

Differential electrophysiological effects of brain-derived neurotrophic factor on dorsal horn neurons following chronic spinal cord hemisection injury in the rat

Bryan C. Hains^{a,b}, William D. Willis^a, Claire E. Hulsebosch^{a,*}

^aDepartment of Anatomy and Neurosciences, and Marine Biomedical Institute, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-1043, USA

^bDepartment of Neurology, and PVA-EPVA Center for Neuroscience and Regeneration Research, Yale University School of Medicine, West Haven, CT 06516, USA

Received 24 October 2001; received in revised form 19 December 2001; accepted 20 December 2001

Abstract

To assess the role of brain-derived neurotrophic factor (BDNF) in nociceptive processing after chronic lateral spinal cord hemisection injury (SCI) at T13, we studied the effects of BDNF on evoked activity of dorsal horn wide dynamic range (WDR) neurons. Evoked responses of WDR cells ($n = 34$ total) at L3–L5 were characterized electrophysiologically after spinal administration of vehicle, or BDNF (10 μ g). In hemisected animals, application of BDNF to the surface of the cord resulted in reductions in evoked activity in 24 of 32 cells (75%), and enhancement of evoked activity in eight of 32 (25%) cells. Phosphate-buffered saline-receiving animals demonstrated evoked response rates of between 75 and 93 Hz, while BDNF(–) cells had evoked rates from between 20 and 41 Hz, and BDNF(+) activities were between 80 and 119 Hz, significant changes of 76 and 124%, respectively. Effects were bilateral and differences in sidedness were not observed. These results further implicate BDNF in nociceptive processing, but suggest a complex role after chronic SCI. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Brain-derived neurotrophic factor; Wide dynamic range; Chronic central pain; Spinal cord injury

In previous studies, we and others have demonstrated that following lateral spinal cord hemisection injury (SCI) at T13, rats develop behavioral signs consistent with chronic central pain [2] as well as increased responsiveness of wide dynamic range (WDR) dorsal horn projection neurons to innocuous and noxious peripheral stimuli [5,17]. This hyperexcitability is often referred to as central sensitization and may be demonstrated electrophysiologically by changes in background activity (BK), increased responsiveness to peripheral stimulation, and acute changes in receptive field size and the presence of afterdischarges [21]. Following such an injury, transplantation of cells that secrete brain-derived neurotrophic factor (BDNF) can attenuate nociceptive behaviors [6] and cellular hyperexcitability [7] to peripheral stimuli.

Neurotrophic factors such as nerve growth factor, BDNF, and neurotrophin-3 play a significant role in the develop-

ment and maintenance of sensory neurons responsive to temperature and tactile pain [10,20]. However, the precise role of BDNF in modulation of pain-related behaviors is uncertain.

Although some studies demonstrate that BDNF is pronociceptive [9,12], other studies indicate that BDNF is antinociceptive [3,14,18]. The present study tests the direct effects of BDNF on WDR cells in the dorsal horn in response to peripheral stimulation after spinal cord injury. We use a chronic T13 spinal hemisection preparation to demonstrate the effects of BDNF on modulation of nociceptive processing after such injury when pain-like behaviors are evident. Extracellular single-unit recordings were made in the dorsal horn at L3–L5 to characterize the electrophysiological activity of WDR neurons following topical BDNF application. In particular, we examined background and activity evoked by a variety of innocuous and noxious peripheral stimuli (brush, press, pinch, graded von Frey filaments, 47 °C, 32 °C) under these conditions.

Male Sprague–Dawley rats ($n = 10$), 100–125 g, were anesthetized with sodium pentobarbital (40 mg/kg i.p.)

* Corresponding author. Tel.: +1-409-772-5193; fax: +1-409-772-3222.

E-mail address: cehulseb@utmb.edu (C.E. Hulsebosch).

and the T13 spinal cord segment was hemisected using a #11 scalpel blade without damage to the posterior vessel or its branches. Postoperative treatments included saline (2.0 cc s.c.) and penicillin-G (0.35 ml/kg i.m.).

Electrophysiological recordings were made from animals 28–35 days following hemisection when rats exhibited mechanical allodynia and thermal hyperalgesia bilaterally (data not shown). The activity from 44 cells was recorded in both vehicle (six cells/side of cord) and BDNF-treated animals (16 cells/side of cord). Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and supplemented (5 mg/kg per h) through a jugular vein catheter. Animals were fixed in a stereotaxic frame and the spinal cord was exposed from T12–L6 and covered with warm mineral oil.

WDR cells were classified by their responses to brush, pressure and pinch. Cells were isolated in the L3–L5 segments medial to the dorsal root entry zone up to a depth of 1000 μm . Extracellular single-unit recordings were made with a low-impedance insulated tungsten (12 M Ω) microelectrode (A-M Systems, Carlsborg, WA). Once a cell was identified, BK was measured followed by cutaneous receptive field mapping. Mechanical stimuli were applied to the lateral and ventral surfaces of the hindpaw: (1), brushing (BR) the skin with a cotton brush; (2), pressure (PR), by attaching a large arterial clip with a weak grip to a fold of the skin (144 g/mm²); and (3), pinching (PI), by applying a small arterial clip with a strong grip to a fold of skin (583 g/mm²). Next, various strength von Frey filaments (3.84, 9.96 and 204.1 mN) and 47, 22 and 7 °C thermal stimuli consisting of a heated, ambient temperature or cooled steel probe (surface area 1 cm²) were applied. Stimuli were applied serially for 20 s, separated by 20 s of baseline activity. Electrical signals were amplified and fed into a window discriminator, displayed on analog and digital storage oscilloscopes, processed by a data collection system (CED 1401 + ; Cambridge Instruments, Cambridge, UK; 486 PC, Dell, Austin, TX), and stored on a computer. Records were analyzed off-line with Spike 2 software (v3.13, Cambridge Electronic Design, Cambridge, UK).

Recombinant human BDNF (10 μg ; Chemicon, Temecula, CA) was dissolved in 2.0 μl sterile 0.9% phosphate-buffered saline, pH 7.4–7.6 (PBS). Drug delivery was performed by soaking solutions onto Kimwipes pledgets (2 \times 2 mm) which were placed on the dorsal aspect of the cord. Recordings began 5 min following drug application. PBS vehicle control pledgets were applied in the same fashion before or after washout to confirm the return of the pre-drug response. At least 90 min elapsed between each application to allow for washout, after which time the cell's activity returned to pre-drug levels.

Statistical tests were evaluated at the alpha level significance of 0.05, by two-tailed analyses using parametric tests. Significance of the effects of BDNF was determined using the paired Student's *t*-test or two-sample Student's *t*-test.

Sampled cells were distributed in both superficial and deep dorsal horn laminae I–V up to a depth of 1000 μm .

The mediolateral distribution of recorded units extended from the dorsal median septum to the edge of the dorso-lateral funiculus, and the units were concentrated medially in the spinal cord at L3–L5, a region receiving a major input from the plantar (lateral–ventral) aspect of the hindpaw. Cell phenotypes were not restricted to particular depths; mean depths were 645 and 722 μm for cells inhibited by BDNF, termed BDNF(–), and cells excited by BDNF, termed BDNF(+). Cells of either phenotype were not related to either ipsilateral or contralateral sides of the cord. On the ipsilateral side, three of 16 sampled cells were excited by BDNF, and on the contralateral side, five of 16 were excited by BDNF. Thirteen of 16 sampled cells were inhibited BDNF on the ipsilateral side, and on the contralateral side, 11 of 16 were inhibited by BDNF.

Application of BDNF to the dorsal surface of the spinal cord resulted in rapid alterations in the evoked responses of lumbar dorsal horn WDR neurons. Discharge activity to peripheral stimulation changed within 2–5 min of drug application, and this decrease persisted for at least 30 min. Maximum responses were recorded from between 5 and 25 min. Following this period of drug action, pledgets containing drug were removed and responses returned to pre-drug levels after washout, which typically took 45 min.

Representative peristimulus time histograms (spikes/1 s bin) are shown in Fig. 1 for neurons sampled from intact and chronically-hemisected animals. When compared with uninjured controls, WDR neurons in hemisected animals

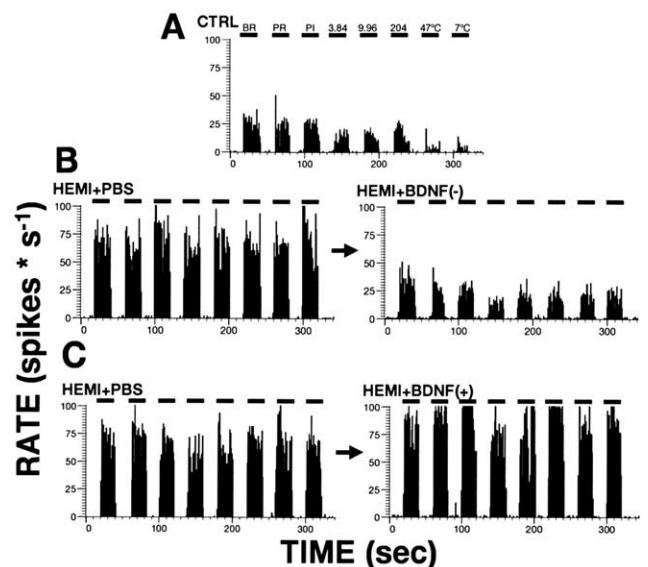


Fig. 1. Representative peristimulus time histograms (spikes/1 s bin) of WDR cells from intact controls and on the ipsilateral side in chronically-hemisected animals to innocuous and noxious mechanical and thermal stimuli (brush, press, pinch, 3.84, 9.96, and 204.1 mN calibrated von Frey filaments, 47 °C, 7 °C) recorded from L3–L5 after cord surface application of PBS vehicle (A) or 10 μg BDNF (B,C). Stimuli were applied for 20 s which was followed by 20 s of ongoing BK.

show increased evoked activity to all peripheral stimuli following vehicle delivery, irrespective of sidedness of the lateral hemisection injury. Evoked activity of WDR cells was increased on both ipsilateral and contralateral sides of the cord, and of the same magnitude.

Evoked activity of representative cells following BDNF application is shown in Fig. 1. In hemisected animals, application of BDNF (10 μ g) to the surface of the cord resulted in reductions in evoked activity in 24 of 32 cells (75%), and enhancement of evoked activity in eight of 32 (25%) cells tested. No changes in BK were evident in either BDNF(+) or BDNF(-) cells. Responsiveness to all types of peripheral stimuli was reduced or enhanced consistently, but in BDNF(-) cells, the percentage of change was greater. Following this period of drug action, pledgets were removed and rates decreased to pre-drug levels after washout by 60 min.

BDNF(+) and BDNF(-) activities were significantly

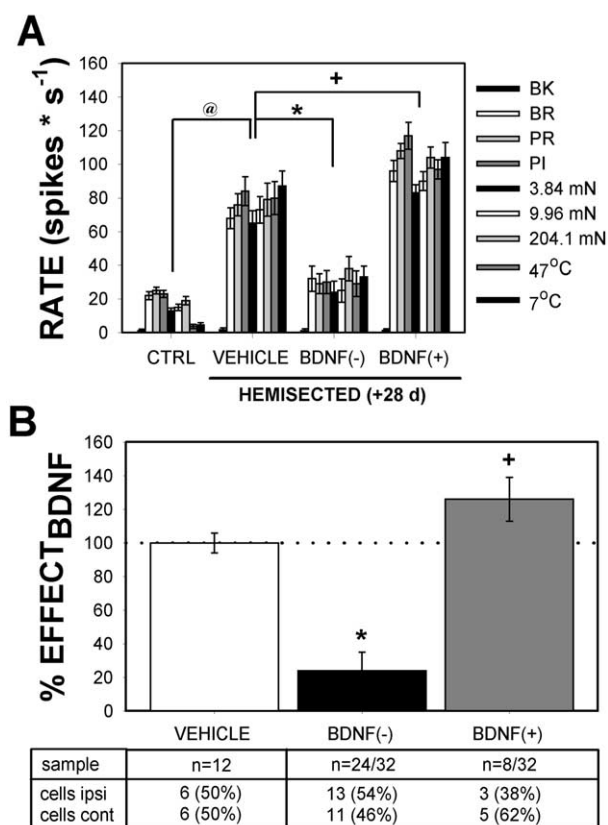


Fig. 2. Quantification of discharge rates (mean \pm SD spikes/1 s bin) of WDR cells recorded in hemisected animals after receiving surface application of vehicle ($n = 12$ cells) or 10 μ g BDNF ($n = 32$ cells) to brush, press, pinch, 3.84, 9.96 and 204.1 mN calibrated von Frey filaments, 47 and 32 $^{\circ}$ C stimulation (A). Surface application of BDNF resulted in significant ($*P < 0.05$) decreases of up to 76% in evoked activity to all peripheral stimuli in 24/32 sampled cells (BDNF(-)), however, BDNF application produced significantly ($+P < 0.05$) increased activity in 8/32 cells sampled, up to 124% of control (BDNF(+)). (B). Number of cells in each response group and ipsilateral/contralateral distribution are also shown.

($P < 0.05$) different from those of vehicle-treated animals and represent 76 and 126% of controls, respectively for BDNF(-) and BDNF(+) cells (Fig. 2). In no case did we observe any significant difference in background or after-discharge activity, or in regularity of spontaneous interspike intervals.

In this study, we show a surprising bilateral increase in responsiveness to natural peripheral stimuli of WDR cells after chronic SCI [5] and differential modulation by topically applied BDNF. Properties of the WDR neurons, such as their graded sensitivity to mechanical and thermal stimuli, capability of development of sustained hyperexcitability, referred to as central sensitization [21], and the similarity in responses to stimulation and pain sensation in humans [11], suggests their importance in nociceptive information processing.

BDNF is expressed by primary sensory neurons, and it colocalizes with calcitonin gene-related peptide-containing arborizations of afferent nociceptive C-fiber central terminals in the superficial dorsal horn [22]. In this configuration, it could be released by activation of nociceptive afferents [4] to modulate responses to sensory stimuli. The role of BDNF as antinociceptive or pronociceptive is controversial. Several reports suggest that BDNF may be pronociceptive. In a peripheral inflammation model, endogenous BDNF increases *N*-methyl-D-aspartate receptor-mediated excitability [9] and BDNF sequestration blocks behavioral sensitivity to tactile stimuli [12]. After nerve injury, BDNF is able to reduce GABA_A-mediated conductances in cutaneous afferent dorsal root ganglion cells [16]. An antinociceptive function for BDNF has been proposed, supported by evidence that midbrain [18] and intracerebroventricular [3] infusion increases hotplate withdrawal latency, and chronic systemic delivery attenuates mechanical allodynia after chronic nerve constriction injury [14]. It may also be the case that the differential effects of BDNF are dose-dependant [14] and as such, contradictory results from various studies need to be interpreted carefully. Here we show for the first time inhibition and excitation of spinal nociceptive neurons by BDNF.

It will be important to determine the mechanistic basis of the differential responses to BDNF. Since chronic spinal hemisection results in interruption of ipsilateral descending modulatory pathways, some of which are serotonergic [8], and in supersensitivity to serotonin (5-HT) in dorsal horn neurons [5], it is possible that BDNF may act through a serotonergic mechanism as suggested by others [1]. Mechanisms include increased BDNF synthesis and release of 5-HT from serotonergic neurons [13], as well as reduction of 5-HT transporter function (uptake) by BDNF [15]. Additionally, BDNF has been proposed to modulate 5-HT levels through increasing steady state tryptophan hydroxylase mRNA levels [19].

In summary, the electrophysiological evidence presented here demonstrates a differential modulation of hyperexcitability after chronic SCI by BDNF. These data explain, in

part, the differing roles of BDNF in acute and chronic pain paradigms from a variety of laboratories, and differences in response within a single paradigm. Clearly, more work is needed to elucidate further the exact nature of BDNF in nociceptive processing.

The authors wish to thank Ms Debbie Pavlu for her secretarial assistance. Work supported by the Spinal Cord Research Foundation of the Paralyzed Veterans of America, the Kent Waldrep National Paralysis Foundation, Mission Connect of TIRR-Houston, NIH NS11255 and NS39161.

- [1] Celada, P., Siuciak, J.A., Tran, T.M., Altar, C.A. and Tepper, J.M., Local infusion of brain-derived neurotrophic factor modifies the firing pattern of dorsal raphe serotonergic neurons, *Brain Res.*, 712 (1996) 293–298.
- [2] Christensen, M.D. and Hulsebosch, C.E., Chronic central pain after spinal cord injury, *J. Neurotrauma*, 14 (1997) 517–537.
- [3] Cirulli, F., Berry, A. and Alleva, E., Intracerebroventricular administration of brain-derived neurotrophic factor in adult rats affects analgesia and spontaneous behavior but not memory retention in a Morris Water Maze task, *Neurosci. Lett.*, 287 (2000) 207–210.
- [4] Griesbeck, O., Canossa, M., Campana, G., Gartner, A., Hoener, M.C., Nawa, H., Kolbeck, R. and Thoenen, H., Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity, *Microsc. Res. Tech.*, 45 (1999) 262–275.
- [5] Hains, B.C., Johnson, K.M., McAdoo, D.J., Eaton, M.J. and Hulsebosch, C.E., Serotonin agents modulate hyperexcitability of wide dynamic range dorsal horn neurons after chronic spinal cord hemisection injury in rat, *J. Neurophys.* (2001) in press.
- [6] Hains, B.C., Fullwood, S.D., Eaton, M.J. and Hulsebosch, C.E., Engraftment of immortalized serotonergic neurons enhances locomotor function and attenuates chronic central pain following spinal hemisection injury in the rat, *Exp. Neurol.*, 171 (2001) 361–378.
- [7] Hains, B.C., Johnson, K.A., Eaton, M.J., Willis, W.D. and Hulsebosch, C.E., Engraftment of serotonergic neural precursors amends hyperexcitability and phenotype shifts of dorsal horn neurons after spinal hemisection in rat, *J. Neurosci.*, (2001) in press.
- [8] Hains, B.C., Everhart, A.W. and Hulsebosch, C.E., Serotonin and serotonin transporter mediated behavioral deficits after spinal hemisection are modulated by intrathecal 5-HT, antagonists, and reuptake inhibitors, *Exp. Neurol.*, (2001) in press.
- [9] Kerr, B.J., Bradbury, E.J., Bennett, D.L.H., Trivedi, P.M., Dassan, P., French, J., Shelton, D.B., McMahon, S.B. and Thompson, W.E.N., Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord, *J. Neurosci.*, 19 (1999) 6138–6148.
- [10] Klein, R., Role of neurotrophins in mouse neuronal development, *FASEB J.*, 8 (1994) 738–744.
- [11] Loeser, J.D., Ward, A.A. and White, L.E., Chronic deafferentation of human spinal cord neurons, *J. Neurosurg.*, 29 (1968) 48–50.
- [12] Mannion, R.J., Costigan, M., Decosterd, I., Amaya, F., Ma, Q.P., Holstege, J.C., Ji, R.R., Acheson, A., Lindsay, R.M., Wilkinson, G.A. and Woolf, C.J., Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity, *Proc. Natl. Acad. Sci. USA*, 96 (1999) 9385–9390.
- [13] Martin-Iverson, M.T., Todd, K.G. and Altar, C.A., Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine, *J. Neurosci.*, 14 (1994) 1262–1270.
- [14] Miki, K., Fukuoka, T., Tokunaga, A., Kondo, E., Dai, Y. and Noguchi, K., Differential effect of brain-derived neurotrophic factor on high-threshold mechanosensitivity in a rat neuropathic pain model, *Neurosci. Lett.*, 278 (2000) 85–88.
- [15] Mossner, R., Daniel, S., Albert, D., Heils, A., Okladnova, O., Schmitt, A. and Lesch, K.P., Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF), *Neurochem. Int.*, 36 (2000) 197–202.
- [16] Oyelese, A.A., Rizzo, M.A., Waxman, S.G. and Kocsis, J.D., Differential effects of NGF and BDNF on axotomy-induced changes in GABA(A)-receptor-mediated conductance and sodium currents in cutaneous afferent neurons, *J. Neurophysiol.*, 78 (1997) 31–42.
- [17] Paik, K.S., Gwak, Y.S., Yeon, D.S., Leem, J.W. and Nam, T.S., Modulation of response properties of spinal dorsal horn neurons following spinal cord injury in rat, *Soc. Neurosci. Abstr.*, 26 (2000) 1690.
- [18] Siuciak, J.A., Wong, V., Pearsall, D., Wiegand, S.J. and Lindsay, R.M., BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception, *Eur. J. Neurosci.*, 7 (1995) 663–670.
- [19] Siuciak, J.A., Clark, M.S., Rind, H.B., Whittemore, S.R. and Russo, A.F., BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain, *J. Neurosci. Res.*, 52 (1998) 149–158.
- [20] Thompson, S.W., Bennett, D.L., Kerr, B.J., Bradbury, E.J. and McMahon, S.B., Brain-derived neurotrophic factor is an endogenous modulator of nociceptive responses in the spinal cord, *Proc. Natl. Acad. Sci. USA*, 96 (1999) 7714–7718.
- [21] Willis, W.D., Central sensitization and plasticity following intense noxious stimulation, *Basic and Clinical Aspects of Chronic Abdominal Pain*, Elsevier, Amsterdam, 1993, pp. 201–217.
- [22] Zhou, X.F. and Rush, R.A., Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons, *Neuroscience*, 74 (1996) 945–951.