Brain, Behavior, and Immunity 64 (2017) 59–64

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Short Communication

Constriction of the buccal branch of the facial nerve produces unilateral craniofacial allodynia

Susannah S. Lewis [a,1], Peter M. Grace [a,b,1,2], Mark R. Hutchinson [b,c], Steven F. Maier [a], Linda R. Watkins [a,*]

[a] Department of Psychology & Neuroscience, University of Colorado, Boulder, USA
[b] School of Medicine, University of Adelaide, Adelaide, Australia
[c] Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, Adelaide, Australia

ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 2 December 2016
Accepted 5 December 2016
Available online 18 December 2016

Keywords:
Orofacial
Muscle
Glia
Hyperalgesia
Mirror-image pain

ABSTRACT

Despite pain being a sensory experience, studies of spinal cord ventral root damage have demonstrated that motor neuron injury can induce neuropathic pain. Whether injury of cranial motor nerves can also produce nociceptive hypersensitivity has not been addressed. Herein, we demonstrate that chronic constriction injury (CCI) of the buccal branch of the facial nerve results in long-lasting, unilateral allodynia in the rat. An anterograde and retrograde tracer (3000 MW tetramethylrhodamine-conjugated dextran) was not transported to the trigeminal ganglion when applied to the injury site, but was transported to the facial nucleus, indicating that this nerve branch is not composed of trigeminal sensory neurons. Finally, intracisterna magna injection of interleukin-1 (IL-1) receptor antagonist reversed allodynia, implicating the pro-inflammatory cytokine IL-1 in the maintenance of neuropathic pain induced by facial nerve CCI. These data extend the prior evidence that selective injury to motor axons can enhance pain to supraspinal circuits by demonstrating that injury of a facial nerve with predominantly motor axons is sufficient for neuropathic pain, and that the resultant pain has a neuroimmune component.

1. Introduction

Peripheral nerve lesions or disease can initiate neuropathic pain, which is responsible for chronic pain in up to 10% of the general population (Treede et al., 2008; van Hecke et al., 2014). Due to the fact that pain is a sensory experience, neuropathic pain is frequently assumed to only follow damage to sensory neurons. However, recent studies have revealed that selective lesion of spinal motor neurons by L5 ventral root transection induces nociceptive hypersensitivity and microglia activation in the spinal dorsal horn, which are both dependent on tumor necrosis factor (TNF) signaling (Li et al., 2002; Sheth et al., 2002; Xu et al., 2006, 2007). Such neuroimmune signaling has a well-documented role in the development of neuropathic pain after injury to mixed (sensory and motor) peripheral nerves (Grace et al., 2014, 2016a). Furthermore, injury of the gastrocnemius-soleus (predominantly motor) nerve results in nociceptive hypersensitivity, and both induces ectopic activity and amplifies evoked action potentials of sciatic nerve and DRG neurons (Kirillova et al., 2011; Michaelis et al., 2000; Zhou et al., 2010). Thus, injury of spinal motor nerves is sufficient for peripheral neuropathic pain.

To date, several models of craniofacial neuropathic pain have been developed, involving lesions of the sensory infraorbital (Eriksson et al., 2005; Vos et al., 1994), or sensory inferior alveolar nerves (Sugiyama et al., 2013). However, it is not yet known whether injury of cranial motor nerves is sufficient to induce neuropathic pain, similar to the spinal system. Uniformity cannot be assumed, given the documented pathophysiological differences between the injured spinal and trigeminal systems. For example, production of spinal dorsal horn interleukin (IL)-6 and sprouting of noradrenergic nerves within the dorsal root ganglia (DRG) occurs after sciatic nerve injury (Latrémolière et al., 2008; McLachlan et al., 1993), but neither occur within the trigeminal ganglia after infraorbital nerve injury (Benoliel et al., 2001; Latrémolière et al., 2008). Furthermore, triptans and calcitonin gene-related peptide (CGRP) receptor antagonists are effective in reversing nociceptive hypersensitivity induced by injury of the infraorbital nerve, but not of the sciatic nerve (Kayser et al., 2002, 2011; Michot et al., 2012, 2015).

Therefore, the goal of this study was to determine whether injury of a motor cranial nerve could produce neuropathic pain.

* Corresponding author at: Department of Psychology, Campus Box 345, University of Colorado at Boulder, Boulder, CO 80309-0345, USA.
E-mail address: linda.watkins@colorado.edu (L.R. Watkins).
[1] Authors contributed equally to this work.
[2] Current address: Department of Critical Care Research, University of Texas MD Anderson Cancer Center, Houston, USA.

http://dx.doi.org/10.1016/j.bbi.2016.12.004
0889-1591/

The facial nerve (cranial nerve VII) of the rat is an excellent candidate to address this question, as it is comprised of motor efferent neurons without a significant somatosensory nerve component from the skin (Nerve, 2013), is readily accessible surgically and there is a well-established protocol for demonstrating facial allodynia in the rat (Ren, 1999). Given the dimorphic role of pro-inflammatory cytokines in craniofacial and spinal neuropathic pain (Latrémolière et al., 2008), the second goal of this study was to determine whether allodynia induced by facial nerve injury could be attenuated by blocking IL-1 signaling.

2. Methods

2.1. Animals

Adult, male, pathogen-free Sprague-Dawley rats (Harlan Labs, Madison, WI) were used for all experiments. Rats (350–400 g at time of surgery) were housed in temperature (23 ± 3 °C) and light (12 h:12 h light:dark; lights on 0700 h) controlled rooms with water and food given *ad libitum*. All habituation and behavioral testing procedures were performed during the light phase of the daily cycle. All procedures were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee. All experimental groups have 6–9 rats per group.

2.2. Facial nerve chronic constriction injury surgery

This novel surgery constricted the buccal branch of the facial nerve. The buccal branch of the facial nerve has the advantage of being readily accessible following a skin incision, allowing for a straightforward surgery with very little damage to tissues surrounding the nerve. All surgical instruments were sterilized prior to use and all surgical procedures were conducted under isoflurane anesthesia.

The buccal branch of the facial nerve was aseptically exposed through a 1 cm skin incision. Great care is necessary when shaving the skin, as damage to whiskers alters subsequent behavioral responses. The buccal facial nerve branch is superficial and visible following a skin incision. The incision was made along the line from the corner of the mouth to the ear, about two-thirds of the way to the ear (Fig. 1). Once exposed, the nerve was kept moist with sterile physiological saline drops and only touched with glass instruments to prevent damage through metal instruments. Borosilicate 6″ glass pipettes (Fisherbrand, Fisher Scientific, Waltham, MA) were molded into a curved 'L' shape approximately 8 mm long at the tip and used to gently manipulate the nerve. These steps were undertaken to minimize the variability in nerve damage between rats.

To isolate the nerve, two nicks (each approximately 0.5 mm) were made into the fascia and muscle surrounding the nerve using the tip of a #11 scalpel blade (Havel, Cincinnati, OH, USA). These small incisions were expanded using a pair of shaped glass pipettes in a spreading motion to gently separate the nerve completely from the surrounding fascia and muscle. The spreading motion, rather than additional scalpel incisions, separated the muscle along muscle fibers and minimized damage and bleeding. Care was taken not to stretch the nerve during the separation of the nerve and musculature.

Once the nerve was isolated from surrounding muscle and connective tissue, three 4-0 chromic gut (Ethicon, Somerville, NJ, USA) ligatures were tied around the nerve with a square knot. Ligatures were tied tightly enough so to not to move along the nerve when gently pushed with forceps, but loose enough not to visibly deform the nerve and spaced approximately 1 mm apart. Again, care was taken not to stretch or deform the nerve during ligation. After ligation, the chromic gut was cut close to the knot and the skin was then sutured closed with 4-0 silk suture (Ethicon, Somerville, NJ, USA). Sham surgeries were as described above, with the exception that no chromic gut sutures were tied around the isolated nerve.

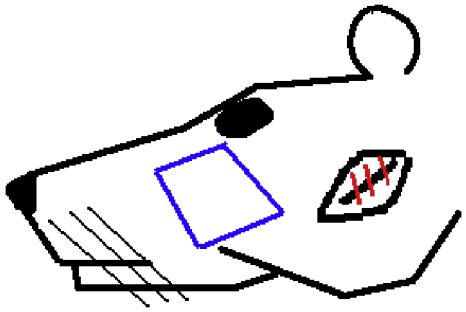

Fig. 1. Approximate size and position of the incision with skin retracted. Three chromic gut ligatures are shown in red. The buccal branch of the facial nerve (straight black line) is readily visible upon skin incision. Area for tactile testing is shown in blue square. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. von Frey test for tactile sensitivity

Assessment of the development and persistence of tactile allodynia was conducted as detailed (Ren, 1999). Briefly, rats were habituated in two 5 min sessions to stand comfortably with their forepaws in a leather glove. This method allows the rats to be completely unrestrained. Calibrated microfilaments (von Frey hairs; Stoelting, Wood Dale, IL, USA) were applied to the hairy skin under the eye by and experimental blind to treatment groups. Microfilaments were applied in 5 quick up-down applications and the number of brisk head withdrawals or aggravated paw swipes recorded as responses.

Microfilaments ranging logarithmically from 1.2 to 75.86 g were applied starting with a mild stimulus of 3.63 g and increasing or decreasing to find the range from 0 out of 5, to 5 out of 5 responses from the rat. Assessments were made prior to and 3, 7, 10, 14, 21, 28, 35, 42 days following facial nerve CCI or sham surgery by an experimenter blind to treatment group. Responses were fitted to a Gaussian integral psychometric function using a maximum-likelihood fitting method as described (Milligan et al., 2000).

2.4. Body weights

Body weights were measured prior to and 3, 7, 10, 14, 21, 28, 35, 42 days following facial nerve CCI or sham surgery by an experimenter blind to treatment group. Measurements were made between 0900 and 1100 h to reduce variability due to circadian changes.

2.5. Neuronal tracing

Although the majority of the constricted nerve is efferent facial nerve axons, it is possible that there may be a small component of afferent trigeminal axons also mixed within the nerve bundle. In order to determine whether any increase in mechanical sensitivity could be due to damage of intermingled trigeminal afferents in the buccal nerve CCI site, a neuronal tracing study was conducted.

Anterograde and retrograde labeling of the facial and trigeminal brainstem nuclei and trigeminal ganglia with the tracer 3000 MW tetramethylrhodamine-conjugated dextran (Invitrogen, Carlsbad, CA, USA) was used to determine origin/terminus of neurons in