Published in final edited form as:

Neurosci Lett. 2007 July 5; 422(1): 54–58.

Increased spinal dynorphin contributes to chronic nicotine-induced mechanical hypersensitivity in the rat

Chris Lough², Tracey Young¹, Renee Parker¹, Shannon Wittenauer¹, and Michelle Vincler^{1,*}

¹*Department of Anesthesiology and the Center for the Study of Pharmacological Plasticity in the Presence of Pain, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA*

²*Department of Biology, Wake Forest University, Winston-Salem, NC 27109, USA*

Abstract

Chronic nicotine administration has been shown previously to produce mechanical hypersensitivity in the rat although the mechanism of this effect is unknown. Rats treated with chronic systemic nicotine 3.6 or 8.6 mg/kg/day for 14–21 days displayed mechanical hypersensitivity coincident with an increase of prodynorphin immunoreactivity and dynorphin content within the spinal cord. The administration of dynorphin antiserum intrathecally significantly attenuated chronic nicotine-induced mechanical hypersensitivity. Our results suggest that chronic nicotine administration produces an increase in spinal dynorphin content and release that contributes to mechanical hypersensitivity.

Keywords

smoking; pain; immunohistochemistry; ELISA

Introduction

Although the impact of smoking on a variety of health-related issues has been studied extensively, the impact of smoking on pain and its treatment have been virtually ignored. Clinical data support a correlation between smoking and the incidence and severity of diabetic neuropathy [5], fibromyalgia [35], and chronic back pain [12;26]. In a recent prospective study, smoking was identified as a “premorbid” factor in the development of chronic, disabling, low back pain [30]. Despite the correlation between smoking and an increased incidence of chronic pain conditions, there have been few systematic investigations of the impact of chronic nicotine exposure on nociceptive systems.

In the rodent, chronic nicotine administration produces numerous effects including a short-lived antinociception [3], an upregulation of nicotinic acetylcholine [7;25] and μ -opioid receptors, and a decrease in met-enkephalin levels [34]. Repeated exposure of rats to cigarette smoke increases tail flick latencies and reduces paw withdrawal thresholds to von Frey filaments [1]. Recently, our laboratory has reported that chronic nicotine increases the

*Corresponding author: Michelle Vincler, Ph.D. Department of Anesthesiology, Medical Center Blvd., Wake Forest University School of Medicine, Winston-Salem, NC 27157. Phone: (336)-716-3452; Fax: (336)-716-6744; email: mvincler@wfubmc.edu

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.

sensitivity of normal rats to mechanical pressure and compounds nerve injury-induced mechanical hypersensitivity following peripheral nerve injury [14].

Although the mechanism of chronic nicotine-induced mechanical hypersensitivity is unknown, the transcription factor, cAMP response element binding protein (CREB), is phosphorylated in the spinal cords of chronic nicotine-treated rats [14]. A considerable amount of evidence has accumulated to demonstrate the importance of prolonged CREB activation in central sensitization [21] and a number of pain-related genes are upregulated by pCREB including the immediate early gene c-fos [16], brain-derived neurotrophic factor (BDNF) [4], calcitonin gene-related peptide (CGRP) [8], the neurokinin 1 receptor [2], and prodynorphin [16].

Spinal dynorphin has been shown to be a critical mediator of chronic pain following peripheral nerve injury and chronic opioid administration [18;32]. Prodynorphin knockout mice fail to develop neuropathic pain following peripheral nerve injury [9] and the administration of chronic opioids produces a paradoxical hypersensitivity that is dependent upon NK-1 expressing spinal cord neurons and descending facilitation [33]. The mechanism of spinal dynorphin A maintenance of neuropathic pain is not dependent on opioids receptors but requires the activation of spinal bradykinin receptors [17].

The present studies were designed to investigate potential mediators of chronic nicotine-induced changes in mechanical pain thresholds in the spinal cord. Prodynorphin immunoreactivity and dynorphin content were measured in the spinal cords of chronic saline or chronic nicotine-treated rats. We also investigated the role of spinal dynorphin release in chronic nicotine-induced mechanical hypersensitivity.

Materials and Methods

For these studies, male Sprague-Dawley rats (200-250g) were used (Harlan, Indianapolis, IN). The animals were housed in pairs with free access to food and water. The procedures and protocols were approved by the Animal Care and Use Committee (Wake Forest University Health Sciences, Winston-Salem, NC, USA).

Osmotic Pump Implantation

Rats for the nicotine dose-response studies were implanted subcutaneously with Alzet® osmotic minipumps calculated to administer saline or nicotine (nicotine bitartrate; Sigma-Aldrich, USA) at a free base concentration of 3.6 mg/kg/day (Alzet® Model 2001) or 8.6 mg/kg/day (Alzet® Model 2ML4). The pumps were stabilized in 0.9% physiological saline for 24 hours at 37° C prior to implantation. Activated pumps were implanted by way of a single skin incision along the midline at mid-thoracic level. Osmotic minipumps delivered saline or nicotine for 14 days prior to sacrificing for immunohistochemical testing or ELISA.

Intrathecal catheterization

Lumbosacral intrathecal catheters were implanted as described previously [28], with slight modifications [24]. Catheters consisted of PE-10 tubing stretched to reduce the overall diameter. Briefly, under halothane anesthesia, an incision was made in the skin of the lower back and a sterile 20 G needle, used as a guide cannula, was inserted between the L5 and L6 vertebrae. A tail flick confirmed entry into the intrathecal space. The stretched PE10 catheter containing a guide wire was gently fed through the needle until the catheter extended 3 cm beyond the tip of the needle to reach the lumbar enlargement. The needle and guide wire were gently removed. A loosely tied knot was made in the catheter and three sutures were used to hold the catheter in place. A small fistula (a modified 1cc syringe hub [24]) was sutured to the

muscle surface and the catheter was fed through the fistula. The remaining externalized catheter was coiled into the fistula and sealed with a small amount of silicon sealant.

Behavioral Testing

Paw withdrawal thresholds (PWTs) to mechanical pressure were measured using an Analgesy-meter (Ugo Basile, Italy). The Analgesy-meter applies a constant rate (16g/sec) of increasing pressure to the animals' hind paws. The cutoff of pressure was set at 250g. Rats underwent 4 training sessions to stabilize baseline responses prior to experimental trials [29]. Paw withdrawal measurements were performed on days 5 and 14 post-osmotic pump implantation. On day 14 post-pump implantation, paw withdrawal thresholds of rats receiving 8.6 mg/kg/day nicotine were measured at least 1 hour prior to the intrathecal administration of undiluted polyclonal anti-dynorphin A [1-17] antiserum (Oncogene, San Diego, CA) or normal rabbit serum (NRS). Paw withdrawal thresholds were measured by an observer blinded to treatment at 30 and 60 minutes post-dynorphin antiserum/NRS administration. Separate groups of rats (n = 6-8) were used for each treatment group.

Immunohistochemistry

Rats were deeply anesthetized with pentobarbital and perfused transcardially with 0.01M PBS + 1% sodium nitrite followed by 4% paraformaldehyde. The lower lumbar spinal cord (L4-L6) was removed and post-fixed in 4% paraformaldehyde for 2-3 hours followed by cryoprotection in 30% sucrose. Tissue was embedded in Tissue-Tek O.C.T. compound and cut transversely on a cryostat at 40 μ M.

Immunohistochemistry was performed on free-floating sections according to standard biotin-streptavidin techniques. Tissue sections were washed with 0.01M PBS + 0.15% Triton-X 100 (PBS+T), incubated in 0.3% H₂O₂ to quench endogenous peroxidase activity, washed with PBS+T, and incubated in 50% ethanol. Non-specific binding was blocked with 1.5% normal goat serum followed by incubation with anti-prodynorphin (1:4000, Neuromics Antibodies, Northfield, MN) antibody overnight at 4°C. Sections were incubated with biotinylated goat anti-rabbit followed by incubation with streptavidin-conjugated horseradish peroxidase. Antibodies were visualized using the enhanced glucose-nickel-diaminobenzidine method [27].

Quantification of immunoreactivity

Six to eight spinal cord slices per animal (n = 6-8) per group were captured on a Leica Axioplan2 microscope equipped with a CCD camera. The number of immunoreactive pixels was measured in a fixed area of laminae I-II and laminae III-V using Sigma Scan (Jandel Scientific Inc., CA). In each fixed area, a fixed threshold at which all immunoreactive fibers and cells were masked with a digitized pixel overlay was applied across all spinal cord sections by an analyst blind to treatment group. Using this quantification method, sections containing more immunoreactive fibers or a greater number of immunoreactive cells resulted in an increased number of digitized pixels required to overlay all immunoreactive objects. Values are expressed as the mean number of pixels/area $\pm$ S.E.M. for each treatment group. This method of quantification has been used previously [19;20].

Dynorphin ELISA

The lower lumbar spinal cord (L4-L6) was removed by rapid dissection. Tissue samples were homogenized on ice in 50 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid, 1% Igepal CA-630 and with a protease inhibitor cocktail at a 1:100 dilution (Mammalian Cell Lysis Kit, Sigma, Saint Louis, MO). After homogenization, samples were incubated for 15 minutes on an orbital shaker at 4°C. The samples were centrifuged at 12,000

× g for 10 minutes, and the supernatant was taken for use in the immunoassay. Immunoassay was performed with an antibody specific for dynorphin A by the use of a commercial enzyme immunoassay kit (Peninsula Laboratories, San Carlos, CA).

Data analysis

Behavioral data was analyzed using one-way ANOVA or two-way repeated measures ANOVA where appropriate. Immunohistochemical data were analyzed using one-way ANOVA or Student's t test where appropriate.

Results

Chronic nicotine produces mechanical hypersensitivity

Consistent with our previously reported results, rats exposed to 3.6 mg/kg/day or 8.6 mg/kg/day nicotine showed a significant decrease in paw withdrawal thresholds (PWTs) to mechanical pressure across days [$F(2,53)=13.5$; $p < 0.001$] after osmotic minipump implantation (Figure 1). Nicotine-induced decrease in PWTs was dose-dependent on Day 5, but this dose-dependency was not apparent on Day 14.

Chronic nicotine increases spinal prodynorphin expression and dynorphin content

Exposure to chronic nicotine for 14 days significantly increased prodynorphin immunoreactivity in the outer (I-II) (Figure 2A) and inner (III-V) (Figure 2B) laminae of the dorsal horn of rat spinal cords compared to chronic saline-treated animals. In the outer laminae (I-II), no difference in prodynorphin immunoreactivity between 3.6 and 8.6 mg/kg/day chronic nicotine was observed. In the deeper laminae (III-IV), however, the increase in prodynorphin immunoreactivity was dose-dependent [$F(2,17)=54.5$; $p < 0.001$]. Examples of nicotine-induced increases in prodynorphin immunoreactivity in both the outer (I-II) and inner (III-V) laminae of the spinal cord dorsal horn are shown in Figure 3. The administration of chronic systemic nicotine (8.6 mg/kg/day) for 14 days significantly increased the content of dynorphin in the rat spinal cord (30.4 ± 0.7 pg/mg saline-treated vs. 36.3 ± 1.3 pg/mg nicotine-treated, $p < 0.05$).

Spinal dynorphin release contributes to nicotine-induced mechanical hypersensitivity

In order to determine whether chronic nicotine-induced spinal dynorphin release contributed to mechanical hypersensitivity, whole dynorphin antiserum was administered to rats treated with 8.6 mg/kg/day nicotine on day 14 post-osmotic pump implantation. The intrathecal administration of normal rabbit serum or of 10 μ l of undiluted anti-dynorphin antiserum did not alter paw withdrawal thresholds over 60 minutes. However, 20 μ l of undiluted anti-dynorphin antiserum significantly increased paw withdrawal thresholds by $14 \pm 5\%$ 60 minutes following intrathecal administration.

Discussion

Our results confirm previous findings from our laboratory that chronic nicotine induces mechanical hypersensitivity following 14 days of administration. We have extended these findings to show that 3.6 mg/kg/day and 8.6 mg/kg/day of chronic nicotine increased the expression of both prodynorphin immunoreactivity and dynorphin content in the lower lumbar spinal cord of the rat. Furthermore, the release of dynorphin in the rat spinal cord contributes to chronic nicotine-induced mechanical hypersensitivity.

As mentioned above, we have previously reported on chronic nicotine-induced mechanical hypersensitivity and our current results support our earlier findings [14]. In contrast, most previous studies have focused on the impact of chronic nicotine on thermal sensitivity. In these

studies, chronic nicotine produces an antinociceptive effect on tail flick and hot plate latencies, but not on thermal paw withdrawal latencies. [1;3;34]. In the hot plate test, tolerance to the antinociceptive effect of nicotine is observed within 1-2 weeks of chronic nicotine exposure [3;34]. Interestingly, cessation of nicotine administration did not produce hyperalgesia to thermal or mechanical stimuli [3].

Chronic nicotine-induced changes in mechanical sensitivity has been measured previously using von Frey filaments and has been reported to either decrease slightly [1] or to be unchanged [3]. However, our studies suggest that chronic nicotine exposure may increase the sensitivity to more robust mechanical stimuli (i.e., pressure) without the development of tolerance to this effect.

The two doses of nicotine used in our studies are similar to doses of nicotine that have been used previously to produce thermal antinociception [3] and to increase the expression of nAChRs in rat brain [25;31]. Our previous studies indicate that chronic administration of 8.6 mg/kg/day produces plasma nicotine and cotinine concentrations similar to those found in moderately heavy smokers [14]. Although lower doses of chronic nicotine have been shown to produce thermal antinociception [34], chronic administration of 1 mg/kg/day and 0.4 mg/kg/day nicotine failed to alter mechanical sensitivity to pressure (unpublished observations).

In humans, smoking elicits an increase in the threshold and tolerance to a variety of pain stimuli including cold pressor, thermal, and ischemic pain [6;11]. However, these reports refer to the acute effects of nicotine consumption with pain threshold and tolerance being measured within 30 minutes of smoking. The antinociceptive effect reported is compared to non-smokers [11] or smokers following the consumption of a “zero nicotine” cigarette. Although these are perfectly valid measurements, they are not necessarily comparable to the results obtained in the current studies. A clinical correlate to the current studies would involve a comparison between pain thresholds of non-smokers and minimally deprived smokers (i.e., no signs of withdrawal). A previous study by Fertig et al. (1986) provides data that show a difference in the sensitivity to cold between smokers and non-smokers. The mean pain threshold to cold pressor pain in smokers who consumed placebo snuff (0 nicotine) is reported to be 11.7 ± 3.4 seconds and pain tolerance is 35.0 ± 16.6 seconds. In ex-smokers (>1 year abstinence), cold pressor pain thresholds in the placebo snuff group were 28.8 ± 8.9 seconds and cold tolerance was 54.2 ± 11.0 seconds. This suggests that minimally deprived smokers have a marked *increased* sensitivity to cold compared to non-smokers. This difference in pain sensitivity cannot be attributed to nicotine withdrawal because no psychological or physical signs of withdrawal were observed [6].

In the present study, prodynorphin and dynorphin levels increased with exposure to chronic nicotine in the dorsal horn, with a significant dose dependent increase in laminae III-V. There has been little research performed on the relationship of nicotine exposure to dynorphin expression. However, repeated nicotine administration has no effect on preprodynorphin concentrations in many brain areas, and actually decreases preprodynorphin in the ventral shell of the nucleus accumbens [22]. In addition to the effects of chronic nicotine on spinal dynorphin expression, dynorphin itself acts a non-competitive, voltage independent antagonist of nicotine-evoked currents in PC12 cells with an IC_{50} of $0.4 \pm 0.03 \mu M$ [13].

Our results with chronic nicotine are similar to the effects of chronic opiate administration. Chronic opiate administration produces a paradoxical hyperalgesia with the development of tolerance to the analgesic effects of oxymorphone [10]. Previous results by our laboratory and others have shown that chronic nicotine administration also produces tolerance to the analgesic effects of nicotine [3;14]. Similar to the effects observed with chronic opioids, analgesic tolerance and hypersensitivity occur via separable mechanisms [14;15]. Opiate and nicotine-

induced analgesic tolerance can be blocked with the administration of non-selective opioid and nicotinic receptor antagonists [23]. However, the hyperalgesic effects of chronic opiate administration are not due to the activation of opiate receptors [15] and tolerance to the antinociceptive effects of subcutaneous nicotine can be blocked with the co-administration of an NMDA receptor antagonist [14].

The antinociceptive effects of intrathecal dynorphin antiserum are similar to what has been reported previously [32;36]. Intrathecal administration of 10 µl of dynorphin antiserum moderately alleviated mechanical allodynia in spinal nerve-ligated rats [36]. The current results failed to observe an effect of 10 µl of dynorphin antiserum using a higher intensity mechanical stimulus, paw pressure. A higher degree of antinociception was observed in mechanically allodynic chronic opiate-treated rats that were administered 200 µg of dynorphin antiserum intrathecally [32].

Taken together the current results demonstrate that chronic nicotine administration increases the sensitivity to mechanical stimuli, prodynorphin immunoreactivity, and dynorphin content within the rat spinal cord. Therefore, it appears that chronic nicotine-induced mechanical hypersensitivity occurs via a different mechanism than peripheral nerve injury and this may underlie the additive effects of nicotine and neuropathic pain observed previously.

Acknowledgments

The authors would like to thank Dr. Josephine Lai for her helpful comments and suggestions. This work was supported via NIH grant P01 NS41386.

Reference List

- [1]. Anderson KL, Pinkerton KE, Uyeminami D, Simons CT, Carstens MI, Carstens E. Antinociception induced by chronic exposure of rats to cigarette smoke. *Neuroscience Letters* 2004;366:86–91. [PubMed: 15265596]
- [2]. Anderson LE, Seybold VS. Phosphorylated cAMP response element binding protein increases in neurokinin-1 receptor-immunoreactive neurons in rat spinal cord in response to formalin-induced nociception. *Neurosci. Lett* 2000;283:29–32. [PubMed: 10729626]
- [3]. Carstens E, Anderson KA, Simons CT, Carstens MI, Jinks SL. Analgesia induced by chronic nicotine infusion in rats: differences by gender and pain test. *Psychopharmacology (Berl)* 2001;157:40–45. [PubMed: 11512041]
- [4]. Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA. cAMP Response Element-Binding Protein Is Essential for the Upregulation of Brain-Derived Neurotrophic Factor Transcription, But Not the Behavioral or Endocrine Responses to Antidepressant Drugs. *J. Neurosci* 2002;22:3262–3268. [PubMed: 11943827]
- [5]. Eliasson B. Cigarette smoking and diabetes. *Prog. Cardiovasc. Dis* 2003;45:405–413. [PubMed: 12704597]
- [6]. Fertig JB, Pomerleau OF, Sanders B. Nicotine-produced antinociception in minimally deprived smokers and ex-smokers. *Addict. Behav* 1986;11:239–248. [PubMed: 3739811]
- [7]. Flores CM, Davila-Garcia MI, Ulrich YM, Kellar KJ. Differential regulation of neuronal nicotinic receptor binding sites following chronic nicotine administration. *J. Neurochem* 1997;69:2216–2219. [PubMed: 9349569]
- [8]. Freeland K, Liu YZ, Latchman DS. Distinct signalling pathways mediate the cAMP response element (CRE)-dependent activation of the calcitonin gene-related peptide gene promoter by cAMP and nerve growth factor. *Biochem. J* 2000;345(Pt 2):233–238. [PubMed: 10620499]
- [9]. Gardell LR, Ibrahim M, Wang R, Wang Z, Ossipov MH, Malan TP Jr, Porreca F, Lai J. Mouse strains that lack spinal dynorphin upregulation after peripheral nerve injury do not develop neuropathic pain. *Neuroscience* 2004;123:43–52. [PubMed: 14667440]

- [10]. Gardell LR, King T, OSSIPOV MH, Rice KC, Lai J, Vanderah TW, Porreca F. Opioid receptor-mediated hyperalgesia and antinociceptive tolerance induced by sustained opiate delivery. *Neuroscience Letters* 2006;396:44–49. [PubMed: 16343768]
- [11]. Girdler SS, Maixner W, Naftel HA, Stewart PW, Moretz RL, Light KC. Cigarette smoking, stress-induced analgesia and pain perception in men and women. *Pain* 2005;114:372–385. [PubMed: 15777863]
- [12]. Goldberg MS, Scott SC, Mayo NE. A review of the association between cigarette smoking and the development of nonspecific back pain and related outcomes. *Spine* 2000;25:995–1014. [PubMed: 10767814]
- [13]. Itoh H, Andoh T, Watanabe I, Sasaki T, Kamiya Y, Okumura F. Dynorphins directly inhibit neuronal nicotinic acetylcholine receptors in PC12 cells. *Eur J Neurosci* 2000;12:1253–1262. [PubMed: 10762354]
- [14]. Josiah DT, Vincler MA. Impact of chronic nicotine on the development and maintenance of neuropathic hypersensitivity in the rat. *Psychopharmacology (Berl)* 2006;188:152–161. [PubMed: 16915382]
- [15]. Juni A, Klein G, Kest B. Morphine hyperalgesia in mice is unrelated to opioid activity, analgesia, or tolerance: Evidence for multiple diverse hyperalgesic systems. *Brain Research* 2006;1070:35–44. [PubMed: 16409995]
- [16]. Kawasaki Y, Kohno T, Zhuang ZY, Brenner GJ, Wang H, Van Der Meer C, Befort K, Woolf CJ, Ji RR. Ionotropic and Metabotropic Receptors, Protein Kinase A, Protein Kinase C, and Src Contribute to C-Fiber-Induced ERK Activation and cAMP Response Element-Binding Protein Phosphorylation in Dorsal Horn Neurons, Leading to Central Sensitization. *J. Neurosci* 2004;24:8310–8321. [PubMed: 15385614]
- [17]. Lai J, Luo MC, Chen Q, Ma S, Gardell LR, Ossipov MH, Porreca F. Dynorphin A activates bradykinin receptors to maintain neuropathic pain. *Nat Neurosci* 2006;9:1534–1540. [PubMed: 17115041]
- [18]. Lai J, OSSIPOV MH, Vanderah TW, MALAN TP Jr. Porreca F. Neuropathic Pain: The Paradox of Dynorphin. *Mol. Interv* 2001;1:160–167. [PubMed: 14993349]
- [19]. Ma W, Eisenach JC. Cyclooxygenase 2 in infiltrating inflammatory cells in injured nerve is universally up-regulated following various types of peripheral nerve injury. *Neuroscience* 2003;121:691–704. [PubMed: 14568029]
- [20]. Ma W, Eisenach JC. Intraplantar injection of a cyclooxygenase inhibitor ketorolac reduces immunoreactivities of substance p, calcitonin gene-related peptide, and dynorphin in the dorsal horn of rats with nerve injury or inflammation. *Neuroscience* 2003;121:681–690. [PubMed: 14568028]
- [21]. Ma W, Hatzis C, Eisenach JC. Intrathecal injection of cAMP response element binding protein (CREB) antisense oligonucleotide attenuates tactile allodynia caused by partial sciatic nerve ligation. *Brain Res* 2003;988:97–104. [PubMed: 14519530]
- [22]. Mathieu-Kia AM, Besson MJ. Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. *Molecular Brain Research* 1998;54:141–151. [PubMed: 9526067]
- [23]. McCallum SE, Caggiula AR, Epstein LH, Saylor S, Ploskina T, Sved AF. Mecamylamine blocks the development of tolerance to nicotine in rats: implications for the mechanisms of tolerance. *Psychopharmacology (Berl)* 1999;141:332–338. [PubMed: 10027516]
- [24]. Milligan ED, Hinde JL, Mehmert KK, Maier SF, Watkins LR. A method for increasing the viability of the external portion of lumbar catheters placed in the spinal subarachnoid space of rats. *Journal of Neuroscience Methods* 1999;90:81–86. [PubMed: 10517276]
- [25]. Nguyen HN, Rasmussen BA, Perry DC. Subtype-selective up-regulation by chronic nicotine of high-affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. *J. Pharmacol. Exp. Ther* 2003;307:1090–1097. [PubMed: 14560040]
- [26]. Shanti BF, Tan G, Shanti IF. Smoking and Low Back Pain. *Practical Pain Management* 2006;6:52–56.
- [27]. Shu SY, Ju G, Fan LZ. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci Lett* 1988;85:169–171. [PubMed: 3374833]

- [28]. Storkson RV, Kjorsvik A, Tjolsen A, Hole K. Lumbar catheterization of the spinal subarachnoid space in the rat. *J. Neurosci. Methods* 1996;65:167–172. [PubMed: 8740594]
- [29]. Taiwo YO,Coderre TJ, Levine JD. The contribution of training to sensitivity in the nociceptive paw- withdrawal test. *Brain Res* 1989;487:148–151. [PubMed: 2752281]
- [30]. Thomas E, Silman AJ, Croft PR, Papageorgiou AC, Jayson MI, Macfarlane GJ. Predicting who develops chronic low back pain in primary care: a prospective study. *BMJ* 1999;318:1662–1667. [PubMed: 10373170]
- [31]. Ulrich YM, Hargreaves KM, Flores CM. A comparison of multiple injections versus continuous infusion of nicotine for producing up-regulation of neuron [3H]-epibatidine binding sites. *Neuropharmacology* 1997;36:1119–1125. [PubMed: 9294978]
- [32]. Vanderah TW, Gardell LR, Burgess SE, Ibrahim M, Dogrul A, Zhong CM, Zhang ET, MALAN TP Jr. OSSIPOV MH, Lai J, Porreca F. Dynorphin Promotes Abnormal Pain and Spinal Opioid Antinociceptive Tolerance. *J. Neurosci* 2000;20:7074–7079. [PubMed: 10995854]
- [33]. Vera-Portocarrero LP, Zhang ET, King T, OSSIPOV MH, Vanderah TW, Lai J, Porreca F. Spinal NK-1 receptor expressing neurons mediate opioid-induced hyperalgesia and antinociceptive tolerance via activation of descending pathways. *Pain*. In Press, Corrected Proof
- [34]. Wewers ME, Dhatt RK, Snively TA, Tejawani GA. The effect of chronic administration of nicotine on antinociception, opioid receptor binding and met-enkephalin levels in rats. *Brain Res* 1999;822:107–113. [PubMed: 10082888]
- [35]. Yunus MB, Arslan S, Aldag JC. Relationship between fibromyalgia features and smoking. *Scand. J Rheumatol* 2002;31:301–305. [PubMed: 12455822]
- [36]. Zhu X, Vincler MA, Parker R, Eisenach JC. Spinal cord dynorphin expression increases, but does not drive microglial prostaglandin production or mechanical hypersensitivity after incisional surgery in rats. *Pain* 2006;125:43–52. [PubMed: 16740361]

Figure 1.

Mechanical paw withdrawal thresholds (PWTs) in rats treated with chronic saline or nicotine.

The mean PWTs to mechanical pressure in grams (g) $\pm$ S.E.M. are shown prior to osmotic pump implantation (Baseline) and 5 and 14 days following pump implantation (n = 6 - 8). * p < 0.05 compared to Saline and Baseline; ^ p < 0.05 compared to Saline.

Figure 2.

Comparison of prodynorphin immunoreactivity in the dorsal horn of the spinal cord in animals treated with 3.6 or 8.6 mg/kg/day nicotine. The mean number of immunoreactive pixels/area $\pm$ S.E.M. is shown for the outer (A) and inner (B) lamina of the spinal dorsal horn. Both doses showed a significant increase in staining for prodynorphin compared to saline-treated animals.

* $p < 0.05$ compared to saline; ** $p < 0.05$ compared to 3.6 mg/kg/day nicotine.

Figure 3.

Comparison of prodynorphin immunoreactivity in the dorsal horn of the spinal cord in animals treated with 8.6 mg/kg/day nicotine or saline. The images illustrate prodynorphin immunoreactivity in the outer laminae (I-II) of chronic saline (A) and chronic nicotine (C) rats and in the inner laminae (III-V) of chronic saline (B) and chronic nicotine (D) rats.