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ALTERED INTRINSIC AND SYNAPTIC PROPERTIES OF LUMBOSACRAL DORSAL HORN NEURONS IN A MOUSE MODEL OF COLITIS

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Abstract—Visceral pain in inflammatory and functional gastrointestinal conditions is a major clinical problem. The exact mechanisms underlying the development of pain, during and after visceral inflammation are unknown. However, clinical and pre-clinical evidence suggests plasticity within the spinal cord dorsal horn is a contributing factor. Here we use an *in vivo* preparation and patch-clamp electrophysiology to test whether the synaptic and intrinsic properties of superficial dorsal horn (SDH) neurons are altered 5 days after the induction of mild colitis in adult male mice (i.e. during acute inflammation of the colon). Whole-cell recordings were made from lumbosacral (L6-S1) superficial dorsal horn neurons (SDH), in animals under isoflurane anesthesia. Noxious colorectal distension (CRD) was used to identify SDH neurons with colonic inputs, while stimulation of the hind paw and tail was employed to assess convergent cutaneous input. Following inflammation, a significantly increased proportion of SDH neurons received *both* colonic and cutaneous inputs, compared to neurons in naïve animals. In addition, the nature and magnitude of responses to CRD and cutaneous stimulation differed in inflamed animals, as was spontaneous excitatory synaptic drive. Conversely, several measures of intrinsic excitability were altered in a manner that would *decrease* SDH network excitability following colitis. We propose that during inflammation, sensitization of colonic afferents results in increased signaling to the SDH. This is accompanied by plasticity in SDH neurons whereby their intrinsic properties are changed to compensate for altered afferent activity. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: *in vivo*, spinal cord, visceral inflammation.

INTRODUCTION

Chronic pain and visceral hypersensitivity are common and debilitating symptoms of several disorders of the gastrointestinal tract, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). IBS is a functional gastrointestinal disorder (FGID) characterized by chronic, recurrent pain and discomfort with low-grade inflammation of the colon mucosa (Verne et al., 2001, 2003; Barbara et al., 2004), whereas IBD describes several chronic inflammatory organic diseases of the gastrointestinal tract. While pain most commonly occurs during disease flare-ups in IBD, 30–50% of patients report IBS-like persistent pain in the absence of active disease (Minderhoud et al., 2004; Farrokhyar et al., 2006; Siegel and MacDermott, 2009). In both conditions, generalized "referred pain" or somatic hyperalgesia is also widely reported by patients (Bernstein et al., 1996; Verne et al., 2001, 2003; Minderhoud et al., 2004). This mixing of visceral and somatic sensory symptoms, or viscerosomatic convergence, is thought to be the result of plasticity within the central nervous system (CNS), particularly in the spinal cord dorsal horn where these sensory pathways can overlap (Ruch, 1961; Farrell et al., 2014b). The precise mechanisms underlying the development and maintenance of chronic visceral and somatic hypersensitivity remain largely unknown (Price et al., 2006; Farrell et al., 2014a,b).

Our previous review of the literature on CNS plasticity in animal models of visceral hypersensitivity pointed to a crucial role for the dorsal horn (Farrell et al., 2014b). For example, experimental rodent models of colonic inflammation can induce visceral and somatic hyperalgesia (Laird et al., 2001b; Lamb et al., 2006). Inflammation also increases neural activation (cFos and pERK), and the release of 'pain' neuropeptides (Substance P and CGRP) within the spinal cord dorsal horn (Lu and Westlund, 2001; Sun and Luo, 2004; Traub et al., 2008; Harrington et al., 2012). In addition, functional studies using *in vivo* extracellular recording have observed that following inflammation, dorsal horn neurons exhibit increased spontaneous

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Abbreviations: AHP, afterhyperpolarization; ANOVA, analysis of variance; AP, action potential; CNS, central nervous system; CRD, colorectal distension; CRD-NR, colorectal distension-nonresponsive; CRD-R, colorectal distension-responsive; DF, delayed firing; EPSP, excitatory post synaptic potential; ES, effect size; HT, high threshold; IB, initial bursting; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IPSP, inhibitory post synaptic potential; LT, low threshold; OR, odds ratio; R_{in}, input resistance; SDH, superficial dorsal horn; SS, single spiking; TF, tonic firing; TNBS, trinitrobenzenesulfonic acid; U, unclassified; WDR, wide dynamic range.

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action potential (AP) discharge (Al-Chaer et al., 2000; Ness and Gebhart, 2001; Qin et al., 2005) and increased AP discharge in response to distension of the colon (Laird et al., 2001a; Wang et al., 2005b). Together, these studies indicate that visceral inflammation alters the activity or output of dorsal horn neurons, however, the changes to the major determinants of neuron output (i.e. their intrinsic and synaptic properties) that occur during visceral inflammation have yet to be characterized during both acute and chronic models of disease.

Dorsal horn interneurons are involved in the complex processing and integration of inputs from peripheral structures like viscera and skin, higher brain centers, and other local circuit interneurons (Graham et al., 2007). It follows that small changes in their output have the capacity to significantly alter sensory signaling to higher centers and ultimately perception. Until recently, it has proved difficult to study the intrinsic and synaptic properties of the small dorsal horn interneurons that receive visceral inputs. We have developed a mouse preparation that allows high resolution *in vivo* patch-clamp recording from dorsal horn neurons in the spinal segments that receive colonic input (L6-S1) (Farrell et al., 2016). Importantly, this preparation enables detailed analysis of the intrinsic and synaptic properties of neurons that respond to mechanical distension of the colorectum. In this study using our *in vivo* mouse preparation we hypothesized that the intrinsic and synaptic properties of superficial dorsal horn (SDH) neurons are altered during acute colonic inflammation.

Our data demonstrate that during mild colonic inflammation, the synaptic properties and responses of SDH neurons to colonic and cutaneous stimulation render this population more excitable. Conversely, several changes in the intrinsic properties including a hyperpolarization of membrane potential and an increase in the excitability of presumed inhibitory interneurons are consistent with a decrease in overall SDH excitability. We propose that during inflammation, sensitization of colonic afferents results in increased signaling to the SDH. This is accompanied by plasticity in SDH neurons, which have altered their intrinsic properties in an attempt to compensate for this increased afferent activity.

EXPERIMENTAL PROCEDURES

All surgical and experimental procedures were approved by the University of Newcastle's Animal Care and Ethics Committee (protocol # A-2012-223). Male mice (C57BL/6, 6–8 weeks old, 15.7–27.4 g) were used for all experiments. The adult mouse preparation we used for spinal cord *in vivo* patch-clamp recording and the data collection protocols have been described previously (Graham et al., 2004a,b; Jobling et al., 2010; Farrell et al., 2016). They are briefly summarized here.

Animal model of colitis

Colonic inflammation was induced using the modified chemically induced trinitrobenzenesulfonic acid (TNBS) model of colitis (Wirtz et al., 2007; Marks et al., 2015). Mice were presensitized by epicutaneous application of 1% TNBS (Sigma Chemicals, St. Louis, MO, USA) in acetone/olive oil solution (4:1). After 7 days, mice were anaesthetized with isoflurane (2%) and received a single dose of 0.2 ml TNBS (2.5% in 50% ethanol) delivered into the colorectum, 4 cm from the anus, via an anal catheter. Mice were suspended upside down for one minute to ensure TNBS remained in the colorectum. Animals were observed daily for changes in body weight, physical appearance and behavior. Electrophysiological experiments were conducted 5 days after the induction of colitis.

Surgery for *in vivo* spinal cord recording

Mice were anaesthetized with isoflurane (2–3%, 2 L/min O_2 induction, 1–2% maintenance) delivered via a nose cone. When deep anesthesia was confirmed via the absence of hind limb and corneal reflexes, the animals were head-fixed and stabilized using custom-made ear bars in a stereotaxic frame (Narishige Corp., Tokyo, Japan). Body temperature was maintained with a heat mat at 37 °C. All spinal surgery was done under a dissecting microscope (Leica Microsystems, Nuslock, Germany). Vertebral clamps on the T13 and L2 vertebral arches stabilized the vertebral column. A L1 laminectomy was performed to expose the L6-S1 spinal cord segments (Harrison et al., 2013). Small incisions were made in the dura and pia so recording pipettes could be lowered into the spinal cord. The surface of the spinal cord was kept moist with warmed (37 °C) artificial cerebrospinal fluid (aCSF) during the experiment. The aCSF contained (in mM): 118 NaCl, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 1 $MgCl_2$, and 2.5 $CaCl_2$. The aCSF was bubbled with 95% O_2–5% CO_2 to maintain pH at 7.3. Animals were overdosed with pentobarbital (200 mg/kg i.p.) when experiments were complete.

Electrophysiology

Recording pipettes (4–7 MΩ) were pulled from thin-walled filamented borosilicate glass capillaries (1.5 mm o.d., 1.17 mm i.d., Harvard Apparatus, Edenbridge, UK) using a micropipette puller (PC-10, Narishige Corp., Tokyo, Japan). A K^+-based internal solution containing (in mM): 135 KGluconate, 6 NaCl, 2 $MgCl_2$, 10 HEPES, 0.1 EGTA, 2 NaATP, and 0.3 NaGTP (adjusted to pH 7.3 with KOH) was used in all experiments. A Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) was used to record all signals. A Luigs & Neumann SM-6 micromanipulator (Ratingen, Germany) was used to guide pipettes into the spinal cord dorsal horn. Recording depth (in microns) was monitored by a digital readout on the micromanipulator. The pipette was positioned at an angle of 65° (to horizontal) and was advanced to a depth of 100 μm to ensure its tip was located in the dorsal horn gray matter (Farrell et al., 2016). Continuous application of positive pressure (~50 kPa) kept the pipette tip clear of debris (Graham et al., 2004a) as it moved through the white matter. Positive pressure was reduced, and the pipette was then advanced in 3-μm steps for a further 200 μm to search for SDH neurons. Our 'searching' for neurons was