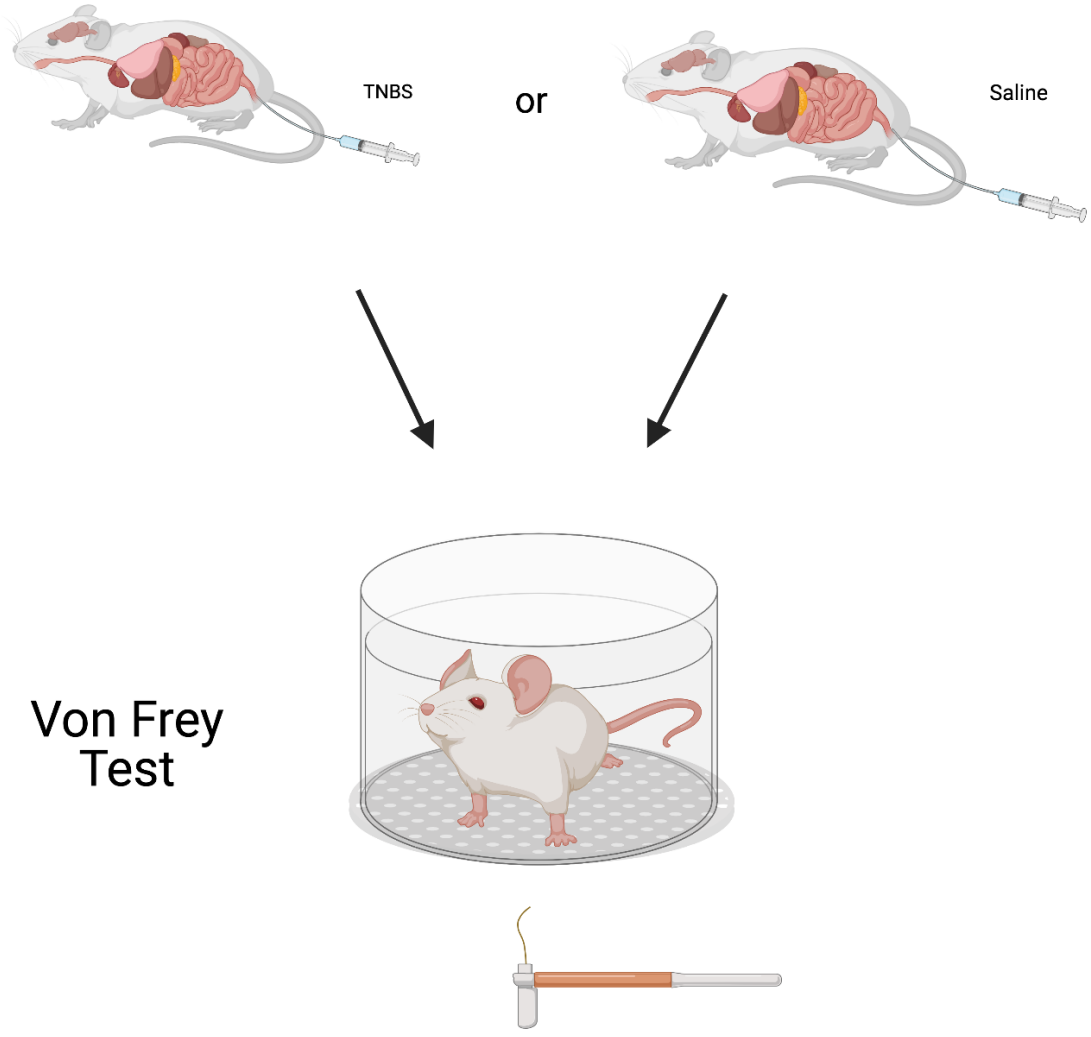
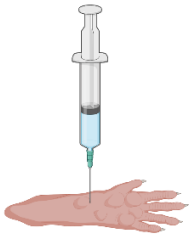
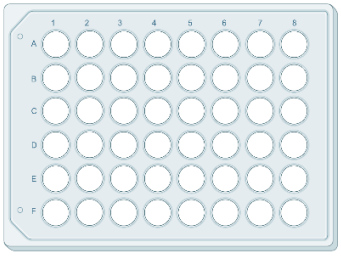


Figure 5: Fast Blue Retrograde Tracing. Mice were administered Fast Blue, and DRG tissues (T13, L1, L2, L3, L4, L5, L6, and S1) were removed from the mice 1 week post-injection.

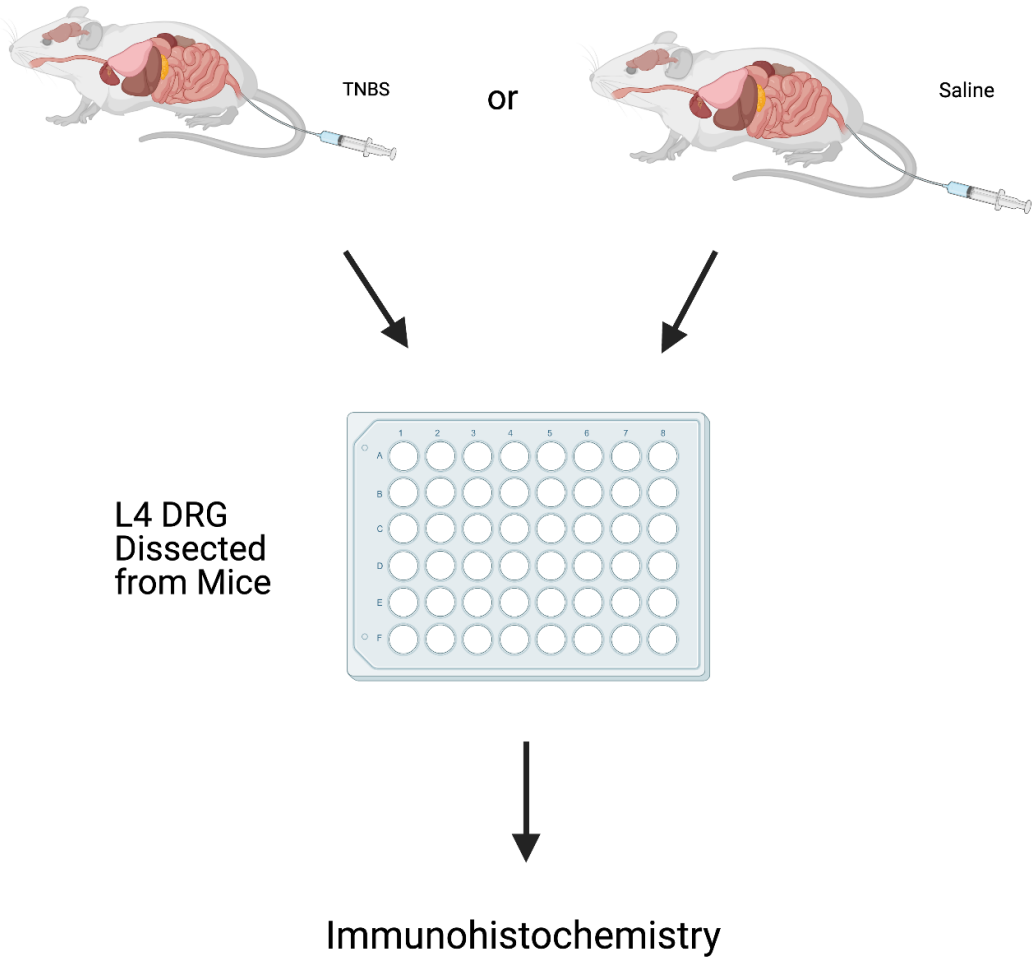


Fast Blue



DRG
Dissected
from Mice

Figure 6: Immunohistochemistry Design. L4 DRG of control and TNBS-administered mice were dissected 2 weeks post-injection. After collection of L4 DRG tissues, immunohistochemistry was conducted for specific cellular mediators.



Results

4.1 TNBS-induced mice develop somatic hypersensitivity which peaks 2 weeks post-TNBS administration

1 week-post TNBS administration, TNBS treated mice began to exhibit a higher number of withdrawal responses relative to control mice once filaments of 0.04-1.0 grams-force were applied. 2 weeks-post TNBS administration, the treated mice exhibited a higher number of withdrawal responses at 0.04-1.0 grams-force than the previous week, and still more than control mice. 3 weeks-post administration the treated and control mice began to have a similar number of withdrawal responses (Figure 7). These results were obtained from two animals per experimental group.

4.2 Fast Blue Retrograde tracing revealed that sensory afferents innervating the hindpaws are localized in L4 DRG

Tissue analysis was conducted 1 week post-Fast Blue administration to the hindpaw, and DRG tissue (T13, L1, L2, L3, L4, L5, L6, and S1) dissection revealed evidence of fast blue primarily in neurons of DRG at spinal levels L4, L5 and L6. Of those, L4 had a significantly higher number of neuronal cells exhibiting presence of the fast blue dye ($p<0.05$). These results were obtained from 4 paws of 2 animals (Figure 8).

4.3 The PI3K/Akt pathway is activated in TNBS-induced somatic hypersensitivity

Immunohistochemical studies were conducted to characterize the role of the PI3K/Akt pathway in development of referred somatic pain. Analysis of images showed that TNBS-treated mice exhibited significantly higher levels of p-Akt in L4 DRG than control mice ($p<0.05$). These results were obtained using 6 animals per experimental group (Figure 9).

4.4 Increased p-Akt co-expression in Piezo2 labeled cells in L4 DRG of TNBS-treated mice

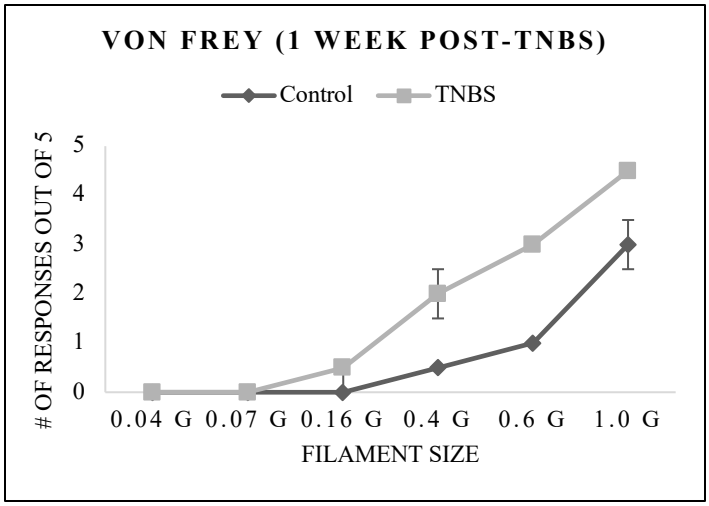
P-Akt colocalization was analyzed in control and TNBS treated Piezo-YFP labeled transgenic mice to investigate the involvement of Akt activation in mechanical sensitization. Findings suggest that the TNBS treated transgenic mouse had greater p-Akt and Piezo colocalization than the control transgenic animal. These results were obtained with one animal per experimental group (Figures 10 and 11).

4.5 TrkB.T1 KO attenuates TNBS-induced increase in p-Akt levels in L4 DRG

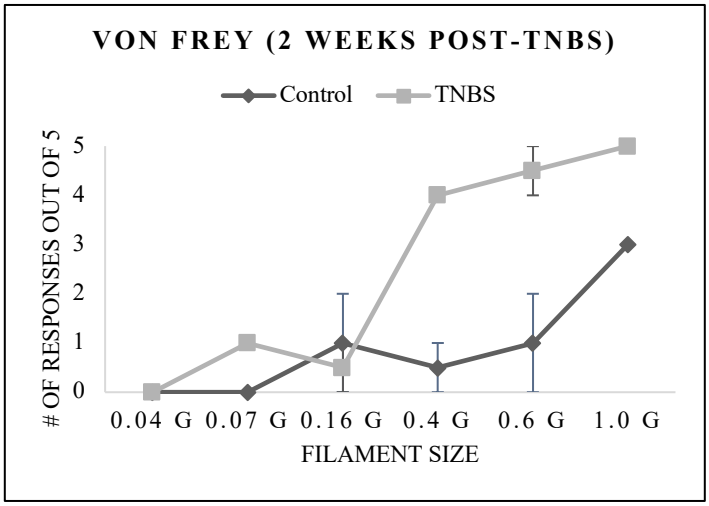
In order to further distinguish the involvement of the PI3K/Akt pathway in cross-sensitization further immunochemistry was conducted with control, TNBS, and TNBS + TrkB.T1 KO mice. Immunohistochemical analysis revealed that p-Akt levels in the L4 DRG of TrkB.T1 KO mice are significantly lower than those in the L4 DRG of TNBS treated mice ($p<0.05$). These results were obtained with 6 animals for the control and TNBS treatment groups, and 3 animals for the TrkB.T1 KO + TNBS treatment group (Figure 12).

Figure 7: Von Frey Test for Mechanical Sensitivity. Graphs display the number of times out of 5 that each mouse exhibited withdrawal response to stimulation for increasing filament sizes (n=2 for both experimental groups) at **A**. 1 week post-TNBS or saline administration **B**. 2 weeks post-TNBS or saline administration **C**. 3 weeks post-TNBS or saline administration

A.



B.



C.

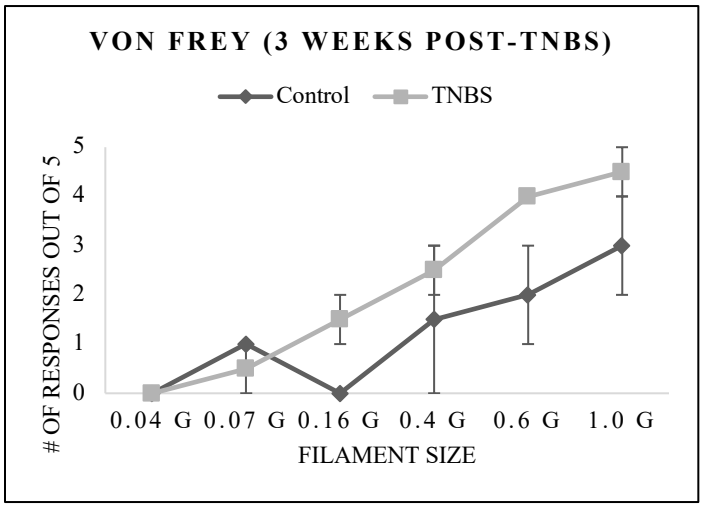
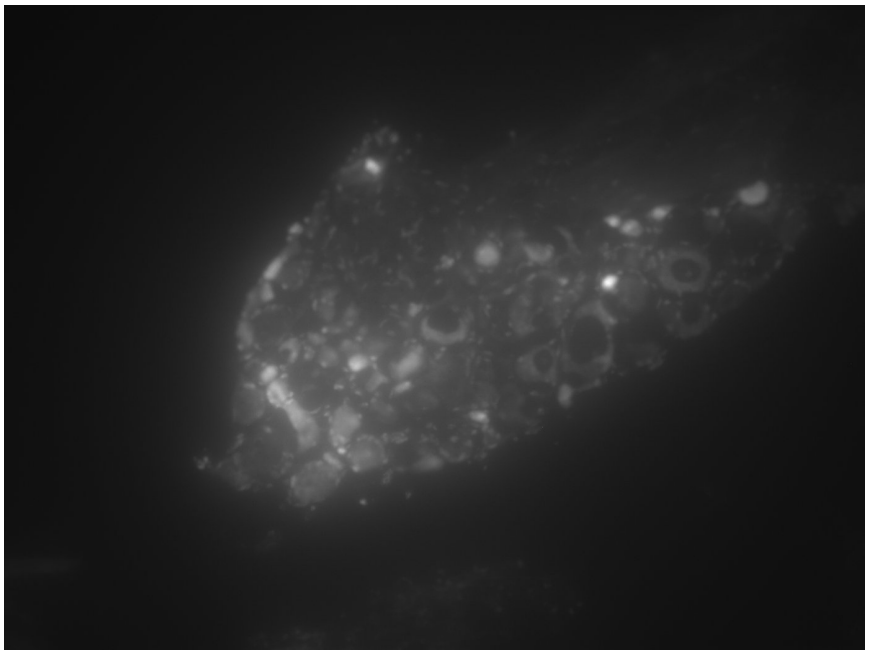


Figure 8: Fast Blue Retrograde Tracing. **A.** Imaging of L4 DRG containing Fast Blue Dye, red arrows point to cells positive for Fast Blue dye **B.** Imaging of sections from each DRG reveal that sensory afferents innervating the hindpaw are primarily localized in L4 DRG (* $p<0.05$)

A.



B.

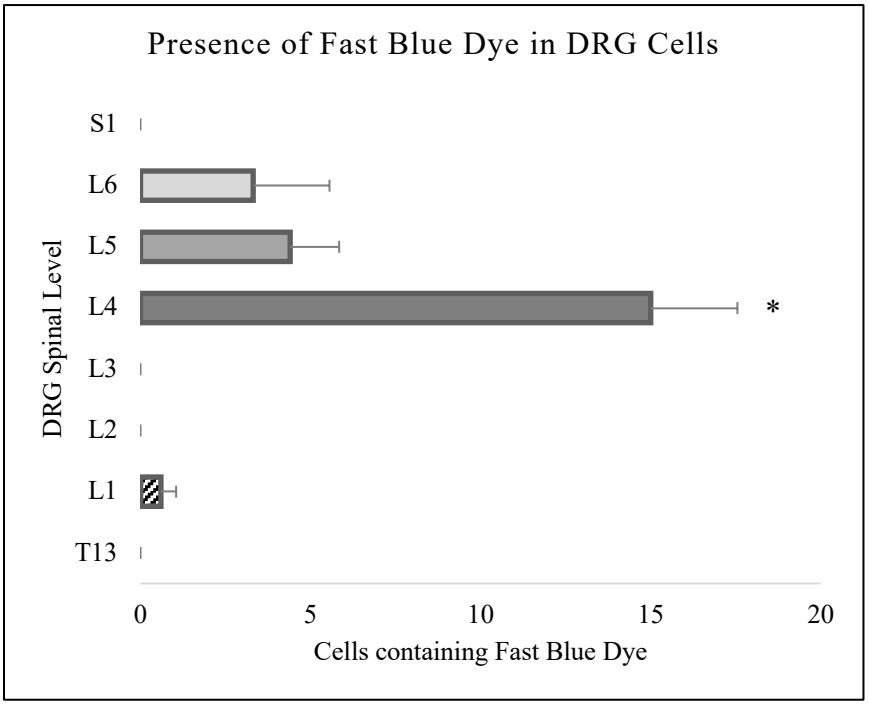
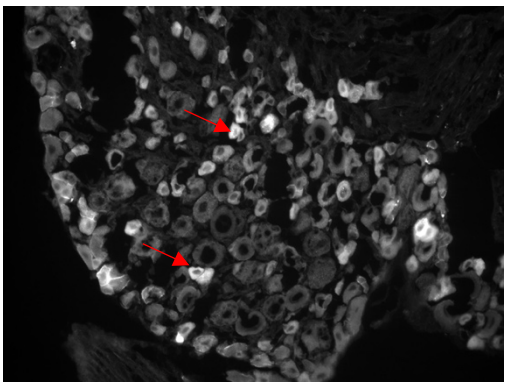
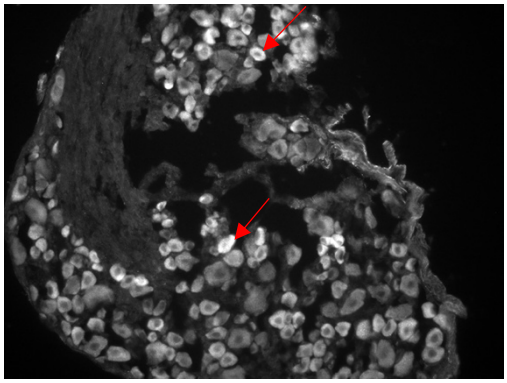


Figure 9: Changes in p-Akt levels after TNBS-induced inflammation. **A.** Images of control versus TNBS-treated DRG **B.** Enlarged image of TNBS treated L4 DRG with arrows pointing to positively stained cells **C.** TNBS-treated mice have increased levels of p-Akt in L4 DRG (n=6, * $p<0.05$)

A.

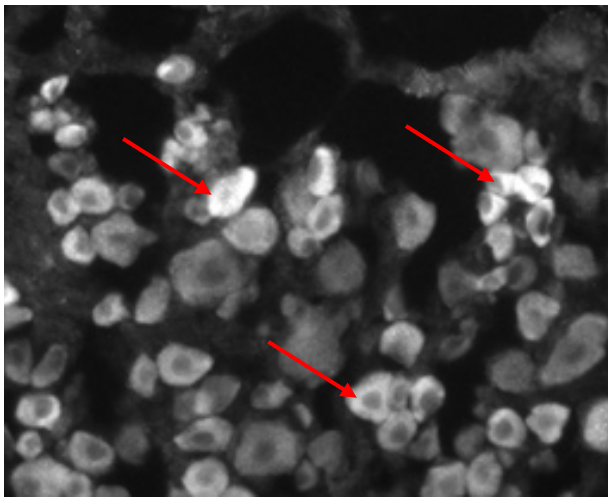


CONTROL



TNBS

B.



TNBS (enlarged)

C.

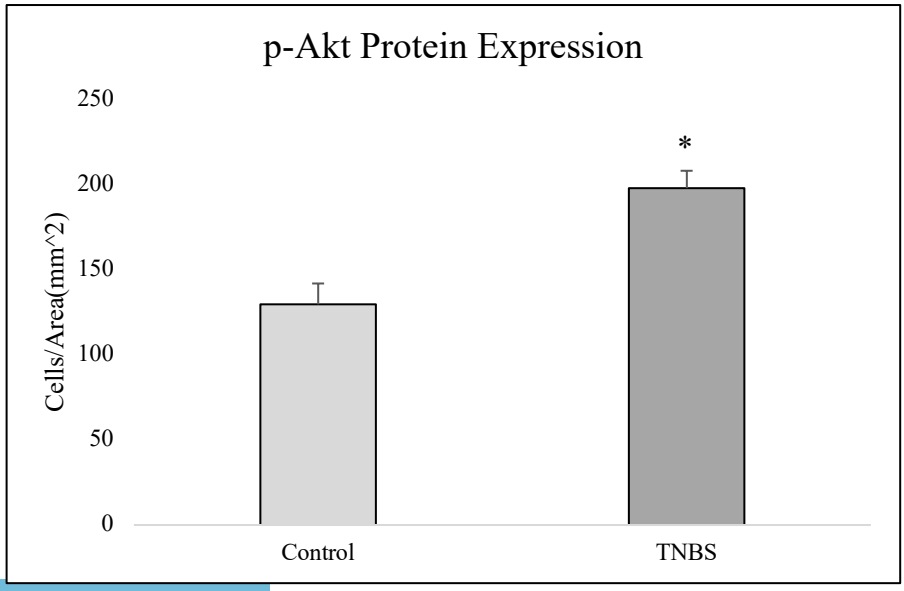


Figure 10: p-Akt expression in Piezo-YFP labeled DRG cells. **A.** Images showing sections of control L4 DRG with Piezo-YFP (green), p-Akt (red), and their colocalization with arrows pointing to positive cells **B.** Images showing sections of TNBS-treated L4 DRG with Piezo-YFP (green), p-Akt (red), and their colocalization with arrows pointing to positive cells

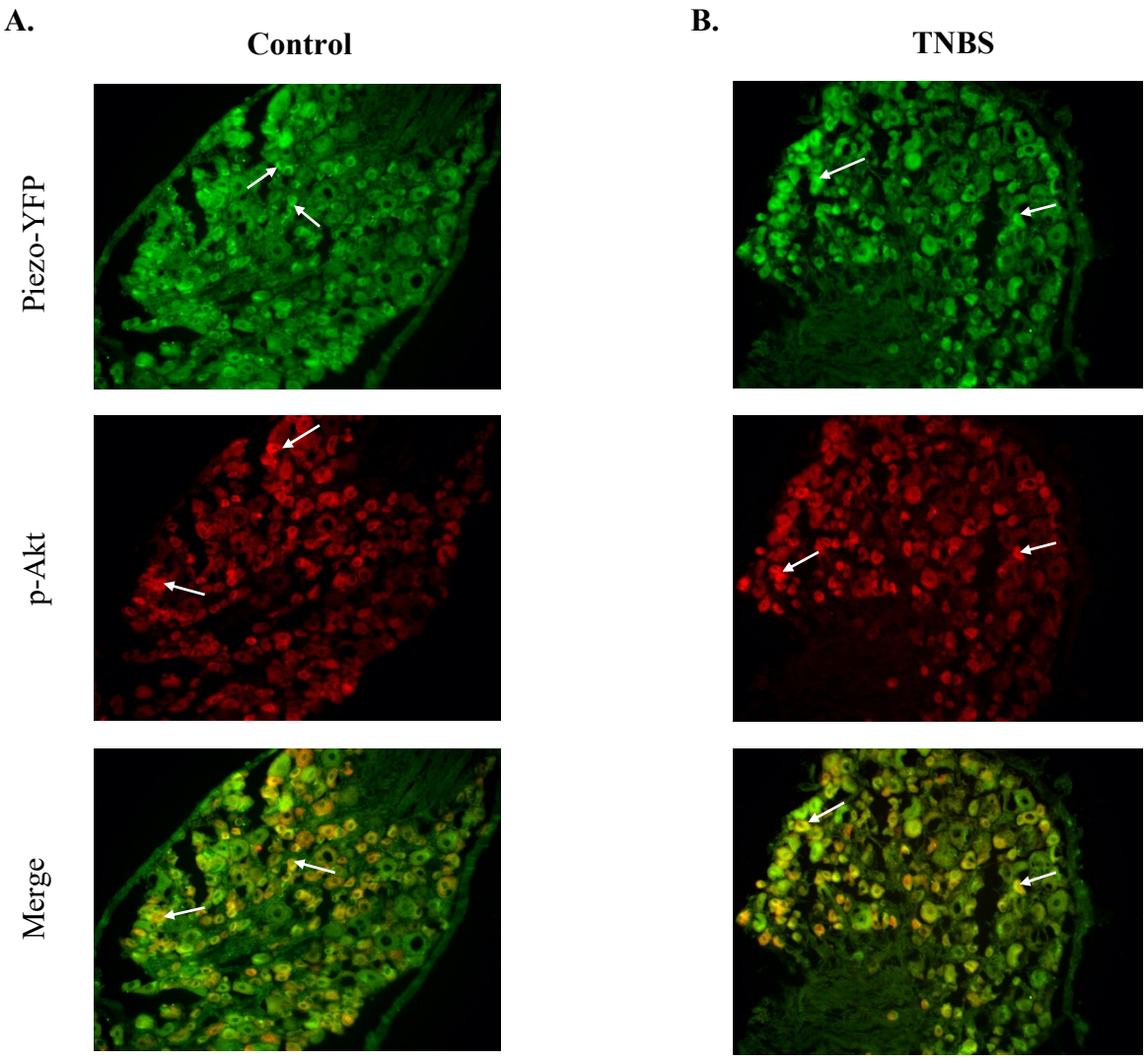
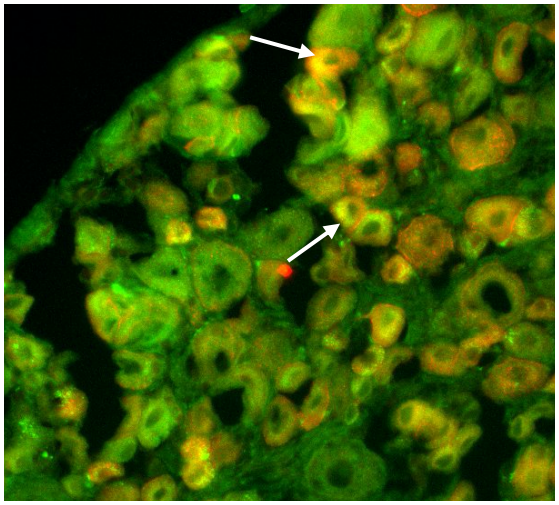
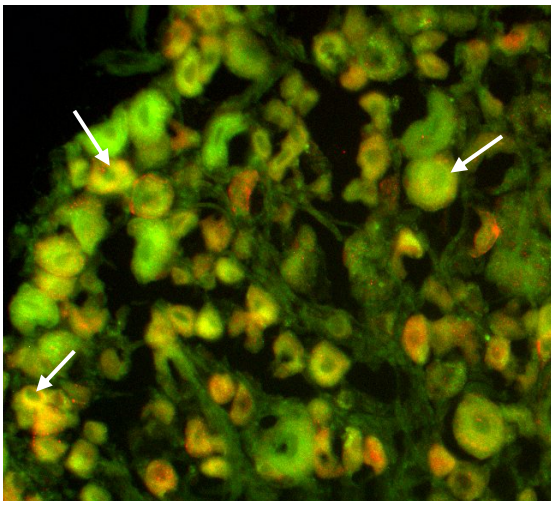


Figure 11: Percentage of Piezo2 cells expressing p-Akt. **A.** Enlarged images of merged control and TNBS-treated L4 DRG, with arrows pointing to cells positive for p-Akt and Piezo colocalization. **B**. Percentage of Piezo-YFP cells expressing p-Akt increases in TNBS-treated L4 DRG (n=1).

A.



Control Merged (enlarged)



Treatment Merged (enlarged)

B.

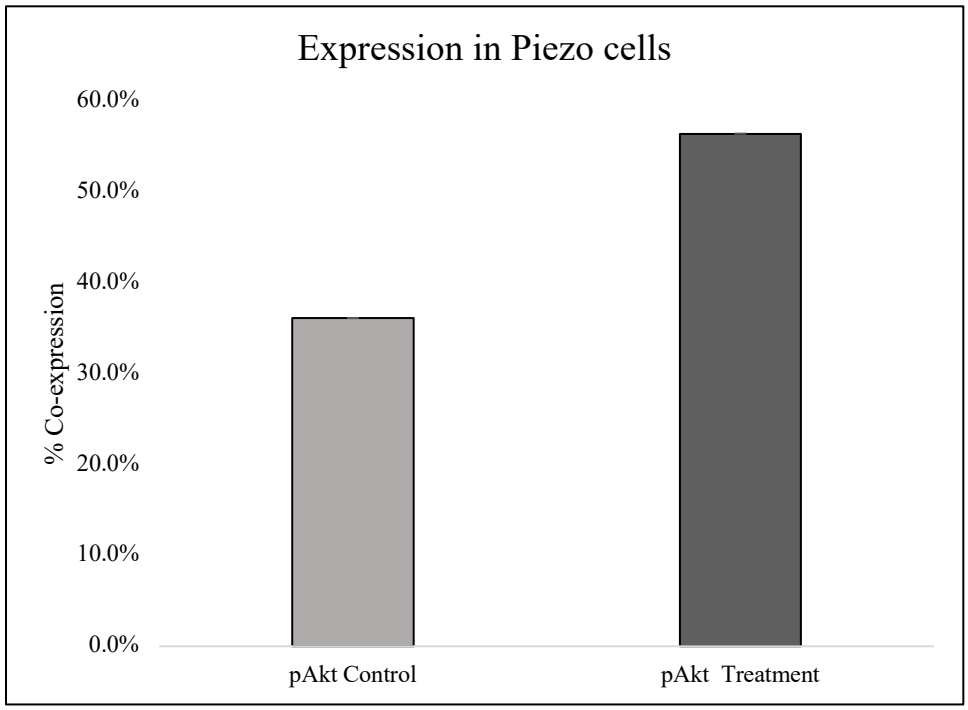
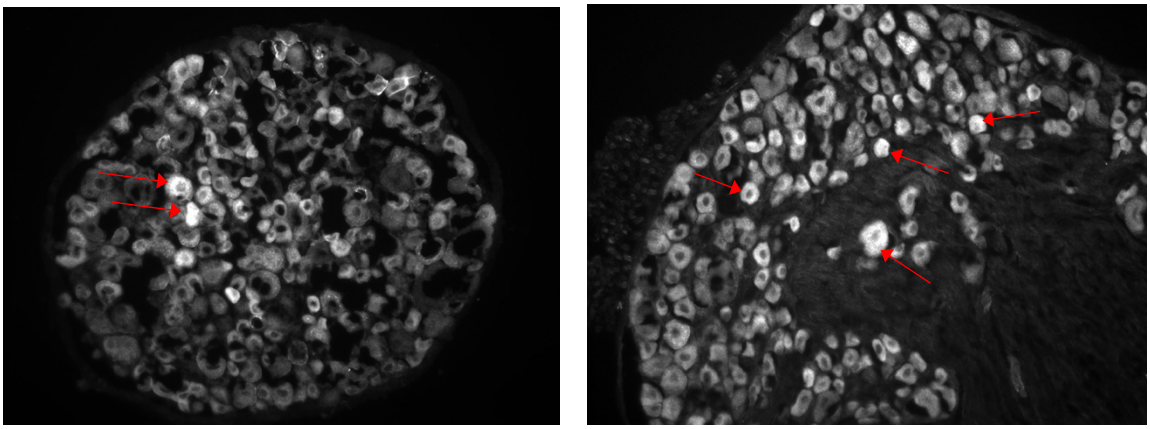
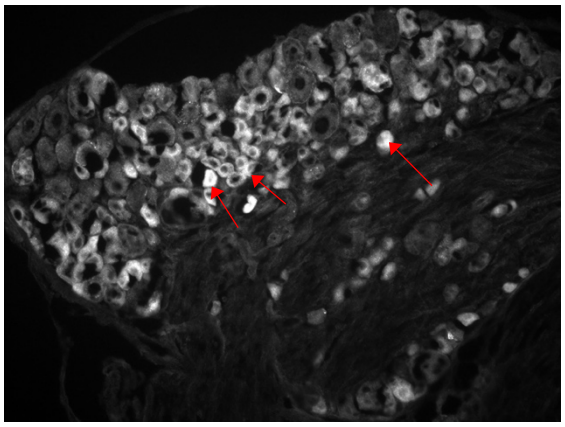


Figure 12: Effect of TrkB.T1 KO on p-Akt expression after TNBS induced inflammation. **A.** Images of L4 DRG from control, TNBS treated, and TNBS + TrkB.T1 KO. **B.** The increase in p-Akt levels in TNBS treated mice is attenuated by TrkB.T1 KO (Control and TNBS: n=6, TrkB.T1 KO: n=3), * $p<0.05$ for TNBS vs control, ** $p<0.05$ for TNBS vs TNBS +TrkB.T1 KO.

A.

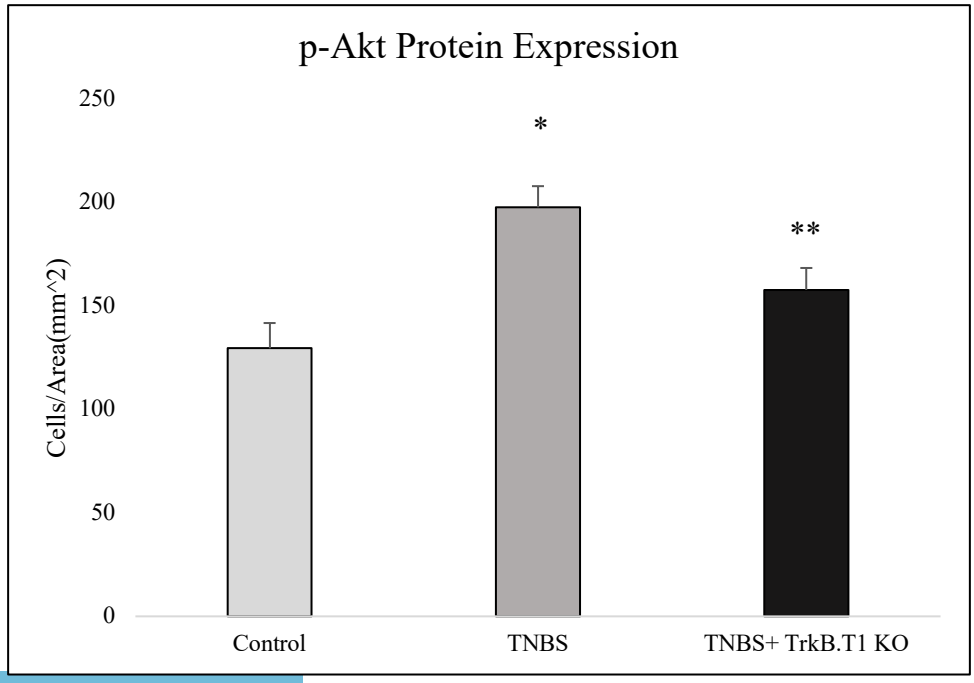


CONTROL TNBS



TNBS + TrkB.T1 KO

B.



Discussion

Inflammatory Bowel Disease (IBD) is a chronic disease affecting millions of people around the world and its prevalence continues to grow. A major contributor to a patient's discomfort is visceral hypersensitivity, which is also experienced by patients with Irritable Bowel Syndrome (IBS). This hypersensitization is attributed to increased inflammatory signaling from primary afferent neurons in the dorsal root ganglion (DRG), and various changes in afferent signaling can lead to the persistence of sensitization. Additionally, a subset of patients with IBD or IBS report development of referred somatic pain, such as neck pain or leg pain, adding to their discomfort (54). The goal of this study is to investigate the underlying cellular mediators which may facilitate the development of such viscerosomatic cross-sensitivity.

In this study, Von Frey tests exhibited that 2,4,6-trinitrobenzene sulfonic acid (TNBS) treated mice began to show mechanical hypersensitivity after one week, and that hypersensitivity peaked two weeks after TNBS administration. Of note, TNBS treated mice also exhibited agitative behavior by attempting to ram their heads into the top of the cage two weeks post-injection, and this was not observed in the control mice. After three weeks, the behavior of the treated mice in response to mechanical stimulation indicated attenuation of their hindpaw hypersensitivity. Retrograde Fast Blue tracing showed that hindpaw innervation primarily takes place through sensory afferents in the L4 dorsal root ganglion (DRG), and this is supported by findings in a previous study (42).

Immunohistochemistry conducted on L4 DRG revealed that phospho-Akt levels were higher in TNBS-treated mice compared to the control, suggesting that the PI3K/Akt pathway is involved in signaling from visceral afferent neurons to those innervating the hindpaw. Previous studies show that an increase in Akt phosphorylation has been noted to occur in L6 DRG of mice with TNBS-induced colitis and the activation of the PI3K/Akt pathway is believed to play a role in spinal central sensitization (37). The increase of phospho-Akt in L4 DRG of TNBS-treated mice noted in this study emphasizes the importance of the PI3K/Akt pathway in spinal cord sensitization and heterosegmental cross-talk. Additionally, colocalization of phospho-Akt and Piezo2 was noted to increase in TNBS-treated animals. Piezo2 is an important gated ion channel expressed by a portion of sensory neurons and is vital for touch sensation. The expression of this channel has been noted to increase in inflammatory conditions, and studies have shown Piezo2 to be a mediator of mechanical sensitization (51). Therefore, the increased colocalization of Piezo2 and phospho-Akt in TNBS-treated mice further supports the role of the PI3K/Akt pathway in referred somatic pain.

The increase in phospho-Akt levels in TNBS-treated mice was found to be partially attenuated in TNBS-treated TrkB.T1 knockout mice. The TrkB.T1 receptor is a truncated form of the TrkB receptor and has a high affinity for BDNF (37). This receptor is upregulated in satellite glial cells (SGCs) during peripheral inflammation, ensuring response to BDNF signaling. The attenuation of phospho-Akt levels in TNBS-treated TrkB.T1 knockout mice indicates that glial cells (SGCs and astrocytes) play a role in somatic hypersensitization. Previous studies examining induced cross-sensitization, specifically of the colon and bladder, have shown that microglia and astrocytes are upregulated in response to inflammation (37,44). These cells start to show high expression of BDNF receptors, and astrocytic TrkB.T1 receptors are known to

induce morphogenesis upon activation. The BDNF increase in sensory neurons can release to the spinal cord therefore inducing increased levels of astrocytes and microglia in the spinal cord, and activation of TrkB.T1 receptors in these non-neuronal cells may allow for the transmission of nociceptive signaling to a distinct DRG via Akt activation.

In conclusion, our findings show that the PI3K/Akt pathway plays a central role in viscerosomatic cross-sensitization. Akt activation is noted to increase in Piezo2 labeled cells, which may be a response indicative of the TNBS-induced somatic mechanical hypersensitivity. BDNF upregulation due to visceral inflammation leads to central sensitization, and activation of the PI3K/Akt pathway via TrkB.T1 receptors allows for the transmission of inflammatory signals through the spinal cord to a heterosegmental DRG, leading to referred somatic pain.

References:

1. Windsor, J.W., Kaplan, G.G (2019). Evolving Epidemiology of IBD. *Curr Gastroenterol Rep* 21, 40.. https://doi.org/10.1007/s11894-019-0705-6

2. Molodecky, N. A., Soon, I. S., Rabi, D. M., Ghali, W. A., Ferris, M., Chernoff, G., Benchimol, E. I., Panaccione, R., Ghosh, S., Barkema, H. W., & Kaplan, G. G. (2012). Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*, *142*(1), 46–e30. https://doi.org/10.1053/j.gastro.2011.10.001

3. Coward, S., Clement, F., Benchimol, E. I., Bernstein, C. N., Avina-Zubieta, J. A., Bitton, A., Carroll, M. W., Hazlewood, G., Jacobson, K., Jelinski, S., Deardon, R., Jones, J. L., Kuenzig, M. E., Leddin, D., McBrien, K. A., Murthy, S. K., Nguyen, G. C., Otley, A. R., Panaccione, R., Rezaie, A., et al (2019). Past and Future Burden of Inflammatory Bowel Diseases Based on Modeling of Population-Based Data. *Gastroenterology*, *156*(5), 1345–1353.e4. https://doi.org/10.1053/j.gastro.2019.01.002

4. Kelsen, J. R., Russo, P., & Sullivan, K. E. (2019). Early-Onset Inflammatory Bowel Disease. *Immunology and allergy clinics of North America*, *39*(1), 63–79. https://doi.org/10.1016/j.iac.2018.08.008

5. Guan Q. (2019). A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease. *Journal of immunology research*, 7247238. https://doi.org/10.1155/2019/7247238

6. McDowell C, Farooq U, Haseeb M. Inflammatory Bowel Disease. [Updated 2020 Jun 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470312/

7. Seyedian, S. S., Nokhostin, F., & Malamir, M. D. (2019). A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *Journal of medicine and life*, *12*(2), 113–122. https://doi.org/10.25122/jml-2018-0075

8. Flynn, S., & Eisenstein, S. (2019). Inflammatory Bowel Disease Presentation and Diagnosis. *The Surgical clinics of North America*, *99*(6), 1051–1062. https://doi.org/10.1016/j.suc.2019.08.001

9. Na, S. Y., & Moon, W. (2019). Perspectives on Current and Novel Treatments for Inflammatory Bowel Disease. *Gut and liver*, *13*(6), 604–616. https://doi.org/10.5009/gnl19019

10. Ng, Q. X., Soh, A., Loke, W., Lim, D. Y., & Yeo, W. S. (2018). The role of inflammation in irritable bowel syndrome (IBS). *Journal of inflammation research*, *11*, 345–349. https://doi.org/10.2147/JIR.S174982

11. Najjar, S. A., Davis, B. M., & Albers, K. M. (2020). Epithelial-Neuronal Communication in the Colon: Implications for Visceral Pain. *Trends in neurosciences*, *43*(3), 170–181. https://doi.org/10.1016/j.tins.2019.12.007

12. Farzaei, M. H., Bahramsoltani, R., Abdollahi, M., & Rahimi, R. (2016). The Role of Visceral Hypersensitivity in Irritable Bowel Syndrome: Pharmacological Targets and Novel Treatments. *Journal of neurogastroenterology and motility*, *22*(4), 558–574. https://doi.org/10.5056/jnm16001

13. Brierley, S. M., Hibberd, T. J., & Spencer, N. J. (2018). Spinal Afferent Innervation of the Colon and Rectum. *Frontiers in cellular neuroscience*, *12*, 467. https://doi.org/10.3389/fncel.2018.00467

14. Deiteren, A., de Wit, A., van der Linden, L., De Man, J. G., Pelckmans, P. A., & De Winter, B. Y. (2016). Irritable bowel syndrome and visceral hypersensitivity : risk factors and pathophysiological mechanisms. *Acta gastro-enterologica Belgica*, *79*(1), 29–38.

15. Farmer, A. D., & Aziz, Q. (2013). Gut pain & visceral hypersensitivity. *British journal of pain*, *7*(1), 39–47. https://doi.org/10.1177/2049463713479229

16. Ahimsadasan N, Reddy V, Kumar A. Neuroanatomy, Dorsal Root Ganglion. [Updated 2021 Jan 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532291/

17. Feng, B., & Guo, T. (2020). Visceral pain from colon and rectum: the mechanotransduction and biomechanics. *Journal of neural transmission*, *127*(4), 415–429. https://doi.org/10.1007/s00702-019-02088-8

18. Grundy, L., Erickson, A., & Brierley, S. M. (2019). Visceral Pain. *Annual review of physiology*, *81*, 261–284. https://doi.org/10.1146/annurev-physiol-020518-114525

19. Feng, B., La, J. H., Schwartz, E. S., Tanaka, T., McMurray, T. P., & Gebhart, G. F. (2012). Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity. *American journal of physiology. Gastrointestinal and liver physiology*, *302*(7), G676–G683. https://doi.org/10.1152/ajpgi.00490.2011

20. Feng, B., La, J. H., Tanaka, T., Schwartz, E. S., McMurray, T. P., & Gebhart, G. F. (2012). Altered colorectal afferent function associated with TNBS-induced visceral hypersensitivity in mice. *American journal of physiology. Gastrointestinal and liver physiology*, *303*(7), G817–G824. https://doi.org/10.1152/ajpgi.00257.2012

21. Sengupta J. N. (2009). Visceral pain: the neurophysiological mechanism. *Handbook of experimental pharmacology*, (194), 31–74. https://doi.org/10.1007/978-3-540-79090-7_2

22. Brierley, S. M., & Linden, D. R. (2014). Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nature reviews. Gastroenterology & hepatology*, *11*(10), 611–627. https://doi.org/10.1038/nrgastro.2014.103

23. Lewin, G. R., & Barde, Y. A. (1996). Physiology of the neurotrophins. *Annual review of neuroscience*, *19*, 289–317. https://doi.org/10.1146/annurev.ne.19.030196.001445

24. Reichardt L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *361*(1473), 1545–1564. https://doi.org/10.1098/rstb.2006.1894

25. López-Pérez, A. E., Nurgali, K., & Abalo, R. (2018). Painful neurotrophins and their role in visceral pain. *Behavioural pharmacology*, *29*(2 and 3-Spec Issue), 120–139. https://doi.org/10.1097/FBP.0000000000000386

26. Bronfman, F. C., Lazo, O. M., Flores, C., & Escudero, C. A. (2014). Spatiotemporal intracellular dynamics of neurotrophin and its receptors. Implications for neurotrophin signaling and neuronal function. *Handbook of experimental pharmacology*, *220*, 33–65. https://doi.org/10.1007/978-3-642-45106-5_3

27. Qiao L. Y. (2014). Neurotrophin signaling and visceral hypersensitivity. *Frontiers in biology*, *9*(3), 216–224. https://doi.org/10.1007/s11515-014-1304-4

28. Petty, B. G., Cornblath, D. R., Adornato, B. T., Chaudhry, V., Flexner, C., Wachsman, M., Sinicropi, D., Burton, L. E., & Peroutka, S. J. (1994). The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. *Annals of neurology*, *36*(2), 244–246. https://doi.org/10.1002/ana.410360221

29. Donnerer, J., Schuligoi, R., & Stein, C. (1992). Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience*, *49*(3), 693–698. https://doi.org/10.1016/0306-4522(92)90237-v

30. Xu, X. J., Zhang, Y. L., Liu, L., Pan, L., & Yao, S. K. (2017). Increased expression of nerve growth factor correlates with visceral hypersensitivity and impaired gut barrier function in diarrhoea-predominant irritable bowel syndrome: a preliminary explorative study. *Alimentary pharmacology & therapeutics*, *45*(1), 100–114. https://doi.org/10.1111/apt.13848

31. Prato, V., Taberner, F. J., Hockley, J., Callejo, G., Arcourt, A., Tazir, B., Hammer, L., Schad, P., Heppenstall, P. A., Smith, E. S., & Lechner, S. G. (2017). Functional and Molecular Characterization of Mechanoinsensitive "Silent" Nociceptors. *Cell reports*, *21*(11), 3102–3115. https://doi.org/10.1016/j.celrep.2017.11.066

32. Scadding JW (1999). Complex regional pain syndrome. In: Wall PD, Melzack R, editors. *Textbook of pain.* 4th ed. Churchill Livingstone; Edinburgh: pp. 835–49

33. Qiao, L. Y., Gulick, M. A., Bowers, J., Kuemmerle, J. F., & Grider, J. R. (2008). Differential changes in brain-derived neurotrophic factor and extracellular signal-regulated kinase in rat primary afferent pathways with colitis. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*, *20*(8), 928–938. https://doi.org/10.1111/j.1365-2982.2008.01119.x

34. Shen, S., Al-Thumairy, H. W., Hashmi, F., & Qiao, L. Y. (2017). Regulation of transient receptor potential cation channel subfamily V1 protein synthesis by the phosphoinositide 3-kinase/Akt pathway in colonic hypersensitivity. *Experimental neurology*, *295*, 104–115. https://doi.org/10.1016/j.expneurol.2017.06.007

35. Qiao, L. Y., & Grider, J. R. (2007). Up-regulation of calcitonin gene-related peptide and receptor tyrosine kinase TrkB in rat bladder afferent neurons following TNBS colitis. *Experimental neurology*, *204*(2), 667–679. https://doi.org/10.1016/j.expneurol.2006.12.024

36. Hashmi, F., Liu, M., Shen, S., & Qiao, L. Y. (2016). EXPRESS: Phospholipase C gamma mediates endogenous brain-derived neurotrophic factor - regulated calcitonin gene-related peptide expression in colitis - induced visceral pain. *Molecular pain*, *12*, 1744806916657088. https://doi.org/10.1177/1744806916657088

37. Qiao, L. Y., & Tiwari, N. (2020). Spinal neuron-glia-immune interaction in cross-organ sensitization. *American journal of physiology. Gastrointestinal and liver physiology*, *319*(6), G748–G760. https://doi.org/10.1152/ajpgi.00323.2020

38. Alagiri, M., Chottiner, S., Ratner, V., Slade, D., & Hanno, P. M. (1997). Interstitial cystitis: unexplained associations with other chronic disease and pain syndromes. *Urology*, *49*(5A Suppl), 52–57. https://doi.org/10.1016/s0090-4295(99)80332-x

39. Grundy, L., & Brierley, S. M. (2018). Cross-organ sensitization between the colon and bladder: to pee or not to pee?. *American journal of physiology. Gastrointestinal and liver physiology*, *314*(3), G301–G308. https://doi.org/10.1152/ajpgi.00272.2017

40. Xia, C. M., Gulick, M. A., Yu, S. J., Grider, J. R., Murthy, K. S., Kuemmerle, J. F., Akbarali, H. I., & Qiao, L. Y. (2012). Up-regulation of brain-derived neurotrophic factor in primary afferent pathway regulates colon-to-bladder cross-sensitization in rat. *Journal of neuroinflammation*, *9*, 30. https://doi.org/10.1186/1742-2094-9-30

41. Huang, T. Y., Belzer, V., & Hanani, M. (2010). Gap junctions in dorsal root ganglia: possible contribution to visceral pain. *European journal of pain (London, England)*, *14*(1), . https://doi.org/10.1016/j.ejpain.2009.02.005

42. Gao, X., Han, S., Huang, Q., He, S. Q., Ford, N. C., Zheng, Q., Chen, Z., Yu, S., Dong, X., & Guan, Y. (2021). Calcium imaging in population of dorsal root ganglion neurons

unravels novel mechanisms of visceral pain sensitization and referred somatic hypersensitivity. *Pain*, *162*(4), 1068–1081. https://doi.org/10.1097/j.pain.0000000000002096

43. Amir, R., & Devor, M. (1996). Chemically mediated cross-excitation in rat dorsal root ganglia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *16*(15), 4733–4741. https://doi.org/10.1523/JNEUROSCI.16-15-04733.1996

44. Lucarini, E., Parisio, C., Branca, J., Segnani, C., Ippolito, C., Pellegrini, C., Antonioli, L., Fornai, M., Micheli, L., Pacini, A., Bernardini, N., Blandizzi, C., Ghelardini, C., & Di Cesare Mannelli, L. (2020). Deepening the Mechanisms of Visceral Pain Persistence: An Evaluation of the Gut-Spinal Cord Relationship. *Cells*, *9*(8), 1772. https://doi.org/10.3390/cells9081772

45. Chen, W. S., Xu, P. Z., Gottlob, K., Chen, M. L., Sokol, K., Shiyanova, T., Roninson, I., Weng, W., Suzuki, R., Tobe, K., Kadowaki, T., & Hay, N. (2001). Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes & development*, *15*(17), 2203–2208. https://doi.org/10.1101/gad.913901

46. Li, Q., Winston, J. H., & Sarna, S. K. (2016). Noninflammatory upregulation of nerve growth factor underlies gastric hypersensitivity induced by neonatal colon inflammation. *American journal of physiology. Regulatory, integrative and comparative physiology*, *310*(3), R235–R242. https://doi.org/10.1152/ajpregu.00342.2015

47. Kay, J. C., Xia, C. M., Liu, M., Shen, S., Yu, S. J., Chung, C., & Qiao, L. Y. (2013). Endogenous PI3K/Akt and NMDAR act independently in the regulation of CREB activity in lumbosacral spinal cord in cystitis. *Experimental neurology*, *250*, 366–375. https://doi.org/10.1016/j.expneurol.2013.10.015

48. Carim-Todd, L., Bath, K. G., Fulgenzi, G., Yanpallewar, S., Jing, D., Barrick, C. A., Becker, J., Buckley, H., Dorsey, S. G., Lee, F. S., & Tessarollo, L. (2009). Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(3), 678–685. https://doi.org/10.1523/JNEUROSCI.5060-08.2009

49. Cao, T., Matyas, J. J., Renn, C. L., Faden, A. I., Dorsey, S. G., & Wu, J. (2020). Function and Mechanisms of Truncated BDNF Receptor TrkB.T1 in Neuropathic Pain. *Cells*, *9*(5), 1194. https://doi.org/10.3390/cells9051194

50. Volkers, L., Mechioukhi, Y., & Coste, B. (2015). Piezo channels: from structure to function. *Pflugers Archiv : European journal of physiology*, *467*(1), 95–99. https://doi.org/10.1007/s00424-014-1578-z

51. Murthy, S. E., Loud, M. C., Daou, I., Marshall, K. L., Schwaller, F., Kühnemund, J., Francisco, A. G., Keenan, W. T., Dubin, A. E., Lewin, G. R., & Patapoutian, A. (2018). The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. *Science translational medicine*, *10*(462), eaat9897. https://doi.org/10.1126/scitranslmed.aat9897

52. Ranade, S. S., Woo, S. H., Dubin, A. E., Moshourab, R. A., Wetzel, C., Petrus, M., Mathur, J., Bégay, V., Coste, B., Mainquist, J., Wilson, A. J., Francisco, A. G., Reddy, K., Qiu, Z., Wood, J. N., Lewin, G. R., & Patapoutian, A. (2014). Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature*, *516*(7529), 121–125. https://doi.org/10.1038/nature13980

53. Yang, J., Zhang, J., Yang, H., Li, K., Lei, X., & Xu, C. (2016). The potential role of Piezo2 in the mediation of visceral sensation. *Neuroscience letters*, *630*, 158–163. https://doi.org/10.1016/j.neulet.2016.07.058

54. Zhou, Q., & Verne, G. N. (2011). New insights into visceral hypersensitivity--clinical implications in IBS. *Nature reviews. Gastroenterology & hepatology*, *8*(6), 349–355. https://doi.org/10.1038/nrgastro.2011.83

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