A systematic review of the evidence for CNS plasticity in animal models of inflammatory-mediated gastrointestinal pain

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Abstract

Background: Abdominal pain frequently accompanies inflammatory disorders of the gastrointestinal tract (GIT) and animal models of visceral inflammation have been developed to explore the role of the CNS in this process. Here we summarize the evidence from animal studies for CNS plasticity following GIT inflammation.

Methods: A systematic review was conducted to identify studies that: 1) used inflammation of GIT organs, 2) assessed pain or visceral hypersensitivity, and 3) presented evidence of CNS involvement. 208 articles were identified and 79 were eligible for analysis.

Results: Rats were most widely used (76%). Most studies used adult animals (42%) with a bias towards males (74%). Colitis was the most frequently employed model (78%) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) the preferred inflammatory agent (33%). Behavioral (58%), anatomical/molecular (44%) and physiological (24%) approaches were used alone or in combination to assess CNS involvement during or after GIT inflammation. Measurement times varied widely (< 1hr - > 2wks after inflammation). Blinded outcomes were employed in 42% of studies, randomization in 10% and evidence of visceral inflammation in 54%. Only 3 studies fulfilled our criteria for high methodological quality and no study reported sample size calculations.

Conclusions: The included studies provide strong behavioral, anatomical and physiological evidence for CNS plasticity following GIT inflammation, specifically in the spinal cord dorsal horn. This evidence includes altered visceromotor responses and indices of referred pain, elevated neural activation and peptide content, and increased neuronal excitability. This evidence supports continued use of this approach for pre-clinical studies, however, there is substantial scope to improve study design.

Introduction

Severe abdominal pain and visceral hypersensitivity are debilitating symptoms of inflammatory disorders of the gastrointestinal tract (GIT), such as inflammatory bowel disease (IBD) and pancreatitis[1-4]. GIT inflammation, however, does not appear to be the sole factor responsible for pain in patients with these conditions. This is based on the observation that 30-50% of IBD patients in clinical remission continue to experience abdominal pain[5-7], which is often sufficient to warrant ongoing use of narcotic medication[8-10]. Likewise, patients with chronic pancreatitis frequently suffer abdominal pain despite successful endoscopic or surgical interventions[11,12]. Recent articles have highlighted major issues in chronic pain management in these distinct conditions, including the lack of effective pharmacological intervention and decreased patient quality of life due to the development of depression and anxiety[7,13-15]. Interestingly, pancreatitis and other heptopancreatobiliary disorders are a common extraintestinal manifestation of IBD[16,17]. Therefore, a review of chronic pain management in the entire gastrointestinal system is warranted.

The mechanisms responsible for the development of chronic abdominal pain as a consequence of inflammation in the GIT are still largely unknown. Clinically, there is evidence that altered central nervous system (CNS) function (plasticity) plays a role in the development of the chronic pain state that can accompany IBD and pancreatitis[13,18-20]. A large proportion of IBD patients in clinical remission not only continue to experience chronic abdominal pain despite the lack of active inflammation in the gut[5-7], but also frequently experience "referred pain," whereby poorly localized pain from the viscera is referred to other body locations[20-24]. This phenomenon is thought to occur when there is overlap between visceral and somatic sensory pathways within the CNS[25]. Likewise, reorganization of CNS pathways has been documented in patients with chronic pancreatitis[4]. Therefore, CNS dysfunction appears to play an important role in both the development and maintenance of chronic pain in inflammatory conditions of the GIT.

In response to this need for a better understanding of the central mechanisms underlying visceral-inflammation related pain, animal models have been developed to mimic IBD, pancreatitis and other painful conditions of the GIT. These models generally involve chemically induced inflammation of a target organ and subsequent assessment of CNS

properties/function during acute inflammation or when inflammation has resolved. In this review, we summarize the current pre-clinical evidence for altered CNS function in animal models of visceral pain as a consequence of GIT inflammation. This review highlights a number of areas where study design could be improved to enhance the integration of findings across studies and laboratories. Finally, we identify a number of areas where additional data are required to improve our understanding of the central mechanisms that underlie inflammatory-mediated GIT pain.

Methods

Literature search

A systematic literature search was conducted using Ovid SP databases (Embase and Medline). The details of our search strategy are provided in Table 1. Once the search was completed, duplicates were removed, and abstracts of the remaining articles were assessed. Retrieved records were screened for relevance prior to inclusion. Papers that did not fit the inclusion criteria were noted, but not analyzed further.

Inclusion criteria

Original research articles were included based on the following criteria: 1) the animal model involved inflammation, 2) inflammation occurred in at least one of the following organs of the gastrointestinal tract: esophagus, stomach, liver, pancreas, gallbladder, appendix, small or large intestine, 3) studies demonstrated the presence of pain, or visceral hypersensitivity, or examined factors that could contribute to pain or visceral hypersensitivity, and 4) the measures employed were considered evidence of CNS involvement in visceral pain processing. All searches were limited to English-language journal articles published before July 12, 2012.

Exclusion criteria

Review articles were excluded, though their reference lists were searched to identify additional studies that met the inclusion criteria. Other articles excluded were dissertations and conference proceedings, studies of cancer or neuropathic pain (i.e. where inflammation

is not the sole cause of “pain”), and studies using the acetic acid writhing test, as this test is not organ-specific.

Study selection

Two investigators (K.E.F and R.J.C) independently evaluated abstracts of identified articles according to the selection criteria, and potentially relevant articles were retrieved in full. In cases of initial disagreement on an article’s eligibility, inclusion was decided after discussion leading to consensus between investigators. Initial agreement between investigators on inclusion of articles was assessed using percentage agreement and the kappa statistic.

Data extraction

The following data were extracted from the included studies: authors, year of publication, animal strain, animal age and sex, gastrointestinal tract section/organ that was inflammed, method of inflammation (including inflammatory agent, route of administration and dose), time points at which measurements were made (hours/days/weeks post-inflammation), techniques used to measure effects of inflammation on CNS function, and the outcomes of these measures. In studies with multiple interventions, only data from uninflamed (control) and inflammed groups were considered for analysis.

Methodological quality assessment of studies

We developed a 10-point checklist to assess the bias of reporting and quality of study design (Fig 2). These 10 points were developed to evaluate key aspects of study design, such as randomization of animals into experimental groups, and to assess how thoroughly study objectives were reported and measured. Studies were deemed to be of higher quality if they reported randomization of animals into experimental groups, blinded analysis, and objective evidence of visceral inflammation (via histology, Evan’s Blue extravasation and/or myeloperoxidase (MPO) analysis). With these criteria met, it can be assumed that all findings are unbiased in regards to animal groups, and that visceral inflammation is present and thus the cause of altered CNS properties.

Results

Selection of studies

The process followed for article selection is presented in Figure 1. A total of 208 articles were identified. Of these, 112 were excluded because they did not meet the inclusion criteria or contained aspects of the exclusion criteria, while a further 33 were excluded because they were reviews. A total of 79 articles were eligible for detailed analysis, including an additional 16 from review article reference lists. There was a high level of agreement on inclusion/exclusion[26,27] between the two investigators who screened the retrieved articles (% agreement = 0.92, kappa score = 0.82).

Characteristics of included studies

The characteristics of studies that identified and investigated CNS involvement in the processing of gastrointestinal inflammatory pain were assessed to determine whether the study design of existing literature contained any consistency. These study characteristics are shown in Tables 2 and 3. Rats were the most widely utilized species in models of gastrointestinal tract inflammation (76%), followed by mice (22%), cats (1%[28]), and rabbits (1%[29]). Visceral inflammation was induced in animals considered to be adults (by the authors) in 33 studies (42%), and in neonates in one study (1%[30]). Surprisingly, 45 studies (57%) reported animal weight but not age. There was a sex bias across studies, where 58 studies used males (74%), while females were used in 12 (15%). Eight studies (10%) used both sexes, and sex was not reported in one study (1%[29]).

Colitis was the most frequently used model of visceral inflammation (78%), followed by pancreatitis (19%). By comparison, inflammation of the esophagus (1%[28]), stomach (1%[31]) and gallbladder (1%[29]) were rarely employed. A variety of chemical agents have been utilized to induce inflammation (see Tables 2 and 3). Trinitrobenzene sulfonic acid (TNBS) was most commonly used in models of colitis (33%), and cerulein (29%) in models of pancreatitis. Other inflammatory agents employed included mustard oil, zymosan, turpentine, and acetic acid. Finally, the time points after the induction of inflammation at which outcome measures were made varied widely from <1 hour (22%), 1-24 hours (31%), 2-7 days (23%), 8-14 days (11%), and >2 weeks (13%). Therefore, there is great variance in both the characteristics of

the models of gastrointestinal inflammation and the time points after the induction of inflammation at which measurements of CNS properties were made.

Methodological quality/risk of bias

The methodological quality of included studies was assessed using our 10-point checklist, and these results are shown in Figure 2. Only 10% of studies reported randomization of animals into experimental groups[31-38]. Blinded outcome measures were undertaken in 42% of studies, and objective evidence of visceral inflammation (histology, MPO assay or Evan's Blue extravasation) was obtained in 54% of studies (Tables 2 and 3). Two studies did not report the dose of the inflammatory agent used: one of these modelled pancreatitis[39], and the other cystic pain[29]. No study reported sample size calculations.

Only three studies (4%) were considered to be of high methodological quality according to our criteria[33,34,37] (see Methods: *Methodological quality assessment of studies)*. Each of these three studies employed models of colitis. Two studies used the chemical dextran sodium sulphate (DSS) as an inflammatory agent, and examined the long-term effects of visceral inflammation (49 days post DSS withdrawal) on neural activation in the spinal cord and referred hypersensitivity[33,34]. The immediate early gene product, c-Fos, was employed as a marker of neural activation. The third study employed the TNBS model of colitis, and measured the visceromotor response (VMR) and referred bladder hypersensitivity one week after induction of inflammation[37].

Evidence for altered CNS function as a measure of gastrointestinal pain

The experimental approaches used to assess altered CNS pain processing mechanisms in the identified studies can be divided into three major categories: behavioral, anatomical/molecular and physiological.

Behavioral approaches

In pre-clinical models of visceral inflammation, confirming that an animal is in pain or at least exhibits visceral hypersensitivity is essential before mechanisms that drive pain can be explored. This is often inferred on the basis of patterns of behavior. Of the included studies, 58% examined behavioral responses after visceral inflammation. These behaviors include

the visceromotor response (VMR) and measures of referred pain via sensory threshold testing (e.g., von Frey hairs).

Ness and Gebhart (1988) have published a detailed description of the VMR[40]. The VMR is a reflex that acts via a spinal cord-brainstem-spinal cord loop, and therefore implicates the CNS in the regulation of involuntary behavioral responses to pain[40,41]. Only 29% of included studies assessed VMR after visceral inflammation. 21 out of 28 of these studies were undertaken in models of colitis[25,30,42-60], where the VMR was elicited by colorectal distension (CRD). Each study reported an increase in the magnitude of responses to distension, and/or a decrease in the distension pressure required to elicit a given response amplitude/intensity during or after visceral inflammation[25,36,42-48,52,53,55-63]. Two studies used a model of gastric inflammation[31], and recorded spontaneous abdominal contractions during pancreatitis as an index of pain[64].

Pain originating in viscera is often "referred" to other body regions, such as skin or even other viscera[21]. Such referred pain is thought to occur because of "overlap" between visceral and somatic sensory pathways within the CNS[25,62,65]. Referred pain after visceral inflammation was measured in 23% of the included studies. Models of colitis were used in 10% of studies and most (7%) measured paw and/or abdominal hypersensitivity via von Frey or heat withdrawal latency. The remainder examined referred hypersensitivity of the bladder[66] or urethral sphincter hypersensitivity during bladder distension[67]. The non-colitis studies (13%) assessed referred hypersensitivity, via von Frey or heat beam application to the abdomen, in models of pancreatitis.

Von Frey testing has consistently shown that animals "withdraw" their limbs at lower thresholds during inflammation ***and*** after recovery from both colitis and pancreatitis[32-34,61-63,68-79]. In contrast, responses to plantar heat are more varied, with some studies reporting a decrease in paw withdrawal latency (i.e. increased sensitivity)[36,62,80], no change[61], or even an increase in latency (decreased sensitivity)[81]. Similarly, assessments of viscero-visceral cross talk in models of colitis have shown reduced micturition latency[37,62,66] and increased urethral sphincter responses to electrical stimulation[67]. Both are indicative of bladder over-reactivity.

Only 6% of studies used a combination of VMR and referred pain testing to assess CNS involvement after inflammation[36,37,61-63]. The data from these studies did not differ from single-assessment approaches and are included in the above description.

Anatomical/molecular approaches

It is clear that visceral inflammation can result in long-term abdominal pain and referred hyperalgesia in both experimental and clinical settings[20,62,63,73,82,83]. However the precise mechanisms underlying these changes are poorly understood. It has been suggested that remodelling of CNS nociceptive networks is largely responsible[84-86] though until recently, this has been relatively unexplored in models of visceral inflammation. Immunohistochemistry (IHC) has been employed to investigate the properties of spinal cord and brainstem neurons during and after visceral inflammation[29,33-35,45,46,48,63,64,68,75,77,79,80,86-97]. This approach is based on the premise that structural remodeling of neural networks in the CNS underlies the exaggerated reflex behaviors observed after visceral inflammation[84-86]. Likewise, molecular analyses have been utilized to investigate altered expression of key proteins and signaling molecules in the CNS after visceral inflammation.

Markers of neural activation

Markers of neural activation in the spinal cord and brainstem during visceral inflammation are commonly used to evaluate CNS involvement in pain processing. There are two markers widely used in the literature: c-Fos, and phosphorylated ERK (pERK)[98]. c-Fos or pERK[98] IHC were used in 23% of studies to assess CNS involvement in nociceptive processing after visceral inflammation. Most used c-Fos (20%), and expression of either marker was consistently increased[29,33-35,45,63,64,68,80,86-88,90,92-94,96,97]. Neural activation was maximal in spinal cord segments T13-L1 and L6-S2 in models of colitis[33-35,45,48,63,86,87,93,94,96,97], T3-L1 in pancreatitis[64,68,80,88,90,92] and T6 for gallbladder inflammation[29]. Labeling was observed in lamina I-VII and/or X, reflecting the diffuse termination of visceral afferents in the spinal cord compared to their cutaneous counterparts[29,33-35,45,63,64,68,80,86-88,90,92-94,96,97]. c-Fos labeling was also elevated in brainstem regions, such as the periaqueductal grey, dorsal raphe nucleus, pontine parabrachial nucleus, locus coeruleus and the nucleus of the solitary tract after colonic[93] and pancreatic[80] inflammation. These nuclei are well known nociceptive centers, which provide descending inhibition to the spinal cord[99].

Gene or protein expression of c-Fos and pERK in the spinal cord homogenates were measured in 6% of studies after visceral inflammation[29,49-51,69]. Three studies[49-51] observed increased c-Fos mRNA in models of colitis, while two[29,69] reported increased pERK mRNA and protein levels after colitis[69] or gallbladder inflammation[29]. Notably, one study showed that increased nuclear expression of pERK was accompanied by the development of referred pain after colitis[69].

c-Fos and pERK IHC were combined with neuroanatomical tracing in two studies in order to identify the ***specific*** neural populations/pathways activated by visceral inflammation[86,97]. Colonic central afferent terminals were retrogradely labeled and identified along with pERK in the dorsal horn after colitis and CRD. The density of adjacent labeled terminals and pERK positive neurons in the dorsal horn increased and labeling expanded from superficial to deep dorsal horn layers after inflammation[86]. Similarly, spinal neurons projecting to the parabrachial nucleus were retrogradely labeled with fluorogold before colitis and CRD. Fluorogold and c-Fos double labeling was then examined in spinal cord segments known to receive colonic inputs. Notably, double-labeled neurons were more prevalent (increased by 15%) in thoracolumbar versus lumbosacral spinal cord. This suggests thoracolumbar segments play an important role in the processing of visceral inflammation[97].

Markers of pain-associated neuropeptides

The neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), and the SP receptor (neurokinin 1; NK1), have long been associated with the induction and maintenance of various chronic pain states[73,100-105], and transmission of nociceptive signals to higher centers along the pain neuroaxis[106,107]. The expression of SP and CGRP were examined in 6% of studies using IHC[35,77,79,86,93]. Increases in the density and intensity of SP and CGRP labeling in the spinal cord dorsal horn have been documented in models of colitis[35,86,93] and pancreatitis[77,79]. Furthermore, increases in SP and CGRP coexisted with increased pain behaviors in 4% of studies[77,79,93], suggesting a role for these neuropeptides in the development of long-term and referred hyperalgesia. Similarly, spinal cord expression of the NK1 receptor was examined in 3 studies[89,91,95] in models of colitis. This work showed that both NK1 receptor-expressing neuron incidence and NK1 receptor internalization increased. Such internalization is considered an indicator of neuronal activation[89,91]. Likewise, de novo

expression of the NK1 receptor in dorsal column (DC) projection neurons was observed after colitis[95].

Spinal cord mRNA or protein expression of neuropeptides and inflammatory markers were examined in 5%[49,51,108,109] of studies employing colitis. Increased mRNA and protein expression of SP[108,109] and NK1 and NK2 receptors[49,51] have been documented after colitis. Likewise, CGRP expression was examined in four studies. Increases[49,108], decreases[109] or no change[51] in expression were reported. Spinal cord mRNA expression of inflammatory markers, cNOS, iNOS, IL-1β, TNF-α, COX-1 and COX-2 were increased in two studies[49,51] after the resolution of colitis. Two studies also showed a correlation between increases in neuropeptides and inflammatory markers and enhanced behavioral responses to CRD[49,51].

Finally, one study[109] examined SP and CGRP expression in the bladder after colonic inflammation. Increased SP and CGRP mRNA and protein were reported in the bladder for up to 30 days after the induction of colitis[109], and this was associated with neurogenic inflammation of the bladder.

Other factors contributing to CNS processing of visceral pain

Additional anatomical changes within the CNS after visceral inflammation have been explored in a further five studies (6%)[46,48,75,86,110]. 3% of these examined spinal cord expression of nNOS and NADPH to investigate the role of the nitric oxide (NO) cascade after colitis[46,48]. Increased nNOS and NADPH expression was shown in the spinal cord, and was accompanied by an enhanced VMR. This exaggerated response was attenuated using intrathecal NOS inhibitors[46]. One study showed that pancreatitis caused microglial activation within the dorsal horn. This was correlated with the development of referred pain, and reversed by the microglia inhibitor minocycline[75].

Utilizing Evan's blue plasma extravasation, one study showed (anatomically) that acute inflammation of the colon could induce cross-organ inflammation[110]. Colitis resulted in plasma extravasation in the bladder and uterine horns. These cross-organ effects were eliminated after cutting the hypogastric nerve. This intervention did not, however, prevent colon extravasation after bladder inflammation, suggesting central mechanisms regulate cross-organ sensitisation[110]. Notably, estrus stage appeared to modulate these effects.

One study examined the role of phosphorylation in visceral pain using western blot analysis after colitis[38]. PKC-γ and –ε were activated via membrane translocation in lumbosacral spinal cord segments and this activation correlated with spontaneous pain behaviors[38]. Importantly, PKC has been shown to activate ERK and increase dorsal horn neuron excitability via modulation of A-type potassium channels[111]. Finally, one study showed that protein expression of GluR6, a subunit of the excitatory glutamate receptor, was increased in the sacral spinal cord after colitis, and is correlated with increased spontaneous pain behaviors. Suppression of GluR6 in the spinal cord using antisense oligodeoxynucleotides attenuated these pain behaviors[112].

Physiological evidence

Extracellular recording

Behavioural and anatomical/molecular evidence from animal studies have shown altered CNS properties after visceral inflammation. However, using these approaches, it is unclear whether cellular physiology drives these functional changes. 16 studies (18%) have examined *in vivo* responses of CNS neurons during and after visceral inflammation using *extracellular* recording techniques[28,30,38,55,56,63,113-122]. This approach allows action potential (AP) discharge to be assessed before and after inflammation. In most of these studies (11/16), recordings were made from spinal cord neurons. Acute or previous colitis increased background or spontaneous activity in recorded neurons in 13% of studies[30,38,56,63,114,115,118-121]. Similarly, 16% of studies reported increased action potential (AP) discharge after both CRD and pelvic nerve stimulation, and a decreased activation threshold for AP generation after colitis[30,56,63,113-117,119,120,122], and esophageal inflammation[28].

Other studies have examined the effect of visceral inflammation on the temporal characteristics of AP discharge. Two populations of dorsal horn neurons (abrupt and sustained firing) have been described according to the way they respond to CRD[123,124]. Two studies reported *decreased* activity in abrupt dorsal horn lumbosacral neurons after colitis[56,63], and another two reported *increased* activity in abrupt thoracolumbar neurons[63,122]. Similarly, two studies reported increased activity of sustained neurons in the lumbosacral spinal cord[56,122]. In contrast, one study reported a decreased proportion of neurons *inhibited* by CRD in the thoracolumbar spinal cord[122]. This supports the notion that

spinal cord processing after inflammation is somehow enhanced in thoracolumbar versus lumbosacral spinal cord as documented in anatomical studies[97].

Referred pain

The development of referred hypersensitivity in both somatic and visceral structures has been frequently observed in animal models of visceral inflammation, and in patients[20,22-25]. Electrophysiological assessment of viscero-somatic convergence in the spinal dorsal horn was conducted in three studies. After colitis, more neurons exhibited colonic-somatic convergence: i.e. they responded to both CRD and somatic pinch/brush. In addition, the sensitivity and size of peripheral somatic receptive fields increased[30,117,119]. Another study demonstrated viscero-visceral convergence in dorsal horn neurons using bladder distension after colitis[121]. AP discharge in neurons excited by bladder distension was increased, while the duration of AP inhibition in neurons normally inhibited by bladder distension decreased[121]. These changes were observed in both convergent and non-convergent colon-bladder neurons, suggesting widespread sensitization of dorsal horn neurons[121].

Cross-sensitization of bladder afferents, presumably by dorsal horn viscero-visceral convergence during colitis was demonstrated in two studies (3%) using single unit pelvic nerve recordings[66,125]. Bladder afferents showed increased spontaneous activity during colitis. This subsided after inflammation had resolved, but the responses of pelvic nerve afferent fibres to bladder distension and intravesical capsaicin increased during and after resolution of colitis. A proportion of fibres were also sensitive to intravesical bradykinin and SP[66,125].

Activation of affective brain centers during visceral inflammation

The activation of nociceptive brainstem sites and cortical structures was recently explored using functional magnetic resonance imaging (fMRI) in a model of pancreatitis[39]. The study reported increased fMRI signaling in the periaqueductal grey (PAG), dorsal raphe nucleus (DR), rostral ventromedial medulla (RVM), and the lateral thalamus one week after the induction of inflammation. Increased fMRI signaling was also observed in limbic or "affective" brain regions, such as the amygdala, parietal cortex, and

midcingulate/retrosplenial cortices[39]. In most cases, these increases were attenuated by morphine, implicating pain directly in subcortical and cortical activation.

Discussion

This systematic review demonstrates that considerable behavioral, anatomical and physiological evidence exists for CNS plasticity, particularly in the dorsal horn of the spinal cord, following visceral inflammation. This evidence suggests that our evolving understanding of how visceral inflammation can alter processing in the CNS offers potential for new therapeutic strategies to manage patients with inflammatory pain from GIT organs. In this review, we have identified aspects of study design that would improve the quality of future studies. Importantly there is a need for additional studies that are relevant to explaining patients' inflammation-based GIT pain and that explore mechanisms underlying plasticity in the spinal cord during and after visceral inflammation.

Quality of pre-clinical studies

Only three studies were deemed high quality[33,34,37] according to our criteria (reported randomization of animals into experimental groups, blinded analysis, and objective evidence of visceral inflammation). Failure to randomize into control and treatment groups was a major limitation in many studies and less than half the studies employed blinded outcome assessments. Sample size calculations and the appropriate statistical powering were not reported in any of the studies assessed. Although many of the studies may have been appropriately powered for the main outcome variables/endpoints they reported, there is much less certainty regarding secondary variables. Future studies should report sample size calculations for primary outcome variables, report the actual p values for all measures, and explore the use of effect size calculations to provide more objective indications of the magnitude of reported differences. These recommendations for improving study design are in agreement with recent articles that have highlighted significant flaws in the experimental design of animal studies[126,127]. Lack of randomization, blinding, poor statistical power and inappropriate statistical analysis has been identified as the major factors contributing to bias and false positive results[126,127].

Other aspects of study design

Rats have been the main species used in pre-clinical studies to date, however, the use of mice has increased over the past decade. Animal age and/or age-range should be more accurately reported because it is known that the properties of spinal neurons involved in pain processing change with age[128]. These oversights potentially introduce uncertainty about the effects of intervention-induced plasticity versus changes associated with developmental mechanisms or interactions between the two. There is also more scope for studies that investigate the effects of early-life events[30,129,130] on long term nociceptive processing, and the effects of repeated inflammatory exposure on pain vulnerability. This is particularly relevant due to the increasing incidence of IBD in childhood and the potential effects of disease on their developing nervous system {Benchimol, 2011 #15}.

Male animals were used in almost three quarters of studies. There is clear evidence in the clinical literature that nociceptive processing differs between the sexes, at least for somatic pain[131-134]. The data for *visceral* pain disorders are, however, less clear. For example, there are no sex-related differences in baseline rectal discomfort or noxious rectrosigmoid distension in IBS patients[135,136], however, ovarian hormone levels influence abdominal pain in both IBD and IBS patients[137,138]. Unfortunately, the pre-clinical literature has not shed much light on the effect of sex on central visceral pain processing. Only 10% of studies used both sexes. One reported that estrus stage enhanced CNS-mediated cross-organ inflammation[110]. These data are consistent with studies showing ovarian hormones can affect responses to visceral stimulation, such as CRD[139,140]. Importantly, one of these studies demonstrated that changes were mediated by spinal estrogen receptors[139]. Therefore, future studies on CNS visceral pain-processing mechanisms should be mindful of sex-related confounds, compare males/females, and report estrus stage.

A wide variety of noxious agents and doses have been used to induce visceral inflammation. There is a clear need to for studies that compare the effects of different agents so the impact of specific inflammatory agents are known and can be subsequently manipulated. Similarly, there is a need to undertake studies that explore the effect of the dose of inflammatory agent. Doses are often selected to ensure an effect and to reduce variability in results. This approach may be experimentally satisfying, however, to better approximate the clinical situation variation in the induction of pain is required to better understand the manner in which pain develops in humans with differing degrees of GIT dysfunction.

Perhaps one of the most clinically relevant and troubling findings for the translation of pre-clinical work is ***when*** CNS outcomes are measured after visceral inflammation. This varied greatly, from as little as 20 mins to > 2 weeks. This is important as chronic inflammatory conditions such as IBD or functional gastrointestinal disorders involve periods of remission and relapse[141,142] and IBD patients often continue to experience abdominal pain during periods of remission[5,6,142]. Thus, there is a pressing need for a time series analysis using pre clinical models.

A minority of studies employing models of colitis (20% of studies) measured CNS outcomes ***after*** inflammation had resolved[30,33,34,43,49,51,59,60,62,63,86,109]. Thus, the *long-term* effects of inflammation on CNS plasticity are relatively unexplored. In addition, studies that measured long-term CNS changes only inflamed the colon once. It is therefore unclear whether the time courses employed in pre-clinical studies accurately reflect inflammatory bowel conditions. To improve clinical relevance, future studies should consider inflaming the colon more than once, to mimic the natural cyclical progression of IBD relapse and remission {Stenson, 2009 #156}.

Pre-clinical models of pancreatitis have, however, assessed CNS outcomes at time points that closely mirror disease progression. Patients with acute pancreatitis are generally hospitalised for <10 days[143] and 73% of the included studies measured CNS outcomes within two weeks of inflammation. Chronic pancreatitis is, however, a progressive disease often caused by alcohol abuse[144-147]. One study attempted to model the long-term induction of chronic pancreatitis using alcohol and a high fat diet for 10 weeks[80]. Thus, pre-clinical models of pancreatitis provide an example where knowledge of the clinical pathology can be utilized to provide more relevant data in terms of mechanisms underlying altered CNS function after visceral inflammation.

Evidence for CNS plasticity

Anatomical and molecular studies have consistently demonstrated changes in the spinal cord following visceral inflammation. Most assessed immediate early gene expression (c-Fos and p-ERK). Others used SP and CGRP expression because they are found in 70-90% of visceral afferents[148]. Importantly both c-Fos and p-ERK expression is attenuated with analgesics[149,150] suggesting both markers are reliable indicators of elevated neural activation

after visceral insults. Notably, pERK expression correlates strongly with pain behaviors, as targeting of pERK with MEK inhibitors reduces both spinal cord expression of pERK and cutaneous hypersensitivity[29,69,110,139] after visceral inflammation. Likewise elevated SP and CGRP expression correlated with increased pain behaviors in three studies[77,79,93], suggesting these central changes may contribute to the development of persistent and referred hyperalgesia. SP and CGRP have also been implicated in neurogenic inflammation of the bladder after resolution of colitis[109]. Release of neuropeptides in the bladder may be explained by cross-organ sensitization due to convergence of colonic and bladder afferents onto neurons in the spinal cord dorsal horn. After colonic nociceptive signals converge with bladder afferents in the spinal cord, excitation can be relayed to the bladder by a phenomenon known as the dorsal root reflex, where an AP develops in the spinal cord and is propagated to peripheral structures[151]. This would result in peripheral release of SP and CGRP in the bladder, and produce pain. Together, these anatomical studies provide strong evidence for CNS plasticity following visceral inflammation.

Three major behavioral changes were observed ***during*** or ***after*** recovery from visceral inflammation: increased VMR; referred hypersensitivity to somatic/skin areas; and sensitization of other viscera. These changes likely represent central sensitization[152], due to the convergence of visceral and somatic afferents in the spinal cord dorsal horn[25,65,153]. Withdrawal responses to mechanical stimuli (i.e. von Frey hairs) following visceral inflammation consistently show hypersensitivity. However, responses to plantar heat vary, with some studies reporting increased sensitivity[36,62,80], decreased sensitivity[81] or no change[61]. The mechanisms underlying the varied responses to mechanical and thermal stimuli are not known. In one study[61], where both mechanical and thermal responses were assessed, referred mechanical hyperalgesia was observed, but responses to heat were unchanged after colonic inflammation. This suggests referred hypersensitivity may be stimulus (or modality) specific and it would be interesting to determine whether visceral and mechanosensitive somatic afferents preferentially converge on dorsal horn neurons[61].

Extracellular recording has clearly demonstrated that visceral inflammation can alter the excitability, AP discharge patterns and level of viscero-somatic convergence in spinal neurons. Although these functional data support the notion of central sensitization in spinal circuits after a visceral insult, the identity of the neurons involved and the detailed mechanisms responsible for their altered output are unknown. Moreover, extracellular

recording tends to introduce bias towards tonically active neurons[113], or those that respond to a stimulus (somatic brush/pinch or CRD) with sustained *excitatory* responses[116,117,120]. Additionally, only two major firing patterns, *abrupt* and *sustained*, have been identified in the dorsal horn[123,124] using extracellular recording. This contrasts with the diversity of AP discharge patterns observed in animal studies employing both *in vitro* and *in vivo* intracellular recording[154,155]. Thus, our knowledge of the spinal neuron populations involved in central plasticity is limited.

Finally, if new CNS-acting therapies are to be developed for visceral pain we must understand what actually drives altered neuronal excitability. It has long been recognized that neuron output (in the form of AP discharge) depends on the combined action of excitatory or inhibitory synaptic inputs and intrinsic properties. Importantly, changes in either synaptic inputs, or intrinsic properties can alter neuron output[156]. Such changes in the intrinsic properties of *sensory* neurons (DRGs) have been demonstrated after TNBS-induced colitis using whole-cell patch clamp electrophysiology[157,158]. At present, the detailed synaptic and intrinsic properties of spinal neurons that receive inputs from the gastrointestinal tract are uncharacterized, under either normal or inflammatory conditions. Future studies aimed at filling this knowledge gap are now required to determine the neurochemical phenotype, morphology, connectivity, intrinsic and synaptic properties of spinal neurons involved in the central plasticity that clearly accompanies inflammation of the GIT.

In summary, chronic pain management in patients with inflammatory diseases of the GIT is lacking. The virtual absence of pharmacological agents that can successfully relieve pain has highlighted the need for further research into the causes of chronic pain. This review has outlined various CNS properties that are altered by visceral inflammation. However, due to the flaws in animal study design, it may be difficult for these results to become translational. Therefore, future studies must ensure that key oversights in study design and data reporting are resolved to improve the translational efficacy of animal work.

Conflict of Interest Statement

The authors report no conflict of interest.

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Figure Legends

Figure 1. Flow diagram for literature searching and screening. 208 articles were identified using Embase and Medline databases. Articles were included/excluded based on relevance, resulting in the inclusion of 63 articles. Reference lists of reviews were also scanned, leading to inclusion of a further 16 articles. A total of 79 articles were included for further analysis.

Figure 2. Methodological quality of included studies. A 10-point checklist was developed to assess study-design quality of the included articles. Dark grey bars indicate the proportion of articles that met each criterion; light grey bars indicate the proportion of studies that did not. Numbers of studies that meet each criterion are shown on each bar.

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