Published in final edited form as:

Brain Behav Immun. 2017 August ; 64: 59–64. doi:10.1016/j.bbi.2016.12.004.

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

Susannah S. Lewis^{1,#}, Peter M. Grace^{1,2,4,#}, Mark R. Hutchinson^{2,3}, Steven F. Maier¹, and Linda R. Watkins^{1,*}

¹Department of Psychology & Neuroscience, University of Colorado, Boulder, USA

²School of Medicine, University of Adelaide, Adelaide, Australia

³Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, Adelaide, Australia

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 (3000MW 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.

Keywords

Orofacial; muscle; glia; hyperalgesia; mirror-image pain

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.,

*Corresponding author: Department of Psychology, Campus Box 345, University of Colorado at Boulder, Boulder, Colorado, USA 80309-0345, linda.watkins@colorado.edu, Fax: (303) 492-2967, Phone: (303) 492-7034.

⁴Current address: Department of Critical Care Research, University of Texas MD, Anderson Cancer Center, Houston, USA

[#]Authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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., 2016a, 2014). 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 (Latrémoière et al., 2008; McLachlan et al., 1993), but neither occur within the trigeminal ganglia after infraorbital nerve injury (Benoliel et al., 2001; Latrémoière 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. 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 (Latrémoière 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 hr) 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 (Figure 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.

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 minute 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 3.63g 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 0900–1100 hr 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 3000MW tetramethylrhodamine-conjugated dextran (Invitrogen, Carlsbad, CA, USA) was used to determine origin/terminus of neurons in the constricted region. The nerve was first exposed and isolated identically to that described above. Using a method adapted from May and Hill (2006), the nerve was then transected and parafilm placed under the nerve to isolate it from surrounding tissues. A Q-tip was used to apply DMSO to the cut end of the nerve to increase dextran penetration. Dextran granules were then placed on the nerve, held in place with a small dab of petroleum jelly and the parafilm sealed around the nerve with superglue. This method allowed the dextran to be applied to the nerve for an extended period of time without contaminating nearby tissues, which are innervated by other cranial nerves. By transecting the nerve, all axons in the nerve were exposed to the retrograde tracer.

At 1, 3, 4, 5, 6 or 7 days after dextran placement ($n = 2/\text{timepoint}$), rats were deeply anesthetized with sodium pentobarbital (50 mg/kg i.p.) and transcardially perfused, first with a saline flush, and then with 4% paraformaldehyde to fix the tissue. Brains and trigeminal ganglia were harvested and cryoprotected in 30% sucrose. Brains and ganglia were then frozen in dry-ice chilled isopentane and sliced in 50 μm sections in a cryostat. The entire trigeminal ganglion was sectioned, and approximately one out of every 10 sections were stained. Sections were mounted on gelatin coated slides and fluorescence examined immediately on an Olympus BX61 fluorescence microscope (Olympus America, Center Valley, PA) using Microsuite software (Olympus America).

2.6. Drug administration

The effect of proinflammatory cytokines on facial nerve CCI was assessed using interleukin-1 receptor antagonist (IL-1ra, Amgen, Thousand Oaks, CA) administered intracisterna magna (i.c.m.). IL-1ra or equivolume sterile, endotoxin free saline was administered 21 and 28 days after facial nerve CCI or sham surgery. Mechanical allodynia was assessed 45 minutes following i.c.m. injection to account for the relatively short cerebrospinal fluid half life of IL-1ra (Milligan et al., 2005).

I.c.m. injections were percutaneously performed as previously described (Frank et al., 2010), using polyethylene-60 (PE60) tubing attached to a 30 gauge 3/8" hypodermic needle. Each rat was briefly anesthetized with isoflurane and a small patch at the nape of the neck was shaved and scrubbed with 70% ethyl alcohol. The rat was then placed in ventral recumbancy on a box with the head positioned beyond the end of the box such that the head bent downward at a 90° angle to the body, allowing easier access to the cisterna magna. The 30 gauge needle was percutaneously inserted into the cisterna magna and a 10 µl injection of either 1 µl of 100 µg IL-1ra plus 8 µl saline vehicle separated by 1 µl air, or 9 µl saline vehicle plus 1 µl air. Injections were given slowly over a 30 second period. A dose of 100 µg IL-1ra was chosen based on prior reports that the same dose intrathecally reversed neuropathic pain induced by sciatic CCI (Grace et al., 2016b) and inflammatory neuropathy (Milligan et al., 2003), and this same dose i.c.m. blocked stress-induced enhancement of pro-inflammatory responses by brain nuclei (Johnson et al., 2004).

2.7. Statistics

Mechanical allodynia was analyzed as the interpolated 50% thresholds (absolute threshold). One-way analysis of variance followed by the Tukey post hoc test was used to confirm that there were no baseline differences in absolute thresholds between treatment groups. Differences between treatment groups were determined using 2-way analysis of variance, followed by the Sidak post hoc test, with a correction for repeated measures for mechanical allodynia. $P < 0.05$ was considered significant, and all data are expressed as mean $\pm$ SEM.

3. Results

3.1. Buccal branch CCI produces unilateral craniofacial allodynia

There were no pre-surgical baseline differences between the either surgery group on either side of the face ($F_{3,24} = 0.69$, $P > 0.05$). CCI of the buccal branch of the facial nerve produced significant orofacial allodynia ipsilateral to the site of injury from day 10 through day 35 after surgery (Figure 2; Time x Treatment: $F_{7,84} = 3.86$, $P < 0.01$; Time: $F_{7,84} = 4.99$, $P < 0.001$; Treatment: $F_{1,12} = 28.93$, $P < 0.001$). Post hoc tests showed a significant decrease in the CCI group compared to Shams ipsilateral to facial nerve CCI at every time point tested after surgery, until testing was concluded at day 42 ($P < 0.05$). No significant allodynia developed contralateral to the site of injury (Time x Treatment: $F_{7,96} = 0.53$, $P = 0.8$).

At no point in the six week duration of allodynia was there a significant difference in body weight gain between the facial nerve CCI and sham animals (Treatment: $F_{8,95} = 0.58$, $P =$

1.0, data not shown). No noticeable changes in whisking behavior or eyeblink reflex were subjectively observed following the ligation of the facial nerve.

3.2. No trigeminal afferents were detected at the site of constriction

To test whether injury of a small contingent of sensory nerves in the facial nerve could have accounted for the robust allodynia, trigeminal afferents were labelled with the antero- and retrograde tracer 3kD tetramethylrhodamine-conjugated dextran. This dye has previously produced robust central nervous system cell body labeling of peripheral gustatory sensory nerves (May and Hill, 2006), and tibial and common fibular motor nerves (English et al., 2009). Strong labeling of neurons in the facial nucleus was found 6 days following dextran placement (Figure 3) with weaker labeling present 5 and 7 days following dextran placement. At no time point (1, 3, 4, 5, 6 or 7 days following dextran placement at the site of transection) was fluorescent labeling detected in the trigeminal ganglion or at any level of the brainstem trigeminal nuclei beyond that seen in an animal without dextran placement. These data indicate that there are no detected trigeminal sensory afferents in the surgical site of the facial nerve.

3.3. IL-1ra reverses established allodynia following facial nerve CCI

Numerous studies have convincingly shown that an increase in neuroinflammation in the dorsal spinal cord importantly contributes to allodynia following sciatic CCI (Grace et al., 2016a, 2014). One of the major neuroinflammatory mediators within spinal cord implicated in creating allodynia is following injury to peripheral sensory/motor mixed nerves is IL-1beta (Grace et al., 2016a, 2014). In contrast, IL-1 has never been implicated in allodynia induced as a consequence of injury to motor axons, either spinally or supraspinally. To determine if IL-1 provides a proinflammatory component necessary to maintain the craniofacial allodynia seen following facial nerve CCI, tactile sensitivity was assessed 45 minutes after i.c.m. IL-1ra, in a within-subjects design described above. There were no baseline differences between the sham and CCI group on either side of the face ($F_{3,23} = 1.10$, $P > 0.05$). There was a significant interaction between surgery and drug treatment (Figure 4; $F_{3,48} = 4.74$, $P < 0.01$), as well as a main effect of treatment ($F_{3,48} = 13.57$, $P < 0.001$), but not of time ($F_{1,48} = 1.42$, $P = 0.2$). Post hoc tests showed that the facial CCI surgery produced a robust allodynia prior to the saline and IL-1ra injections on day 21 and 28 post surgery compared to sham treated animals ($P < 0.05$). The allodynia remained unchanged after an i.c.m. saline injection. However, IL-1ra reversed established craniofacial allodynia, relative to control treatment after facial CCI ($P < 0.01$), with no significant difference between CCI rats treated with IL-1ra and sham treated animals. IL-1ra had no impact on contralateral mechanical thresholds, which were not altered by facial nerve CCI (data not shown).

4. Discussion

These studies present the first evidence that constriction injury to a cranial nerve with predominantly efferent motor neurons can produce reliable and prolonged tactile allodynia. Notably, contralateral allodynia was absent after buccal branch CCI, which contrasts with that reported for some models of sciatic nerve injury (Grace et al., 2010; Milligan et al.,

2003). The allodynia measured in this study was transiently reversed with an intracisterna magna injection of IL-1ra, suggesting a role for central nervous system inflammation in the generation of the allodynia.

To our knowledge, all other craniofacial neuropathic pain models involve damage of sensory nerves (Eriksson et al., 2005; Sugiyama et al., 2013; Vos et al., 1994). The results obtained here demonstrate that injury to the facial nerve, which we show to be devoid of detected trigeminal somatosensory afferents from the skin, is also sufficient to create neuropathic pain. These data parallel and importantly extend studies performed in the motor gastrocnemius-soleus nerve (Kirillova et al., 2011; Michaelis et al., 2000; Zhou et al., 2010) and the motor ventral root (Li et al., 2002; Sheth et al., 2002; Xu et al., 2006, 2007), and highlight a common consequence of damage of nerves that innervate muscles in the cephalic and spinal systems. Injury of these motor nerves also induces nociceptive hypersensitivity, and spontaneous activity in uninjured DRG sensory neurons (Kirillova et al., 2011; Michaelis et al., 2000; Xu et al., 2006, 2007; Zhou et al., 2010). The facial region below the eye, where hypersensitivity was detected, is innervated by the V2 branch of the trigeminal nerve (Nerve, 2013). The trigeminal and facial nerves are not mixed, but both project to the brainstem. This extra-territorial allodynia may therefore be mediated by central sensitization, rather than by Wallerian degeneration of motor neurons, as occurs in the spinal system (Gaudet et al., 2011; Xu et al., 2006, 2007). Future studies may seek to confirm these results in cephalic nerves composed solely of efferent fibers, such as the oculomotor nerve.

Our data also point to the involvement of pro-inflammatory cytokines in neuropathic pain induced by facial nerve CCI. While TNF has previously been implicated in allodynia resultant from injury to motor axons (Li et al., 2002; Sheth et al., 2002; Xu et al., 2006, 2007), no prior study of allodynia in response to motor damage has examined IL-1. Here, IL-1ra reversed allodynia at 21 and 28 days post-surgery, indicating a role for IL-1 in neuropathic pain maintenance, most likely via release within brainstem sites. IL-1 may have a common role in mediating nociceptive hypersensitivity after craniofacial and sciatic nerve injury (Grace et al., 2014), unlike IL-6 (Latrémoière et al., 2008). There are several known mechanisms by which IL-1 may increase neuronal excitability in nociceptive pathways (Grace et al., 2016a, 2014), including phosphorylation of postsynaptic NR1 NMDA receptor subunits (Zhang et al., 2008), and down-regulation of both the astrocyte glutamate transporter GLT-1 (Yan et al., 2014) and neuronal G protein-coupled receptor kinase 2 (an enzymatic regulator of G protein-coupled receptor homologous desensitization, that protects against overstimulation) (Kleibeuker et al., 2008). IL-1 is elevated in the brainstem and contributes to extra-territorial pain after trigeminal nerve injury (Chai et al., 2012; Takahashi et al., 2011), and this report adds to others demonstrating a causal role for this cytokine in craniofacial neuropathic pain (Won et al., 2014). Future studies may investigate whether activated glial cells or recruited immune cells are associated with this nerve injury model, and are responsible for production of IL-1.

In conclusion, this study demonstrates that injury to the facial nerve, which is predominantly composed of motor neurons, is sufficient to induce neuropathic pain in rat. This finding is also supported by the clinical literature, as pain is a principal complaint of Bell's palsy—an idiopathic paralysis of the facial nerve (De Seta et al., 2014). Our data predict that

neuroimmune signaling contributes to nociceptive hypersensitivity after facial nerve injury, and is a possible therapeutic target for craniofacial neuropathic pain.

Acknowledgments

The authors declare that there are no conflicts of interest. Funding from NIH R01 DE021966. Peter M Grace was a NHMRC (Australia) CJ Martin Fellow [ID: 1054091] and American Australian Association Sir Keith Murdoch Fellow. Mark R. Hutchinson was a NHMRC (Australia) CJ Martin Fellow (ID 465423; 2007–2010) and an Australian Research Council Research Fellow (DP110100297). The authors are grateful to Drs. Dianna Bartel and Thomas Finger (University of Colorado Denver) for their assistance with the neuronal tracing protocol.

References

- Benoliel R, Eliav E, Tal M. No sympathetic nerve sprouting in rat trigeminal ganglion following painful and non-painful infraorbital nerve neuropathy. *Neurosci Lett*. 2001; 297:151–154. [PubMed: 11137750]
- Chai B, Guo W, Wei F, Dubner R, Ren K. Trigeminal-rostral ventromedial medulla circuitry is involved in orofacial hyperalgesia contralateral to tissue injury. *Mol Pain*. 2012; 8:78.doi: 10.1186/1744-8069-8-78 [PubMed: 23092240]
- De Seta D, Mancini P, Minni A, Prosperini L, De Seta E, Attanasio G, Covelli E, De Carlo A, Filipo R. Bell's palsy: symptoms preceding and accompanying the facial paresis. *ScientificWorldJournal*. 2014; 2014:801971.doi: 10.1155/2014/801971 [PubMed: 25544960]
- English AW, Cucoranu D, Mulligan A, Sabatier M. Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. *J Comp Neurol*. 2009; 517:245–255. DOI: 10.1002/cne.22149 [PubMed: 19731339]
- Eriksson J, Jablonski A, Persson AK, Hao JX, Kouya PF, Wiesenfeld-Hallin Z, Xu XJ, Fried K. Behavioral changes and trigeminal ganglion sodium channel regulation in an orofacial neuropathic pain model. *Pain*. 2005; 119:82–94. DOI: 10.1016/j.pain.2005.09.019 [PubMed: 16297558]
- Frank MG, Barrientos RM, Hein AM, Biedenkapp JC, Watkins LR, Maier SF. IL-1RA blocks E. coli-induced suppression of Arc and long-term memory in aged F344xBN F1 rats. *Brain Behav Immun*. 2010; 24:254–262. DOI: 10.1016/j.bbi.2009.10.005 [PubMed: 19822205]
- Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation*. 2011; 8:110.doi: 10.1186/1742-2094-8-110 [PubMed: 21878126]
- Grace PM, Gaudet AD, Staikopoulos V, Maier SF, Hutchinson MR, Salvemini D, Watkins LR. Nitroxidative Signaling Mechanisms in Pathological Pain. *Trends Neurosci*. 2016a; doi: 10.1016/j.tins.2016.10.003
- Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. *Nat Rev Immunol*. 2014; 14:217–231. DOI: 10.1038/nri3621 [PubMed: 24577438]
- Grace PM, Hutchinson MR, Manavis J, Somogyi AA, Rolan PE. A novel animal model of graded neuropathic pain: utility to investigate mechanisms of population heterogeneity. *J Neurosci Methods*. 2010; 193:47–53. DOI: 10.1016/j.jneumeth.2010.08.025 [PubMed: 20817038]
- Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, Fabisiak TJ, Anderson ND, Cheng K, Greene LI, Berkelhammer D, Zhang Y, Ellis AL, Yin HH, Campeau S, Rice KC, Roth BL, Maier SF, Watkins LR. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. *Proc Natl Acad Sci U S A*. 2016b; 113:E3441–3450. DOI: 10.1073/pnas.1602070113 [PubMed: 27247388]
- Johnson JD, O'Connor KA, Watkins LR, Maier SF. The role of IL-1beta in stress-induced sensitization of proinflammatory cytokine and corticosterone responses. *Neuroscience*. 2004; 127:569–577. DOI: 10.1016/j.neuroscience.2004.05.046 [PubMed: 15283957]
- Kayser V, Aubel B, Hamon M, Bourgoin S. The antimigraine 5-HT 1B/1D receptor agonists, sumatriptan, zolmitriptan and dihydroergotamine, attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. *Br J Pharmacol*. 2002; 137:1287–1297. DOI: 10.1038/sj.bjp.0704979 [PubMed: 12466238]

- Kayser V, Latrémolière A, Hamon M, Bourgoin S. N-methyl-D-aspartate receptor-mediated modulations of the anti-allodynic effects of 5-HT_{1B/1D} receptor stimulation in a rat model of trigeminal neuropathic pain. *Eur J Pain Lond Engl*. 2011; 15:451–458. DOI: 10.1016/j.ejpain.2010.09.012
- Kirilova I, Rausch VH, Tode J, Baron R, Jänig W. Mechano- and thermosensitivity of injured muscle afferents. *J Neurophysiol*. 2011; 105:2058–2073. DOI: 10.1152/jn.00938.2010 [PubMed: 21307318]
- Kleibeuker W, Gabay E, Kavelaars A, Zijlstra J, Wolf G, Ziv N, Yirmiya R, Shavit Y, Tal M, Heijnen CJ. IL-1 beta signaling is required for mechanical allodynia induced by nerve injury and for the ensuing reduction in spinal cord neuronal GRK2. *Brain Behav Immun*. 2008; 22:200–208. DOI: 10.1016/j.bbi.2007.07.009 [PubMed: 17869478]
- Latrémolière A, Mauborgne A, Masson J, Bourgoin S, Kayser V, Hamon M, Pohl M. Differential implication of proinflammatory cytokine interleukin-6 in the development of cephalic versus extracephalic neuropathic pain in rats. *J Neurosci Off J Soc Neurosci*. 2008; 28:8489–8501. DOI: 10.1523/JNEUROSCI.2552-08.2008
- Li L, Xian CJ, Zhong JH, Zhou XF. Effect of lumbar 5 ventral root transection on pain behaviors: a novel rat model for neuropathic pain without axotomy of primary sensory neurons. *Exp Neurol*. 2002; 175:23–34. DOI: 10.1006/exnr.2002.7897 [PubMed: 12009757]
- May OL, Hill DL. Gustatory terminal field organization and developmental plasticity in the nucleus of the solitary tract revealed through triple-fluorescence labeling. *J Comp Neurol*. 2006; 497:658–669. DOI: 10.1002/cne.21023 [PubMed: 16739199]
- McLachlan EM, Jänig W, Devor M, Michaelis M. Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia. *Nature*. 1993; 363:543–546. DOI: 10.1038/363543a0 [PubMed: 8505981]
- Michaelis M, Liu X, Jänig W. Axotomized and intact muscle afferents but no skin afferents develop ongoing discharges of dorsal root ganglion origin after peripheral nerve lesion. *J Neurosci Off J Soc Neurosci*. 2000; 20:2742–2748.
- Michot B, Bourgoin S, Viguier F, Hamon M, Kayser V. Differential effects of calcitonin gene-related peptide receptor blockade by olcegepant on mechanical allodynia induced by ligation of the infraorbital nerve vs the sciatic nerve in the rat. *Pain*. 2012; 153:1939–1948. DOI: 10.1016/j.pain.2012.06.009 [PubMed: 22795918]
- Michot B, Kayser V, Hamon M, Bourgoin S. CGRP receptor blockade by MK-8825 alleviates allodynia in infraorbital nerve-ligated rats. *Eur J Pain Lond Engl*. 2015; 19:281–290. DOI: 10.1002/ejp.616
- Milligan ED, Langer SJ, Sloane EM, He L, Wieseler-Frank J, O'Connor K, Martin D, Forsayeth JR, Maier SF, Johnson K, Chavez RA, Leinwand LA, Watkins LR. Controlling pathological pain by adenovirally driven spinal production of the anti-inflammatory cytokine, interleukin-10. *Eur J Neurosci*. 2005; 21:2136–2148. DOI: 10.1111/j.1460-9568.2005.04057.x [PubMed: 15869510]
- Milligan ED, Mehrt KK, Hinde JL, Harvey LO, Martin D, Tracey KJ, Maier SF, Watkins LR. Thermal hyperalgesia and mechanical allodynia produced by intrathecal administration of the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein, gp120. *Brain Res*. 2000; 861:105–116. [PubMed: 10751570]
- Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, Tracey K, Martin D, Maier SF, Watkins LR. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. *J Neurosci Off J Soc Neurosci*. 2003; 23:1026–1040.
- Nerve, O. Cranial Nerves. In: Patestas, MA., Gartner, LP., editors. *A Textbook of Neuroanatomy*. Blackwell Publishing; 2013. p. 253–281.
- Ren K. An improved method for assessing mechanical allodynia in the rat. *Physiol Behav*. 1999; 67:711–716. [PubMed: 10604842]
- Sheth RN, Dorsi MJ, Li Y, Murinson BB, Belzberg AJ, Griffin JW, Meyer RA. Mechanical hyperalgesia after an L5 ventral rhizotomy or an L5 ganglionectomy in the rat. *Pain*. 2002; 96:63–72. [PubMed: 11932062]

- Sugiyama T, Shinoda M, Watase T, Honda K, Ito R, Kaji K, Urata K, Lee J, Ohara K, Takahashi O, Echizenya S, Iwata K. Nitric oxide signaling contributes to ectopic orofacial neuropathic pain. *J Dent Res*. 2013; 92:1113–1117. DOI: 10.1177/0022034513509280 [PubMed: 24130220]
- Takahashi K, Watanabe M, Suekawa Y, Ito G, Inubushi T, Hirose N, Murasaki K, Hiyama S, Uchida T, Tanne K. IL-1beta in the trigeminal subnucleus caudalis contributes to extra-territorial allodynia/hyperalgesia following a trigeminal nerve injury. *Eur J Pain Lond Engl*. 2011; 15:467e1–14. DOI: 10.1016/j.ejpain.2010.10.006
- Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology*. 2008; 70:1630–1635. DOI: 10.1212/01.wnl.0000282763.29778.59 [PubMed: 18003941]
- van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. *Pain*. 2014; 155:654–662. DOI: 10.1016/j.pain.2013.11.013 [PubMed: 24291734]
- Vos BP, Strassman AM, Maciewicz RJ. Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *J Neurosci Off J Soc Neurosci*. 1994; 14:2708–2723.
- Won KA, Kim MJ, Yang KY, Park JS, Lee MK, Park MK, Bae YC, Ahn DK. The glial-neuronal GRK2 pathway participates in the development of trigeminal neuropathic pain in rats. *J Pain Off J Am Pain Soc*. 2014; 15:250–261. DOI: 10.1016/j.jpain.2013.10.013
- Xu JT, Xin WJ, Wei XH, Wu CY, Ge YX, Liu YL, Zang Y, Zhang T, Li YY, Liu XG. p38 activation in uninjured primary afferent neurons and in spinal microglia contributes to the development of neuropathic pain induced by selective motor fiber injury. *Exp Neurol*. 2007; 204:355–365. DOI: 10.1016/j.expneurol.2006.11.016 [PubMed: 17258708]
- Xu JT, Xin WJ, Zang Y, Wu CY, Liu XG. The role of tumor necrosis factor-alpha in the neuropathic pain induced by Lumbar 5 ventral root transection in rat. *Pain*. 2006; 123:306–321. DOI: 10.1016/j.pain.2006.03.011 [PubMed: 16675114]
- Yan X, Yadav R, Gao M, Weng H-R. Interleukin-1 beta enhances endocytosis of glial glutamate transporters in the spinal dorsal horn through activating protein kinase C. *Glia*. 2014; 62:1093–1109. DOI: 10.1002/glia.22665 [PubMed: 24677092]
- Zhang RX, Li A, Liu B, Wang L, Ren K, Zhang H, Berman BM, Lao L. IL-1ra alleviates inflammatory hyperalgesia through preventing phosphorylation of NMDA receptor NR-1 subunit in rats. *Pain*. 2008; 135:232–239. DOI: 10.1016/j.pain.2007.05.023 [PubMed: 17689191]
- Zhou LJ, Ren WJ, Zhong Y, Yang T, Wei XH, Xin WJ, Liu CC, Zhou LH, Li YY, Liu XG. Limited BDNF contributes to the failure of injury to skin afferents to produce a neuropathic pain condition. *Pain*. 2010; 148:148–157. DOI: 10.1016/j.pain.2009.10.032 [PubMed: 19945222]

Research Highlights

- Constriction of the buccal branch of the facial nerve induced allodynia
- Facial allodynia was reversed with interleukin-1 receptor antagonist
- Injury to a predominantly motor nerve induces pain of neuroinflammatory origin

Figure 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.

Figure 2.

Chronic constriction injury of the facial nerve lead to the development of tactile allodynia ipsilateral to the surgery. No significant allodynia was found contralateral to injury. Animals with CCI maintained significant allodynia from 10–35 days after surgery. Allodynia was no longer significant at 42 days post-surgery. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, relative to Sham Ipsilateral. Mean $\pm$ SEM are presented, $n = 6-99/\text{group}$.

Figure 3.

Representative micrographs from dextran staining demonstrate that the injury site did not contain trigeminal sensory afferents. Brain slices from the caudal portion of the trigeminal nucleus through the hindbrain as well as the trigeminal ganglia were examined from 1–7 days following dextran placement at the injury site. The only fluorescence noted was in the facial nucleus 5, 6 and 7 days following dextran placement. Six days was optimal and shown in the above pictures. Micrographs show relative position of the illuminated neurons in the facial nucleus (A, 4x), detailed morphology of illuminated neurons (B) and the lack of staining in the trigeminal ganglia (C).

Figure 4.

Intracisterna magna IL-1 receptor antagonist (IL-1ra; 100 μ g) significantly attenuated the tactile allodynia that developed following facial nerve constriction. Assessments were made prior to (pre-treatment), and 45 minutes after administration (post-treatment). No significant change was noted following i.c.m. saline injections. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mean $\pm$ SEM are presented, $n = 6-9$ /group.