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Glial overexpression of NGF enhances neuropathic pain and adrenergic sprouting into DRG following chronic sciatic constriction in mice

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Abstract

Adrenergic sprouts within axotomized dorsal root ganglia (DRG) may contribute to neuropathic pain, and may arise under the influence of nerve growth factor (NGF). We investigated effects of chronic constriction injury (CCI) on behavior and sprouting in mice in which NGF overexpression is driven by a glial protein (GFAP) promotor. GFAP-NGF mice were naturally hyperresponsive to radiant heat, and had enhanced ipsilateral responses to thermal and mechanical stimulation following CCI compared to wild-type mice. Sympathetic axons were already present in intact DRG of GFAP-NGF mice. Following CCI, sprouting in ipsilateral and to a lesser extent contralateral DRG occurred in both genotypes, but the sprout density 2 weeks post-lesion was much greater in GFAP-NGF mice. These results demonstrate a connection between the endogenous ectopic overexpression of NGF and (1) neuropathic pain behaviour and (2) sympathetic sprouting in the DRG.

Keywords: Nerve growth factor; Allodynia; Glial protein; Neuropathic pain; Sympathetic sprouting; Chronic constriction injury; Mice; Dorsal root ganglia

Nerve growth factor (NGF) is the prototypical member of a family of target-derived neurotrophic molecules (for a review, see [7]). NGF's best known peripheral effects are its abilities to promote survival and neurite outgrowth in sympathetic and sensory neurons, and to modulate gene expression in these and other neuronal populations expressing the NGF-specific tyrosine kinase receptor trkA.

NGF's role in peripheral neuropathic pain has been difficult to define, as it has been proposed to have both alleviating [10] and exacerbating [5] effects in rodent models. Painful chronic sciatic constriction injury (CCI, [1]) leads to invasion of the dorsal root ganglion (DRG) by adrenergic axons, with formation of sympathetic 'baskets' around some DRG neurons [8,9]. CCI-induced sprouting may rely on retrogradely-transported NGF from the degenerating nerve stump via spared axons [8,9]. Support for this hypothesis comes from mice overexpressing NGF in the skin [2], which are not only hypersensitive to peripheral noxious stimulation, but also contain plexuses of sympathetic axons within their trigeminal ganglia.

Here we use a transgenic mouse line in which the expression of NGF is driven by the glial fibrillary acidic protein (GFAP) promotor (GFAP-NGF, [6]). GFAP expression is low in the intact adult central nervous system (CNS), but increases after nerve injury in satellite cells of the DRG [12], and in astrocytes of the spinal cord dorsal horn [4], and thus these mice offer an excellent opportunity to investigate the effects of nerve injury-induced NGF overexpression. Abnormal sympathetic growth into the CNS and trigeminal ganglia has been described previously in intact adult GFAP-NGF mice [6,11].

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These experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care, as enforced by Queen's University. Thirty-two mice were used (16 each of GFAP-NGF and of the C57Bl/6J parental strain, all male). Four animals of each genotype were left unoperated. The remainder received a CCI, performed as previously described [8,9]: in methoxyflurane-anesthetized animals, three 5–0 chromic gut sutures were tied loosely around the sciatic nerve high in the left thigh. Nerve-injured mice remained well-groomed and gained weight normally. At the end of each experiment, mice were re-anesthetized deeply with an overdose of sodium pentobarbital, and perfused through the heart with ice-cooled phosphate-buffered saline (PBS).

The behavioural tests used here have been described elsewhere in detail [8,9]. Thermal allodynia was defined as a reduction in pain threshold to radiant heat, and was characterized by a reduced latency to withdrawal of the injured hindlimb compared to pre-operative measurements. Withdrawal latency to radiant heat (generated by a lamp shone on the sciatic territory of the plantar hindpaw) was measured three times alternately on each hindpaw and an average difference score (uninjured side minus injured side) was calculated for each mouse. Mechanoallodynia (a reduction in pain threshold to mechanical stimulation) was assessed using a calibrated set of von Frey filaments (0.5 g, 0.9 g, 1.4 g, 2.2 g, 3.1 g), which were applied in order of ascending stiffness until a nocifensive withdrawal was elicited. Difference scores were calculated as for thermal allodynia measurements. Behavioral testing was carried out 1 day preoperatively, and at 4, 7 and 14 days post-operatively. Allodynia in each strain was verified using a repeated measures analysis of variance (ANOVA), and comparisons between strains were made using a one-way ANOVA.

At the end of each testing period (0, 4, 7, and 14 days), ipsi and contralateral L5 DRG were removed and immediately frozen. Cryosections (16 μm thick) were dipped five times (1 second/dip) in SPG solution (10.2 g sucrose, 4.8 g sodium phosphate monobasic, 1.5 g glyoxylic acid in 150 ml H_2O, pH 7.5), dried under cool air blowers and baked for 2.5 min at 95–100°C. Images of DRG sections (5/DRG) were digitally captured (DIC-U camera; World Precision Instruments), and sprout density was defined as the fraction of area occupied by blue-green fluorescent fibers, as measured with Mocha Image analysis software (Jandel Scientific).

Preoperatively, there were no significant differences in mechanical responsiveness between GFAP-NGF mice and wild type controls (Fig. 1A). However, GFAP-NGF mice had a significantly reduced withdrawal latency from radiant heat compared to the control genotype, suggesting that excess endogenous NGF enhances thermal nociception in the nerve-intact state. Following CCI, both genotypes developed significant thermal allodynia (Fig. 1B), but withdrawal latency difference scores were significantly greater in the transgenics at 7 and 14 days PO. CCI also induced mechanical allodynia in both groups of mice (Fig. 1C), but again, the magnitude of the difference score was greater in GFAP-NGF mice, at 4, 7 and 14 days PO. No contralateral mechanical or thermal allodynia was observed. These results suggest that excess endogenous NGF enhances both mechanical and thermal allodynia induced by CCI.

In uninjured wild-type mice there were few fluorescent (sympathetic) axons in the DRG (Fig. 2A). This is in contrast to GFAP-NGF ganglia, which contained many sympathetic axons and baskets (Fig. 2B) like those previously

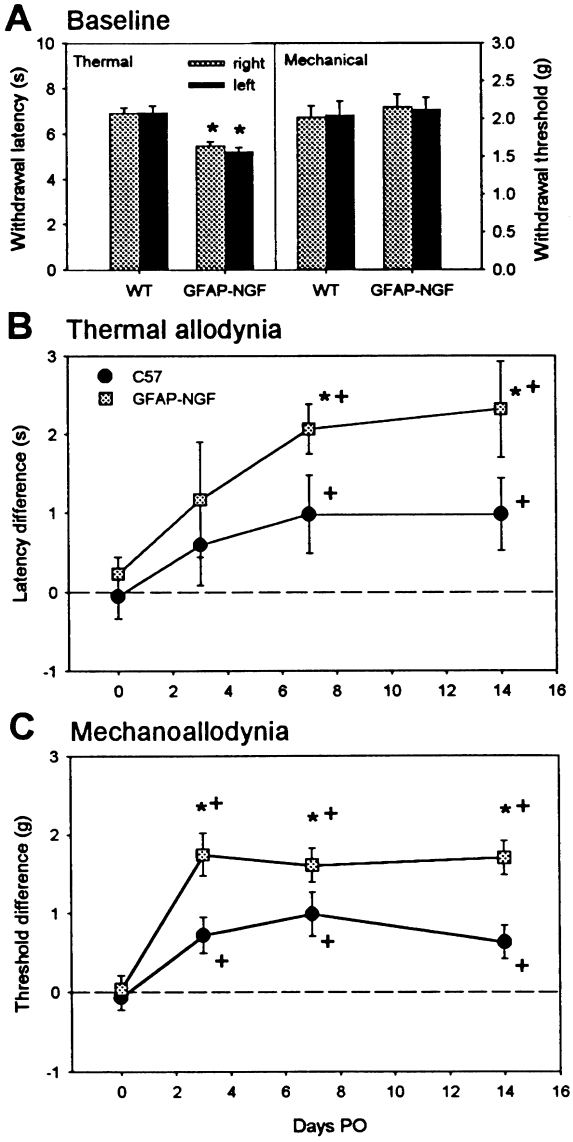

Fig. 1. (A) Baseline sensitivity to thermal and mechanical stimulation in wild-type (WT) and GFAP-NGF transgenic mice. GFAP-NGF mice have significantly reduced withdrawal latencies to radiant heat (*t*-test), but otherwise resemble WT mice. (B,C) Responses to thermal (B) and mechanical (C) stimulation following CCI. Values shown are difference scores (intact minus injured sides). GFAP-NGF mice are significantly more sensitive to both stimulus modalities following CCI compared to WT mice. Asterisks indicate significant differences between strains (one-way ANOVA, $P < 0.05$). Plus (+) signs indicate significant allodynia (ANOVA with repeated measures). At day 0, $n = 16$; day 2, $n = 12$; day 7, $n = 8$; day 14, $n = 4$.

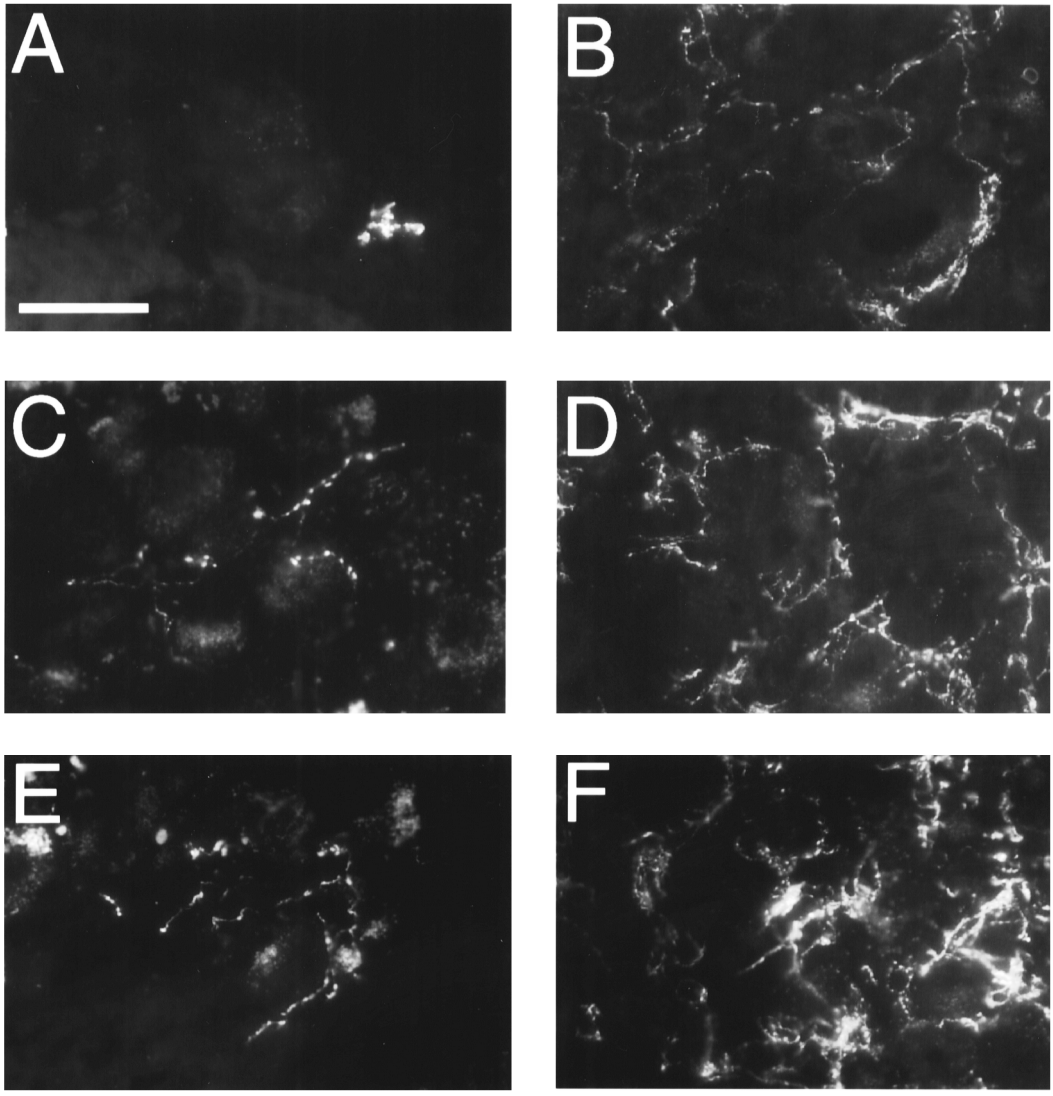

Fig. 2. SPG fluorescence of sympathetic axons within wild-type (WT) (A,C,E) and GFAP-NGF DRG (B,D,F). Few sympathetic axons are visible in WT DRG pre-operatively (A). This is in contrast to unoperated GFAP-NGF DRG, which are well-innervated by sympathetic axons (B). One (C,D) and 2 (E,F) weeks following CCI, sprout density has increased in injured DRG in both WT (C,E) and GFAP-NGF (D,F) mice. Scale bar, 50 μm.

described in the trigeminal ganglion [2,11]. Seven and 14 days following CCI, wild-type DRG were invaded by sympathetic axons, some of which were closely associated with DRG neurons (Fig. 2C,E). CCI induced a dramatic increase in the sympathetic innervation density in GFAP-NGF mice 7 and 14 days post operative (Fig. 2D,F), resulting in a dense plexus of fluorescent axons within the DRG. In both genotypes, innervation density also increased contralaterally (Fig. 3). These results show that excess expression of endogenous NGF following nerve injury leads to profuse sprouting within DRG.

Previous studies from our laboratory have shown that in Wallerian degeneration-deficient mice (in which injury-induced NGF upregulation is impaired) pain behaviour and sympathetic sprouting following CCI are attenuated compared to normal mice [8,9]. These results suggested that a distal stump-derived factor (likely NGF) is responsible. Other evidence implicates NGF's involvement in sympathetic sprouting that follows nerve injury: not only do uninjured trigeminal ganglia of GFAP-NGF mice contain sympathetic baskets [11], but so do these ganglia in mice overexpressing NGF in the skin [2].

Infra-orbital nerve lesion in GFAP-NGF mice leads to increased NGF protein levels in trigeminal ganglia [11], and so by analogy, NGF is likely increased in the DRGs of these mice. Although it is not known whether NGF levels are supranormal in injured nerves of these animals, GFAP increases in the spinal cord following nerve injury [4], so is highly likely that in the GFAP-NGF transgenic mice, the central branches of DRG neurons are in an environment of excess NGF which can be retrogradely transported to the DRG to induce sympathetic sprouting, presumably by a mechanism similar to that occurring in mice overexpressing NGF in the skin [2].

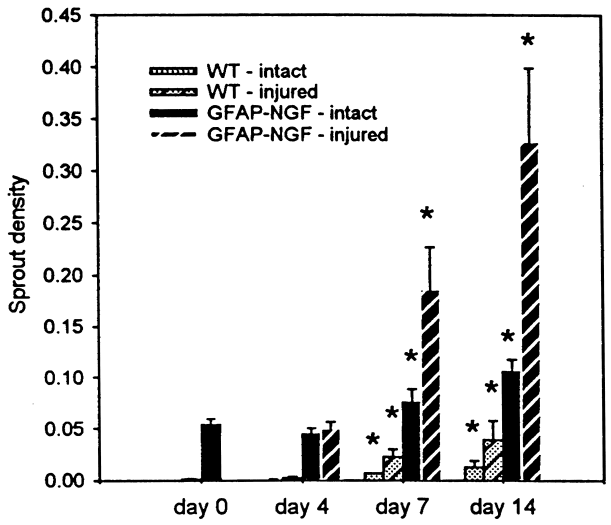

Fig. 3. CCI-induced changes in sympathetic innervation density within DRG. By 1 week post-CCI, sympathetic innervation density has increased in injured (hatched bars) and uninjured contralateral (unhatched bars) DRG of both wild-type (WT, grey bars) and GFAP-NGF DRG (black bars). At all time points, sprout density is significantly greater in GFAP-NGF mice ($n = 4$ per time point) than WT mice ($n = 4$ per time point) (one-way ANOVA, $P < 0.05$), and at all post-operative time points, sprout density is significantly greater in ipsilateral ganglia than in contralateral ganglia (paired *t*-test, $P < 0.05$).

The relationship between NGF, sympathetic sprouting and pain is complex. Uninjured GFAP-NGF mice are allodynic to thermal but not to mechanical stimulation, despite the presence of sympathetic axons and baskets within their DRG. With injury, these mice develop profound hypersensitivity to both thermal and mechanical stimulation, indicating that sympathetic sprouting itself is insufficient to produce the full range of behavioural abnormalities that accompany CCI, and that axotomy-induced changes in sensory neurons are required. Additionally, and for unknown reasons, contralateral increases in sprout density were observed without significant changes in contralateral behaviour.

Though the present experiment demonstrates a link between NGF overexpression and pain, and between NGF overexpression and sympathetic innervation of the DRG following nerve injury, it falls short of linking sympathetic sprouting in the DRG with pain. In fact, despite several reports that hypersensitivity and sympathetic sprouting in DRG are coincident [2,8,9], and that sensory neurons become responsive to adrenergic input following nerve injury [3], further study is required in order to describe fully the precise involvement of sympathetic sprouting in abnormal sensory function.

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