Altered ion channel/receptor expression and function in extrinsic sensory neurons: The cause of and solution to chronic visceral pain?

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Acknowledgements:

A/Prof Stuart Brierley is supported by an NHMRC R.D Wright Fellowship and by NHMRC Australia Project grants (1063803, 1063803, 1049928 and 1049682).

Abstract:

The gastrointestinal tract is unique in that it is innervated by several distinct populations of neurons, whose cell bodies are either intrinsic (enteric, viscerofugal) or extrinsic (sympathetic, sensory afferents) to the wall of the gut. We are usually completely unaware of the continuous, complicated orchestra of functions that these neurons conduct. However, for patients with Inflammatory Bowel Disease (IBD) or functional gastrointestinal disorders, such as Functional Dyspepsia (FD) and Irritable Bowel Syndrome (IBS) altered gastrointestinal motility, discomfort and pain are common, debilitating symptoms. Whilst bouts of inflammation underlie the symptoms associated with IBD, over the past few years there is increased pre-clinical and clinical evidence that infection and inflammation are key risk factors for the development of several functional gastrointestinal disorders, in particular IBS. There is a strong correlation between prior exposure to gut infection and symptom occurrence; with the duration and severity of the initial illness the strongest associated risk factors. This review discusses the current body of evidence for neuroplasticity during inflammation and how in many cases fails to reset back to normal, long after healing of the damaged tissues. Recent evidence suggests that the altered expression and function of key ion channels and receptors within extrinsic sensory neurons play fundamental roles in the aberrant pain sensation associated with these gastrointestinal diseases and disorders.

Introduction:

Neural control of gastrointestinal function is a highly integrated system, comprised of distinct populations of neurons, whose cell bodies are either intrinsic or extrinsic to the gut wall. Neural control involves interactions between; i) local enteric reflexes within the gut wall; ii) reflexes that pass through prevertebral sympathetic ganglia and iii) reflexes that pass to and from the gut via the central nervous system (CNS)[1]. To add further intricacy the gastrointestinal tract is an incredibly complex signalling environment. Neurons are subjected mechanical events such as distension and contraction, whilst being inundated with a constantly changing milieu of endogenous mediators [2]. Inflammation of the gut, either through abnormal immune responses or via gut infection has been consistently demonstrated to cause neuroplasticity and abnormal neuronal function. These profound effects result in disregulated neuronal signalling, abnormal secretion, motility and sensory signalling resulting in the development of diarrhea, constipation, discomfort and pain. The importance of this neuroplasticity is highlighted in a number of highly prevalent organic and functional gastrointestinal disorders. In organic disorders such as Inflammatory Bowel Disease (IBD), which includes Crohn's Disease and Ulcerative Colitis, chronic uncontrolled inflammation of the intestinal mucosa is recognised as the pathogenesis of neuronal dysfunction and correspondingly the presentation of symptoms [3,4]. However, for functional bowel disorders such as Irritable Bowel Syndrome (IBS), where macroscopic mucosal damage is not evident, but symptoms of persistent abdominal pain, discomfort and abnormal bowel function are evident [2]. The underlying source of this neuronal deregulation remained unclear, until the recent association with infectious gastroenteritis. Numerous clinical studies have attributed IBS symptom development to a preceding bout of gastroenteritis induced by pathogens such as *Campylobacter* [5,6], *Escherichia coli* [6], *Salmonella* [7], *Giardia lamblia* [8]. Whilst IBS is multi-factorial and several additional risk factors may also be required for development [9, 10], acute gastroenteritis can trigger IBS symptoms that persist for at least 8 years [11]. In the relatively

short term setting of tissue damage, inflammation is a protective process which facilitates wound healing, however these clinical findings suggests that in these individuals the neuroplasticity induced by infection and inflammation, fails to reset back to normal long after healing of the intestinal tissue. As increasing effort has been directed towards determining the extent of neuroplasiticity that is associated with gut disorders then the number of different models used to investigate it has also increased. Experimental models of gut inflammation have included administration of dextran sodium sulphate (DSS), chemical irritants such as mustard oil and acetic acid, infection with nematodes (e.g. *Trichinella spiralis)* or bacteria (e.g *Citrobacter rodentium*), and haptens such as trinitrobenzene sulphonic acid (TNBS). However, the time course and nature of the resultant inflammation is different between these models and is defined by the different categories of immune cells involved in the response [12, 13]. For example TNBS combines with endogenous proteins and antigens to evoke a transmural Th1-mediated inflammation, whilst DSS is more dependent on innate immunity and is restricted to the mucosa. Furthermore, zymosan which unlike the models described above, does not induce an increase in myeloperoxidase (MPO) activity at any time after intracolonic treatment, does result in a brief monocyte-based inflammation [14]. However, despite these differences an increasing amount of data suggests neuroplasticity can occur in extrinsic sensory afferent neurons, their peripheral and central projections and their resultant communication with the CNS. These changes are likely to further alter communication along the brain-gut axis, and have a profound effect on resultant neuronal plasticity. Given this complexity and that understanding of many of these interactions remain in their infancy; this review will focus on the resultant effects of gut inflammation on neuronal function in the gut-brain pathway and the persistent long term neuroplasticity than remains following resolution of inflammation. Where apparent this review will also highlight the channels, receptors and mediators involved in this process.

Extrinsic sensory afferent pathways innervating the gastrointestinal tract:

Distinct from the enteric and sympathetic nervous systems are the extrinsic sensory innervations of the gastrointestinal tract. These pathways have become one of the most intensely studied areas of neuro-gastroenterology, as they are the first step in generating sensations. In particular they are responsible for signaling nociceptive stimuli from the gut, and ultimately the conscious perception of pain. Therefore, identifying the afferents, mediators and mechanisms involved in this process is crucial in understanding the mechanisms of neuroplasticity underlying inflammatory and chronic visceral pain. Most studies have focused on the innervations of the small intestine, colon and rectum, as these regions are associated with the symptoms of IBD and IBS. The complexity of this intact system means that many of its individual components; afferent endings in the gut wall, cell bodies in the DRG, activation of pathways in the spinal cord and the overall pain-related behavior to gut distension (visceromotor response to colorectal distension) have been studied independently, either *in vitro* or *in vivo*. These studies indicate that mechanisms underlying inflammatory and chronic post-inflammatory visceral pain are varied, but originate from changes in the periphery [15-19]. The peripheral endings of particular afferent subtypes feed into nociceptive pathways within the spinal cord and pain sensing regions in the brain. Whilst pain is an emotive process, the threshold of nociceptors has to be high enough not to interfere with normal physiology, but low enough that it can be evoked before marked tissue damage occurs [20]. In order to achieve this function nociceptive nerve endings express a variety of ion channels and receptors, which regulate neuronal excitability and transduce mechanical or chemical stimuli [21-25]. To add further complexity there are several schools of thought regarding the types of afferent that contribute to nociceptive signaling and therefore inflammatory and chronic pain. These subtypes include low- and high-threshold afferents, and mechanically insensitive 'silent' afferents. However, by definition nociceptors selectively

respond to noxious or potentially tissue damaging stimuli and can be sensitized, or increase their excitability in response to tissue insult or inflammation.

Neuroplasticity in extrinsic sensory afferent pathways innervating the gut.

It is clear that experimentally induced inflammation or infection causes afferent hypersensitivity, neuronal hyper-excitability and correspondingly hyperalgesia and allodynia in whole animal models. Consistent findings of neuroplasticity have been most apparent when studying isolated neuronal cell bodies across different regions of gut and across different experimental models. Most studies utilizing inflammatory (TNBS), nematode (*T. Spiralis, Nippostrongylus brasiliensis*) or bacterial models (*Citrobacter rodentium*) show that neurons innervating the stomach [26-29], small intestine [30-33] and the colon [34-36] display pronounced hyper-excitability after the initial insult. This hyper-excitability is characterized by a decreased threshold for activation, increased firing rate, increases in TTX-resistant Na_V currents and suppression of K_V, I_A and I_K channels (Figure 3). Recent reports indicate a crucial role for $Na_V1.8$ in colonic innervating DRG neurons, with its expression differentially regulated across different time points during colitis [36]. Furthermore, *Nippostrongylus brasiliensis* induced jejunal neuronal hyper-excitability is lost in $Na_V1.8^{-/-}$ mice, but not $Na_V1.9^{-/-}$ mice [30]. Longer term neuroplasticity is also evident as K_V, I_A and I_K currents are reduced in colonic innervating DRG neurons 10 days post-*Citrobacter rodentium* infection, whilst suppression of K_V I_A currents contributes to neuronal hyper-excitability 30 days post-infection [35].

Neuroplasticity of peripheral sensory afferent endings is also evident across a range of different experimental models; however different afferent subtypes, different neuronal pathways and time courses are involved in this process. For example, following *T. spiralis* infection both low- and high-threshold jejunal afferents initially display significant *reductions*

in mechanosensitivity at 14 days post-infection. However, at 28 and 56 days post-infection pronounced mechanical hypersensitivity is now evident [33]. The development of this longer term mechanical hypersensitivity is dependent upon a $P2X_7$ receptor-dependent increase in immune cell IL-1β expression and release. Notably these $P2X_7R$ -/- animals display a clear attenuation of the innate inflammatory response and no post-infectious mechanical hypersensitivity at any time point [37].

DSS-induced colonic inflammation does not induced afferent mechanical hypersensitivity [38], or short or long term hyperalgesia in response to colorectal distension [39]. However, DSS treated animals display increased visceral sensitivity to capsaicin and 5-HT [39,40]. By contrast, TNBS induced colitis causes high-threshold nociceptors to become mechanically sensitized, have reduced activation thresholds, and display hypersensitive responses in inflammatory and post-inflammatory states [41, 42]. This hypersensitivity is particularly apparent in splanchnic afferents with high mechanical activation thresholds, which is partially mediated by TRPA1 [43]. A potential contributing factor is also a reduction in the mechanosensitive K2P channels TREK-1 and TREK-2, as these hyperpolarizing K^+ channels are significantly reduced in splanchnic and pelvic colonic DRG neurons during TNBS inflammation[44]. The extent of this mechanical hypersensitivity in high threshold afferents is greater following recovery from overt tissue damage (28 days post-TNBS) [41, 42]. This hypersensitivity translates to an increased density and sprouting of colonic afferent central terminals in the thoracolumbar spinal cord and an increased number of activated DH neurons in the spinal cord in response to noxious colorectal distension [45]. In contrast, the same investigators have shown TNBS induced mechanical hypersensitivity is not evident during inflammation in afferents with low-thresholds (mucosal, muscular and muscular/mucosal). However, pelvic high-threshold and mucosal afferents only become hypersensitive post-inflammation [41, 42]. Other studies have shown transient, absent or inconsistent effects of

TNBS-induced inflammation on low-threshold distension-sensitive afferents [46-48,49] and transient hypersensitivity during *in vivo* colorectal distension studies [50]. The apparent discrepancy of these findings with TNBS may relate to the severity of mucosal inflammation, which is a predictor for alterations of visceral sensory function in rodents [51] and in humans. However, acute zymosan treatment, which recruits a different immune response, does lead to low-threshold sensitive afferents displaying short and long term hypersensitivity [14, 49], which is partially dependent on TRPV1 [52], ASIC3 [52] and P2X receptors [53]. Inflammatory mediators, TNBS and zymosan treatment can also activate or sensitize two different types of mechanically insensitive afferents (MIAs), also known as 'silent afferents'. One population is silent, responds to chemical stimuli, but doesn't subsequently display mechanosensitivity [54, 55], whilst the other population is sensitized by mediators and develops mechanosensitivity [56]. The proportion of this second type of MIA is increased in a number of inflammatory and post-inflammatory states [14, 49]. Another model utilizing intracolonic administration of deoxycholic acid, an unconjugated secondary bile acid, induces a mild, transient colonic inflammation within 3 days, which resolves within 3 weeks. This causes exaggerated visceromotor responses to colorectal distension, referred pain to mechanical stimulation, and increased dorsal horn neuron activity, which persists for at least 4 weeks [57].

Various stress models have been shown to increase visceral pain sensitivity [58,59]. However, stress, combined with prior acute colitis induced by *C rodentium*, results in exaggerated peripheral nociceptive signaling of colonic afferents, their cell bodies and correspondingly visceromotor reflex thresholds via protease, β-2 adrenergic, glucocorticoid receptor and PAR2 mechanisms [60]. However, such an interaction does not occur with stress and DSS treatment [61], which again may suggest specific neuroimmune interactions in the development of neuroplasticity and chronic colonic hyperalgesia.

One of the most consistent and long-term displays of visceral neuroplasticity occurs following neonatal insult. In these cases neonatal animals receive either mechanical or chemical colonic irritation between post-natal days 8 and 21 and are then tested when they are adults [62]. Colonic irritation in neonates results in chronic visceral hypersensitivity, allodynia and hyperalgesia, associated with central neuronal sensitization, in the absence of identifiable peripheral abnormalities. Evidence exists for TRPV1 [52, 63, 64] and TRPA1 [65] initiating colonic hypersensitivity and TRPV1[64], P2X [66] and TRPA1 [65] maintaining colonic hypersensitivity induced by by neonatal acetic acid or mustard oil colonic irritation. More recent studies indicate similar mechanisms in the upper gut, which may be applicable to Functional Dyspepsia. Gastric irritation in neonates results in chronic gastric hypersensitivity and gastric motor dysfunction in adults, in the absence of detectable gastric pathology [67]. This gastric hypersensitivity in adults can be attenuated by the $GABA_B$ agonist baclofen, although this analgesic affect appears to occur via central rather than peripheral mechanisms [68].

Insights into the mechanisms of neuroplasticity using IBS patient biopsies and samples:

In some subgroups of IBS patient's persistent low-grade inflammation within the gut wall [15, 16] and altered immunological function [18, 69, 70] are evident and may lead to recurrent re-sensitisation of nerve function within the gut [69, 71]. One of the first reports of this interaction demonstrated IBS patients have greater colonic mast cell infiltration and an increased release of key mediators, tryptase and histamine. Crucially these activated mast cells are in closer proximity to nerve fibres in IBS patients, which correlates with the severity and frequency of abdominal pain and discomfort [16]. Correspondingly, supernatants from IBS patient biopsies, but not healthy sbjects, causes activation of afferent nerve endings and their cell bodies, via histamine H1 receptor and serine protease mechanisms [17]. Similar findings have been demonstrated using supernatants from Ulcerative Colitis patients, where application of supernatants enhances the neuronal excitability of colonic sensory DRG neurons. However, in

this case the pro-inflammatory cytokine, TNFα is the key mediator, as it is elevated in Ulcerative Colitis biopsies, and acts at neuronal TNFR1 to modulate K_v and Na_v currents. These findings have increased importance as TNFα and the Ulcerative Colitis supernatants both enhance Na_V currents, and suppress K_V (I_A and I_K) currents [72], which are the same currents that are altered in inflammatory and post-inflammatory states [21].

Changes in IBS patients are also evident in peripheral blood mononuclear cells (PBMCs) [18, 69-71]. In particular several pro-inflammatory cytokines, TNF-α, IL-1β and IL-6, are all increased in PBMC supernatants from diarrhoea-predominant IBS (IBS-D) patients, which correlate with symptoms of pain frequency and intensity [18, 69]. Notably, these supernatants from IBS-D patients evoke pronounced mechanical hypersensitivity in high- and low-threshold splanchnic and pelvic colonic afferents [69, 71]. As these colonic afferents express the receptors for these cytokines they can individually sensitise splanchnic and pelvic colonic afferents to mechanical stimuli [69, 71]. Whilst IL-1β causes direct firing of colonic afferents via a $NaV_{1.7}$ mechanism, TNF-α induces mechanical hypersensitivity, via a TRPA1 dependent mechanism [69]. This is one of numerous interactions that exist between pro-nociceptive mediators and TRP channels, which play key roles in inducing neuronal hypersensitivity and neuroplasticity.

TRP channels: key roles for neuroplasticity.

In addition to its interaction with TNF-α [69], TRPA1 also mediates the mechanical hypersensitivity induced by bradykinin [43], as well as PAR2-induced hyperalgesia [73]. This is important as TRPA1 plays a major role in visceral nociception, as TRPA1 deletion causes pronounced mechanosensory deficits, predominantly in high-threshold colonic afferents [43, 74] and correspondingly reduces visceromotor responses to noxious colorectal distension [43].

Furthermore, activation of TRPA1 by numerous agonists, including mustard oil, cinnamaldehyde and 4-hydroxynonenal, can tune nociceptor responses, inducing pronounced mechanical hypersensitivity [43], and visceral mechanical hyperalgesia [73]. Notably, TRPA1 function is increased during TNBS induced inflammation [43] and TRPA1 deletion markedly reduces TNBS-induced colonic mechanical hyperalgesia [73], suggesting TRPA1 is also a key contributor to inflammatory pain. In addition to these effects on neurons, TRPA1 can also contribute to the inflammatory response itself, via neurogenic inflammation, as activation and sensitization of TRPA1 and release of substance P induces and maintains colitis in mice [75], which correspondingly re-sensitises nociceptors.

Another member of the TRP channel family, TRPV4, also plays a key role in nociception, neuroplasticity and pain. TRPV4 is predominantly expressed in spinal neurons innervating the colon and in the gut only contributes to the mechanosensory function of high-threshold colonic afferents [76]. These changes in colonic neuronal function translate to decreased visceromotor responses to colorectal distension in TRPV4 -/- mice, or in mice with siRNA induced down-regulation of TRPV4 [76-78]. TRPV4 also has a crucial interaction with PAR2, whereby TRPV4 is required for PAR2-induced excitation of colonic afferent neurons and colonic mechanical hyperalgesia [79]. PAR2 is also a key receptor for inducing neuroplasticity, as PAR2 agonists can evoke sustained hyperexcitability of colonic nociceptive neurons by suppressing I_K currents, via a PKC and ERK(1/2) pathway [80]. More recently another key PAR2-dependent mediator has been identified, cathepsin-S, which is activated in macrophages during TNBS colitis and evokes hyperexcitability of colonic nociceptive neurons and visceral hyperalgesia [81]. TRPV4 can also be sensitised by a series of other mediators leading to neuronal hyperexcitability. Pre-exposure of colonic DRG neurons to 5-HT or histamine increases TRPV4 agonist induced responses and increases TRPV4 expression at the plasma membrane via PKC, PLA(2), PLCβ and MAPKK-dependent mechanisms [82]. TRPV4 can

also contribute to the inflammation response itself, by inducing neurogenic inflammation, via activation of neuronal TRPV4 stimulating neuropeptide release from peripheral afferent terminals [83]. Secondly, TRPV4 is also expressed on intestinal epithelial cells, where its activation induces chemokine release and induces colitis [84].

TRPV1 is the most identifiable of the TRP channels and has long been implicated in gut nociception and altered neuronal function. Intra-colonic administration of the TRPV1 agonist, capsaicin, causes pronounced visceral pain [85, 86], whilst TRPV1 -/- mice display decreases in visceromotor responses to colorectal balloon distension [87]. TRPV1 appears to have a transient role in neuroplasticity, with initial increases in TRPV1 expression and function during the height of active colonic inflammation [46, 88-90], which may return to normal levels at later post-inflammatory time points [89]. Correspondingly, TRPV1 deletion or pharmacological blockade partially reverses inflammation induced mechanical hypersensitivity and hyperalgesia [52, 89]. However, a key interaction in this process appears to be via TRPV1 and the G protein-coupled receptor kinase 6 (GRK6) [91]. The pro-inflammatory cytokine IL-1β sensitizes TRPV1, which can be prevented by over-expressing GRK6. Following colitis, TRPV1-induced behavioural pain responses are more pronounced in GRK6 -/- mice than in wild-type mice, suggesting GRK6 can regulate inflammation-induced sensitization hyperalgesia[91].

Neuroplasticity induced by bacterial cell products

Mucosal barrier function is crucial for the overall function of the gastrointestinal tract; however it is disturbed during inflammation associated with IBD [92], whilst alterations and increased epithelial permeability are also evident in the small intestine and colon of IBS patients [93, 94]. These changes may allow bacteria to access the interstitial compartment of the gut and several recent studies have identified that bacterial cell products can profoundly alter gut neuronal function. In the jejunum lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, activates extrinsic sensory afferents [95,96], an effect which is reduced

by a non-selective cannabinoid agonist, an anandamide transport inhibitor, but not by a fatty acid amide hydrolase (FAAH) inhibitor [96]. Interestingly, activation of afferents appears to be specific for certain types of LPS, as luminally applied LPS from *Salmonella typhimurium*, but not LPS from *Escherichia coli,* activates these jejunal afferents [97]. Increased afferent activity and an increased afferent sensitivity to a 5-HT3-receptor agonist following *Salmonella typhimurium* LPS can be blocked via a cyclo-oxygenase or EP1/EP2 mediated mechanism [97]. Afferents innervating the colon can also be activated by LPS. Standard-grade LPS applied acutely for 3 minutes or chronically incubated for 24 hours induces significant increases colonic DRG neuronal excitability [98]. These effects can be mimicked by acute application of bacterial lysate from *Escherichia coli* NLM28, which is exaggerated during DSS induced colitis. However, these effects cannot be blocked in TLR4 -/- mice or be replicated by the use of selected bacterial products activating individual TLRs, suggesting additive or alternate mechanisms may be involved. As ultrapure LPS cannot mimic the hyper-excitability effects of standard-LPS and lysate, but does stimulate TNF-α secretion from acutely dissociated DRG neurons, bacterial cell products may also sensitize colonic afferents via the release of pro-nociceptive cytokines from both immune cells and the neurons themselves. This appears to be evident by intracolonic administration of a toll-like receptor TLR7 activator, which causes inflammation, and induces short term hyperalgesia which is reduced in $Na_V1.9$ -/- mice. As wild-type and -/- mice display similar acute inflammatory responses and similar increases in pro-inflammatory cytokines, this reduction in hyperalgesia in $Na_V1.9$ -/- mice presumably occurs via the loss of neuronal $Na_V1.9$ [99].

<u>Endogenous factors that reduce nociceptor signalling</u>

In addition to the myriad of nociceptive mediators and mechanisms described above, several key anti-nociceptive mechanisms have also been described that can reduce nociceptor signalling and prevent hyperalgesia and allodynia. Protease activated receptor 4 (PAR4)

agonists suppresses the excitability of colonic DRG neurons [100] and significantly reduce the visceromotor response to colorectal distension in whole animal studies [101]. PAR4 is actually co-localised in the same neurons as PAR2 and TRPV4 (discussed above) and correspondingly PAR4 activation attenuates both PAR2 agonist and TRPV4 agonist-induced allodynia and hyperalgesia in response to colorectal distension. Interestingly PAR4 agonist exposure inhibits free intracellular calcium mobilization induced by the pro-nociceptive agonists of PAR2 and TRPV4 [101]. As such the resultant balance between PAR2, PAR4 and TRPV4 activation is likely to determine the resultant effect on nociceptor responsiveness and therefore visceral pain.

Endogenous opioids are also key regulators of anti-nociceptive function [100, 102, 103]. The lack of hyperalgesia and allodynia associated with chronic DSS colitis is actually accompanied by an increase in β-endorphin and μ-opioid receptor expression and CD4 +ve T-cells. This suggests chronic DSS induced-inflammation involves infiltration by lymphocytes, which is accompanied by μ-opioid receptor and β-endorphin up regulation, providing an anti-nociceptive input that restores normal visceral perception [103]. In addition, colonic supernatants from chronic DSS treated mice have a 14-fold increase in β-endorphin levels, and their incubation suppresses the excitability of nociceptive colonic DRG neurons [104]. However, the timing of these effects may be disease specific as different opioid induced effects are evident in IBS. It has recently been shown that supernatants from PBMCs taken from healthy subjects actually inhibit colonic afferent mechanosensitiivity, via a μ-opioid receptor mechanism [69]. Moreover, the number of β-endorphin expressing colonic mucosal lamina propria cells actually decreases in constipation predominant-IBS (C-IBS) patients compared with healthy subjects, suggesting that healthy human immune cells actively secrete β-endorphin, which dampens colonic mechanosensation[69]. As this inhibitory effect from PBMC supernatants is lost in C-IBS patients, and actually switches to sensitisation in diarrhea

predominant (D-IBS) patients, where increases in pro-inflammatory cytokines are evident, these results suggests that resultant neuronal function is a constant balance between pro- and anti-nociceptive mechanisms.

More recently, it was demonstrated that inflammation can induce the function of kappa-opioid receptors, as demonstrated by the inhibitory effects of the agonist asimadoline on colonic nociceptor function[105]. Furthermore, the oxytocin receptor is not expressed in healthy colonic DRG neurons, however its expression is induced following inflammation and oxytocin receptor analogues inhibit colonic nociception in vitro and in vivo in post-inflammatory chronic visceral hypersensitivity models [106].

Conclusions and future perspectives:

Recent studies have clearly demonstrated the capacity of inflammation or infection to cause long term neuroplasticity and the development of gut symptoms. In the absence of a 'perfect' pre-clinical model to replicate the multifactorial nature of many gut disorders, such as IBS, concurrent studies on numerous models have allowed identification of several distinct mechanisms that may potentially underlie neuroplasticity in the clinical setting. Specific immune pathways are recruited in response to different insults, which in turn leads to specific interactions between inflammatory cells, immune cells and neurons. This leads to alterations in neuronal ion channel and receptor expression and function, leading to neuroplasticity. These studies also suggest some commonality in the mechanisms underlying neuroplasticity and that several mechanisms may have to interact to cause pronounced long term neuroplasticity. Crucially, several different therapeutic strategies may exist for the treatment and prevention of gastrointestinal dysfunction. Selective targeting of the individual neuronal populations displaying neuroplasticity is the ultimate goal for patients currently experiencing chronic pain or alterations in gut motility. However, another therapeutic window of

opportunity exists, whereby reducing the initial inflammatory response, for example during the early stages of gastroenteritis, may reduce or prevent subsequent inflammation-induced neuroplasticity. Future research will need to identify how the differing extrinsic and intrinsic neural pathways communicate with one another and the complex interactions that each of them have concurrently with stress mediators, immune responses, enteric/spinal glia and gut microbiota to underlie normal gut physiology. Determining how these interactions are altered during pathophysiology will be crucial in the next phase of understanding the mechanisms of neuroplasticity, which underlie gastrointestinal dysfunction.

Figure 1: Neuroplasticity in extrinsic sensory afferent pathways during and following resolution of gut inflammation.

During inflammation nociceptive sensory afferent endings are hypersensitive, are activated at lower stimulus intensities and displayed enhanced mechanical responsiveness, whilst their cell bodies in the DRG also display hyperexcitability. This translates to increased activation of dorsal horn neurons in the spinal cord and in whole animal studies enhanced pain responses to colorectal distension. Many of these changes are still present or are even enhanced following resolution of inflammation. Nociceptive sensory afferent endings now display even greater mechanical hypersensitivity, and their cell bodies in the DRG remain hyper-excitability. An increased density of colonic afferent central afferent terminals is now evident, as is sprouting of these terminals into different regions of the dorsal horn of the spinal cord. This plasticity results in greater numbers of second order dorsal horn neurons in the spinal cord being activated in response to noxious colorectal distension. There is evidence of enhanced pain responses to colorectal distension, which can be dependent upon the experimental model used and influenced by the severity of the initial insult.

Figure 1: Neuroplasticity in extrinsic sensory afferent pathways during and following resolution of gut inflammation.

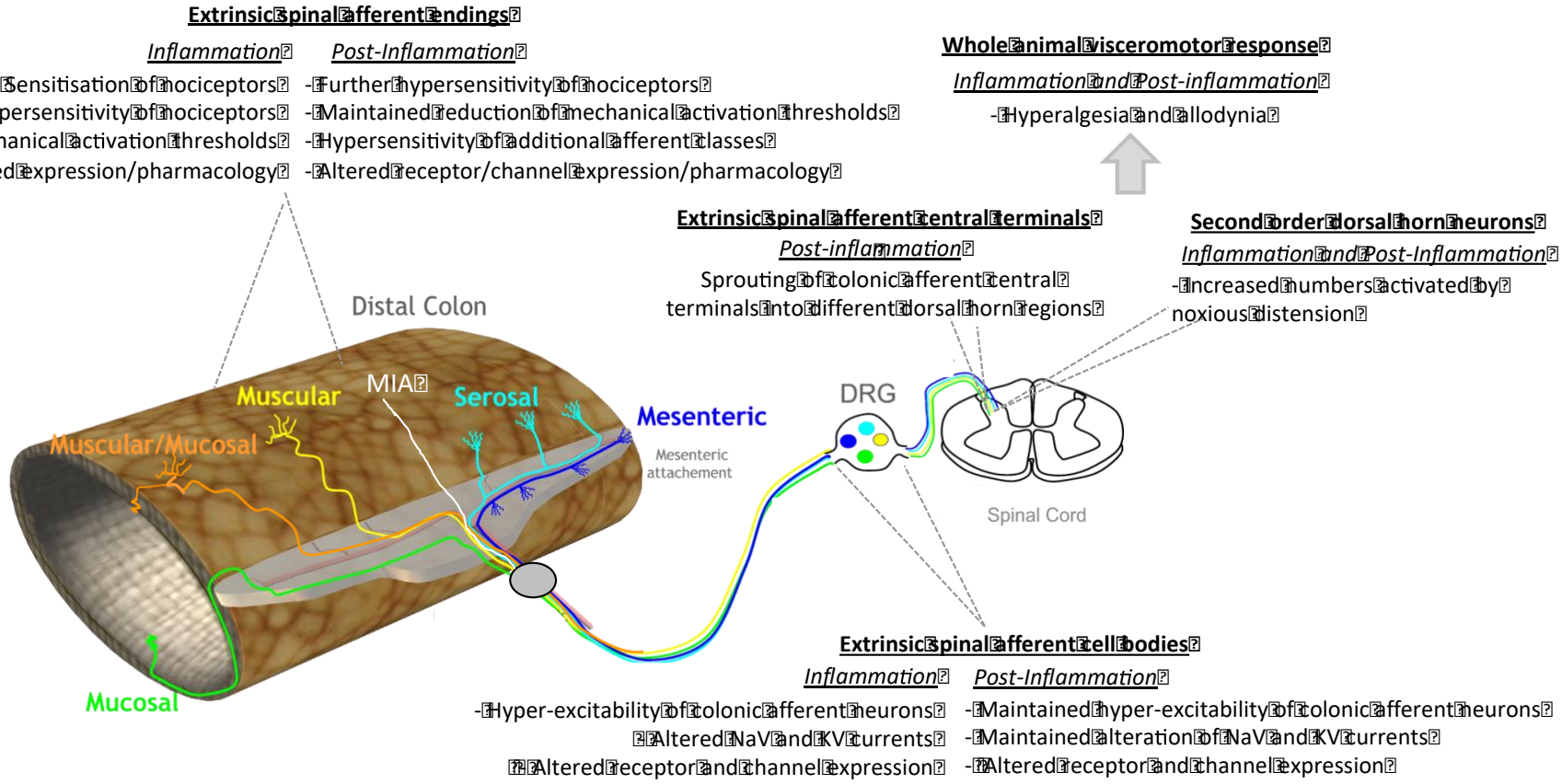


Figure 2: Gut nociception: Interactions between mediator and channel/receptor

Numerous mediators can directly activate gut nociceptors by binding to the numerous cell surface receptors and channels expressed on their peripheral endings. The majority of the channels and receptors identified here result in nociceptor activation, sensitisation and neuronal hypersensitivity and hyperexcitability. Furthermore, once activated the nociceptors themselves can release substance P and CGRP from their peripheral terminals, inducing neurogenic inflammation. TRPA1 and TRPV4 are both implicated in this process. In contrast, activation of another set of channels/receptors results in reduced neuronal excitability and resultant anti-nociceptive effects. Pro-nociceptive mechanisms appear to be up-regulated during inflammatory and post-inflammatory sates, whilst the anti-nociceptive mechanisms are down-regulated. Abbreviations: calcitonin-gene related peptide (CGRP) Transient Receptor Potential (TRP); Acid Sensing Ion Channel (ASIC); Protease activated receptor (PAR), Cannabinoid receptor 1 (CB1), tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), Histamine 1 receptor (H1), Toll-like receptor (TLR), Lipopolysaccharide (LPS), Two-pore domian K^+ (K2P) channel: (TREK).

Figure 2: Gut nociception: interactions between mediator and channel/receptor

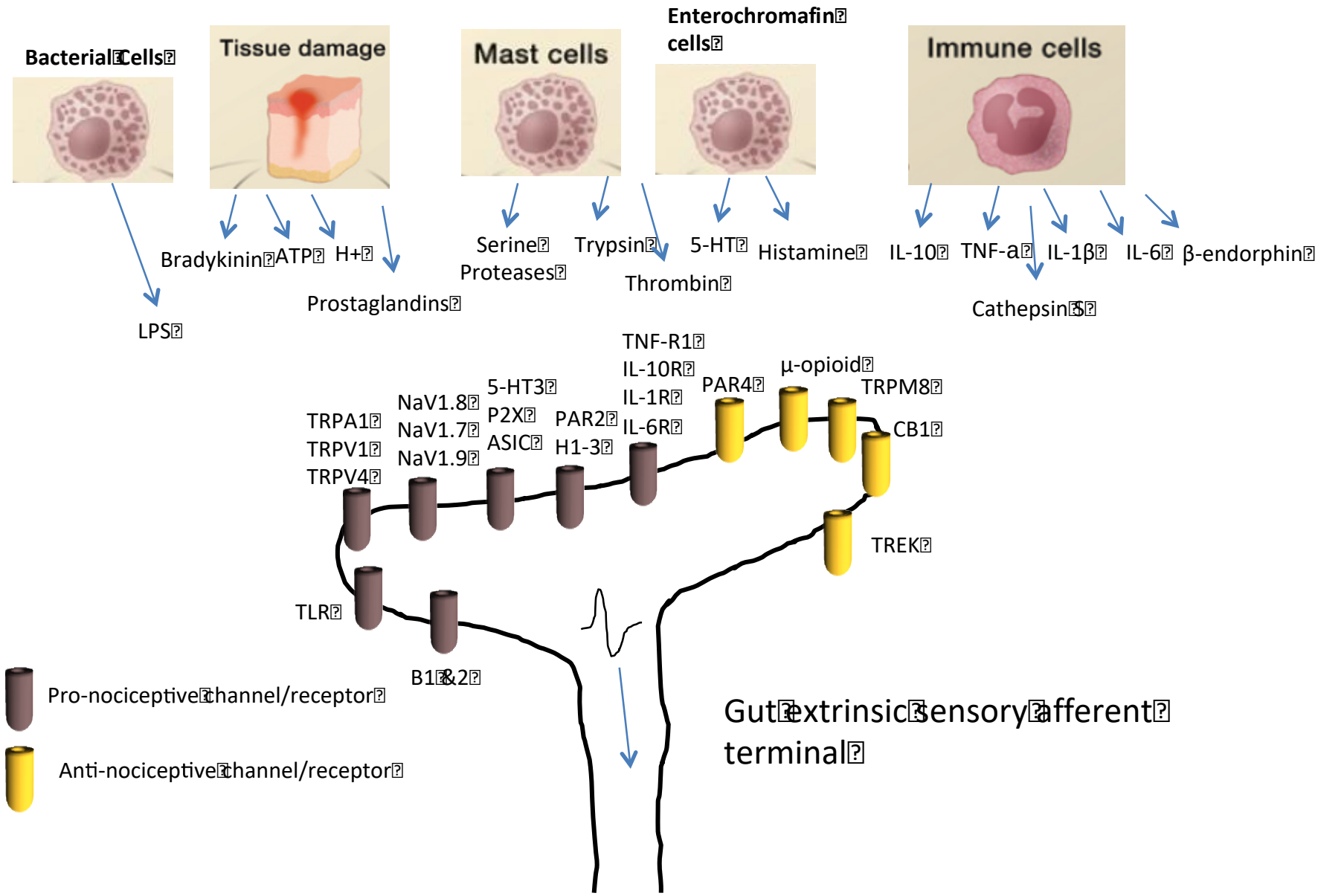
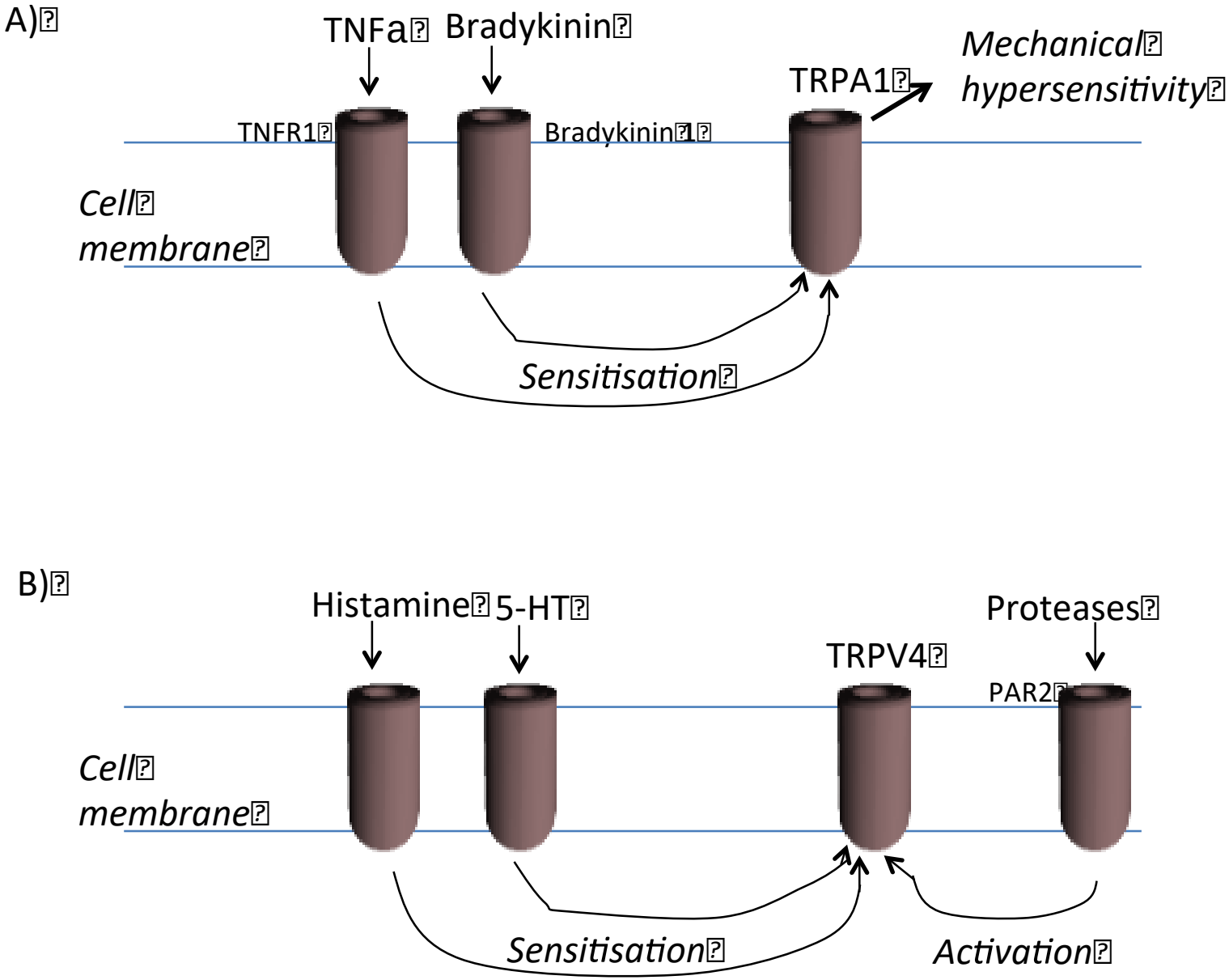


Figure 3: TRP channels are key mediators of visceral afferent hypersensitivity and are downstream targets of receptor activation.

A) Whilst TRPA1 can be activated directly by compounds such a 4-Hydroxynonenal, mustard oil and cinnamaldehyde to induce mechanical hypersensitivity, TRPA1 can also be sensitised by interactions with TNFR1 and bradykinin 1 receptors. Binding of TNFα to TNFR1 and bradykinin to bradykinin 1 respectively can both independently evoke mechanical hypersensitivity of nociceptors by a TRPA1 dependent process. **B)** Similarly, histamine and 5-HT can cause sensitisation of TRPV4, evoking neuronal hypersensitivity. This occurs via mitogen-activated protein kinase kinase (MAPKK) and phospholipase A2 (PLA2)-dependent mechanisms and increased TRPV4 dependent hypersensitivity in response to colorectal distension. By contrast, the interaction between TRPV4 and PAR-2 appears more fundamental, with expression of TRPV4 being required for PAR-2-induced mechanical hyperalgesia and excitation of colonic afferent neurons.

Figure 3: TRP channels are downstream targets of receptor activation



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