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The conditioning lesion effect on sympathetic neurite outgrowth is dependent on gp130 cytokines

H. Hyatt Sachs¹, H. Rohrer², and R.E. Zigmond^{1,*}

¹ Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland OH, USA 44106-4975

² Department of Neurochemistry, Max-Planck Institute for Brain Research, 60528 Frankfurt, Germany

Abstract

Sympathetic neurons, like sensory neurons, increase neurite outgrowth after a conditioning lesion. Studies in leukemia inhibitory factor (LIF) knockout animals showed that the conditioning lesion effect in sensory neurons is dependent in part on this cytokine; however, similar studies on sympathetic neurons revealed no such effect. Comparable studies with sensory neurons taken from mice lacking the related cytokine interleukin-6 (IL-6) have yielded conflicting results. LIF and IL-6 belong to a family of cytokines known as the gp130 family because they act on receptors containing the subunit gp130. In sympathetic ganglia, axotomy leads to increases in mRNA for four of these cytokines (LIF, IL-6, IL-11, and oncostatin M). To test the role of this family of cytokines as a whole in the conditioning lesion response in sympathetic neurons, mice in which gp130 was selectively eliminated in noradrenergic neurons were studied. The postganglionic axons of the SCG were transected, and seven days later the ganglia were removed and neurite outgrowth was measured in explant and dissociated cell cultures. In both systems, neurons from wild type animals showed enhanced growth after a conditioning lesion. In contrast, no enhancement occurred in neurons from mutant animals. This lack of stimulation of outgrowth occurred despite an increase in expression of activating transcription factor 3 (ATF3) in the mutant mice. These studies demonstrate that stimulation of enhanced growth of sympathetic neurons after a conditioning lesion is dependent on gp130 cytokine signaling and is blocked in the absence of signaling by these cytokines in spite of an increase in ATF3.

Keywords

Axotomy; conditioning lesion; cytokine; gp130; interleukin-6; leukemia inhibitory factor; neurite outgrowth; regeneration; superior cervical ganglion; sympathetic

Introduction

The conditioning lesion effect was originally described by McQuarrie and Grafstein (1973) as an increase in the rate of regeneration of fibers in the sciatic nerve following a test lesion, if

*Corresponding author. Richard E. Zigmond, Department of Neurosciences, Case Western Reserve University, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-4975, Telephone: 1 216 368 4614, Fax: 1 216 368 4650, rez@case.edu.

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the nerve had been lesioned several days earlier. This effect was found to hold true for both sensory and motor axons (McQuarrie et al., 1977; McQuarrie, 1978). Subsequently, it was found that a prior lesion *in vivo* also increased the rate of neurite outgrowth observed *in vitro* in explants of sensory ganglia and in cultures of dissociated sensory neurons (Hu-Tsai et al., 1994; Edstrom et al., 1996). Similar increases in neurite outgrowth have recently been shown to occur in sympathetic neurons both in ganglion explants and in dissociated cell cultures (Shoemaker et al., 2005; 2006).

Experiments with sensory neurons have indicated a role in triggering the conditioning lesion effect for cytokines of the gp130 family, specifically leukemia inhibitory factor (LIF) and possibly interleukin (IL)-6. After sciatic nerve injury, LIF is expressed in the nerve distal to the lesion site and immediately proximal to it (Banner and Patterson, 1994; Curtis et al., 1994; Sun and Zigmond, 1996a), and the cytokine is retrogradely transported by sensory axons to their cell bodies in dorsal root ganglia (Hendry et al., 1992; Curtis et al., 1994; Thompson et al., 1997). IL-6 is induced both in sensory neurons and in distal Schwann cells after a sciatic nerve lesion (Bolin et al., 1995; Murphy et al., 1995) but is apparently not retrogradely transported by sensory neurons (Kurek et al., 1996). When the effect of a prior lesion on neurite extension by sensory neurons *in vitro* was compared in LIF $-/-$ and wild type mice, neurite length in the former was shorter than in the latter (Cafferty et al., 2001). Similar experiments in IL-6 $-/-$ mice have produced conflicting results. Cafferty et al. (2004) reported that the growth response after a conditioning lesion was reduced in neurons from mutant animals compared to neurons from wild type animals, whereas Cao et al. (2006) found no such effect.

LIF and IL-6 are also dramatically upregulated in the superior cervical ganglion (SCG) after axotomy of the postganglionic internal and external carotid nerves, along with the related gp130 cytokine IL-11 (Banner and Patterson, 1994; Sun et al., 1994; Boeshore et al., 2004; Habecker et al., 2009). There is also a more modest increase in the cytokine oncostatin M (Habecker et al., 2009). In contrast to the situation with sensory neurons, neurite outgrowth after a conditioning lesion was indistinguishable in sympathetic neurons taken from LIF $-/-$ and wild type mice (Shoemaker et al., 2005).

gp130 cytokines act on a set of multimeric receptors all of which contain the signaling subunit gp130 (Taga, 1997). Stimulation of gp130 leads to the activation of Janus kinase (JAK) and the phosphorylation and activation of signal transducer and activator of transcription (STAT) 3. Following axotomy of neurons in the SCG, phosphoSTAT3 is detectable in these neurons (Rajan et al., 1995; Habecker et al., 2009), consistent with stimulation by one or more of these cytokines.

To examine the cytokine dependence of the conditioning lesion effect in sympathetic neurons further, we examined this effect in mice that have gp130 deleted in neurons that express the norepinephrine synthesizing enzyme dopamine- β -hydroxylase, such as sympathetic neurons (Stanke et al., 2006). Thus, signaling in sympathetic neurons by gp130 cytokines would be expected to be blocked in these animals. The conditioning lesion effect in neurons of the SCG was completely absent in these conditional knockout animals.

Materials and methods

Animals and surgery

Wild type 3–7 month old male and female C57Bl/6J mice were obtained from Jackson Labs, Bar Harbor, ME. Conditional gp130 knockout mice (gp130^{DBH^{Cre}}) were generated by crossing gp130^{fl/fl} animals with animals in which Cre recombinase was expressed under the dopamine- β -hydroxylase (DBH) promoter (Stanke et al., 2006). As shown by Stanke et al. (2006), Cre-mediated recombination takes place in virtually all sympathetic neurons in these animals.

Following intraperitoneal administration of ketamine (100 mg/kg) and xylazine (10 mg/kg), neurons in the SCG of wild type and conditional knockout mice were unilaterally axotomized by transecting the postganglionic internal and external carotid nerves. One week later, mice were sacrificed by CO₂ inhalation, and the ipsilateral (axotomized) and contralateral (sham-operated) ganglia were removed and prepared for explant or dissociated cell culture as previously described (Shoemaker et al., 2005).

Neurite outgrowth and analysis in explants

Twenty four and forty eight hours after being placed in Matrigel (Becton Dickinson, Franklin Lakes, NJ), phase contrast images of the explants were captured using a Zeiss Axiovert 405 M microscope with a 10X objective. Measurements were made using MetaMorph image analysis software (Version 6.2r5, Molecular Devices, Downingtown, PA). The MetaMorph “draw” function was used to draw a straight line from the border of the explant to the leading tip of the 20 longest processes. In each experiment, the minimum number of ganglia measured was three, and the experiment was repeated three times. The results are expressed as the mean $\pm$ standard error of the mean. Following imaging at 48 h, the SCG explants were fixed in 4% paraformaldehyde for 1h.

Neurite outgrowth and analysis of dissociated SCG

We also wanted to look at the response of individual gp130^{DBHcre} neurons to the conditioning lesion. Initially, dissociated SCG cells were grown on glass coverslips as previously described (Shoemaker et al., 2005). However, the gp130^{DBHcre} sham-operated and axotomized neurons failed to grow well under these conditions. We found that the sham-operated gp130 cells grew well on a substrate of control transfected Chinese hamster ovary (CHO) cells (Mukhopadhyay et al., 1994). In these experiments R2-CHO cells [from a stock provided by Dr. M. Filbin (Hunter College, New York)] were cultured to confluence on Nunc 8-well Lab Tek chamber slides (Fisher Scientific, Pittsburgh, PA) for 24h. Three gp130^{DBHcre} mice and three C57Bl/6J mice were unilaterally axotomized. Each ganglion was dissociated according to the procedure described by Orike et al. (2001). The dissociated neurons from each ganglion were added to two wells containing the R2-CHO cells and cultured in F12-Coon's medium (Sigma-Aldrich, St. Louis, MO) containing 3.5% pathocyte-4 bovine serum albumin (MP Biomedicals, Inc., Aurora, Ohio), 340 ng/ml tri-iodo thyronine, 60 ng/ml progesterone, 400 ng/ml L-thyroxine, 38 ng/ml sodium selenite, 16 μ g/ml putrescine, 10 U/ml penicillin, and 10 μ g/ml streptomycin (all from Sigma-Aldrich) for 24 h at 37°C in a humidified atmosphere of 95% air/5% CO₂. Cells were then fixed in 4% paraformaldehyde for 20 min at room temperature, washed in phosphate buffered saline (PBS), blocked with 5% normal donkey serum in 0.3% triton X-100 in PBS, incubated overnight with neuron-specific anti- β III tubulin mAb (1:1000; Promega, Madison, WI) followed by a 1 h incubation in Cy3 conjugated donkey anti-mouse IgG, F(ab')₂ fragment (1:400; Jackson ImmunoResearch Laboratories, West Grove, PA). The slides were coverslipped with Fluoro Gel (Electron Microscopy Sciences, Hatfield, PA). Neurons were visualized on a Leitz epifluorescence microscope (Lieca Microsystems, Inc., Bannockburn, IL), and images were captured with a Hamamatsu ORCA 100 cooled CCD camera (W. Nusbaum, Inc., McHenry, IL) interfaced with C-imaging software (Compix, Inc., Cranberry Township, PA). Using Metamorph software, the longest neuronal process was measured from each β III tubulin immunoreactive (IR) cell that had a process of at least 1.5 times the diameter of the cell body. The mean length $\pm$ standard error of the mean was determined for each group.

Explant Immunohistochemistry

At the end of the culture period, explants were fixed for 1 h in 4% paraformaldehyde (Ted Pella, Redding, CA). In one experiment, the explants were cryoprotected in 30% sucrose, gently

removed from the cover glass and embedded in Tissue-Tek O.C.T medium. Cryostat sections (10 μ m) were cut and mounted on Superfrost®/Plus microscope slides (Fisher Scientific) for immunohistochemistry. The sections were blocked for 1 h in 5% normal donkey serum in 0.3% Triton X-100 in PBS. Sections were incubated overnight with primary rabbit antibodies to either phosphoSTAT3 (Tyr705) (1:100, Cell Signaling Technology, Danvers, MA) or activating transcription factor 3 (ATF3; 1:500, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Sections were washed three times with PBS (5 min each) and then incubated for 45 min with a donkey anti-rabbit Cy3 (1:250, Jackson ImmunoResearch). Sections were then washed in PBS and cover-slipped with Fluoro Gel. For all antibodies used, control sections were run in which the antibody was omitted. No staining resulted with any of these slides (data not shown).

Results

STAT phosphorylation in mutant and wild type ganglia

In a previous study, we examined STAT3 phosphorylation in the SCG 2 days after axotomy in the gp130^{DBHcre} mice as a check that cytokine signaling was absent and found no detectable response in the mutant animals (Habecker et al., 2009). To ensure that this was not simply a delay in response, we examined STAT3 phosphorylation also in the explanted ganglia of wild type and gp130^{DBHcre} mice, both in control ganglia and in ganglia seven days after a conditioning lesion. PhosphoSTAT3 was detectable in 48 h explants of ganglia from wild type animals taken contralateral (Fig. 1A) and even more so taken ipsilateral (Fig. 1B) to the conditioning lesion. On the other hand, no immunostained neurons were seen in explants of ganglia from mutant animals from either side of the animal (Fig. 1C, D).

Conditioning lesion effect in explants from wild type and mutant animals

One week after unilateral axotomy of the SCG, ipsilateral and contralateral ganglia from wild type and mutant animals were placed in explant culture, and neurite outgrowth was measured at 24 and 48 h under phase microscopy. For the SCG from wild type mice, after 24 h in culture, neurite outgrowth was 3.1-fold greater in ganglia that were ipsilateral to the lesion than those contralateral (Fig. 2A). After 48 h in culture, neurite outgrowth was 4.1-fold greater in ipsilateral than in contralateral ganglia (Fig. 2B,C,E). For the ganglia from the gp130^{DBHcre} mice, there was no increase in neurite outgrowth after a conditioning lesion at either 24 or 48 h (Fig. 2A,B,D,F). In addition, the rate of outgrowth in these ganglia from the mutant animals was not significantly different than the outgrowth from the control ganglia from wild type mice (Fig. 2A,B). A stimulation of neurite outgrowth in the conditioned explants of wild type, but not gp130^{DBHcre}, mice was similarly evident after 48 h when the explants were immunostained for β III tubulin (data not shown).

Conditioning lesion effect in cell culture of neurons from wild type and mutant animals

One week after unilateral axotomy of the SCG, neurons were dissociated from ipsilateral and contralateral ganglia from wild type and mutant animals. The neurons were maintained in cell culture for 24 h, fixed and stained for β III tubulin, and neurite outgrowth measured. In cells from wild type animals, the mean length of the longest neurite was about 2-fold greater in neurons isolated from ipsilateral ganglia than in those isolated from contralateral ganglia (Fig. 3A,C,E). In neurons from gp130^{DBHcre} mice, no significant difference in neurite outgrowth was seen in neurons from ipsilateral and contralateral ganglia (Fig. 3B,D,E). Outgrowth of the longest neurite in neurons from the ganglia of the mutant animals was not significantly different from that of neurons from the control (i.e., contralateral) ganglia from wild type animals (Fig. 3E). In addition, no systematic difference was seen in either the number of axonal branches or the number of processes emanating from the neuronal soma.

ATF3 induction in wild type and mutant animals

Sympathetic neurons, like sensory and motor neurons, increase expression of ATF3 immunoreactivity after axotomy (Tsujino et al., 2000; Hyatt Sachs et al., 2007), and it has been proposed that this transcription factor “may mediate the conditioning effect by regulating expression of effectors that increase the intrinsic growth state of ... neurons” (Seijffers et al., 2007). We previously reported that the percentage of sympathetic neurons expressing ATF3 two days after axotomy was not different in wild type and gp130^{DBHcre} mice though there was a somewhat smaller increase in ATF3 mRNA in the latter (Habecker et al., 2009). In the present study, we examined ATF3 expression in both genotypes *in vivo* seven days after a conditioning lesion and in the same explants in which we had measured neurite outgrowth. As shown in the micrographs in Fig. 4, ATF3 immunoreactivity was comparable in wild type and gp130^{DBHcre} mice both prior to and following explantation.

Discussion

Biochemical changes underlying the conditioning lesion in sensory neurons

The conditioning lesion effect was first described over 35 years ago (McQuarrie and Grafstein, 1973), however, certain aspects of this phenomenon have been elucidated only much more recently. Thus, while initially described as an increase in the rate of regeneration of the peripheral process of sensory neurons *in vivo* (McQuarrie et al., 1977), a conditioning lesion is now known also to increase the regeneration of the central process of these neurons following crushing or transection of axons in the dorsal columns (Neumann and Woolf, 1999). Somewhat analogously, while the conditioning lesion was found to increase the outgrowth of sensory and sympathetic neurons *in vitro* on a permissive laminin substrate (Hu-Tsai et al., 1994; Edstrom et al., 1996; Shoemaker et al., 2005), it has subsequently been found to increase neurite outgrowth also on the normally inhibitory substrate of myelin or myelin associated glycoprotein (MAG; Cao et al., 2006).

Along with this broadening of our knowledge of the changes brought about by a conditioning lesion has come the first information concerning the molecular basis for these changes, although whether all of the responses to a conditioning lesion are mediated by the same underlying biochemistry remains to be determined. Up until now most of the molecular information has come from studies on dorsal root ganglion neurons. Following the observation that treatment of these neurons in culture with dibutyryl cAMP blocks the inhibition of neurite outgrowth by myelin and MAG (Cai et al., 1999), it was found that injection of this cyclic AMP analogue directly into the dorsal root ganglion *in vivo* stimulated growth of injured sensory axons in the dorsal columns (Neumann et al., 2002; Qiu et al., 2002) and neurite outgrowth in culture on myelin and MAG (Qiu et al., 2002). Furthermore, it was observed that cAMP levels increase in DRG neurons after a conditioning lesion (Qiu et al., 2002), suggesting that cAMP might be the second messenger mediating these effects of a conditioning lesion.

The stimulation of neurite outgrowth by sensory neurons *in vitro* produced by both a conditioning lesion and by administration of cAMP is dependent on gene transcription (Smith and Skene, 1997; Cai et al., 2002). Among the genes whose expression are increased by both of these manipulations are the gp130 cytokines LIF and IL-6 (Banner and Patterson, 1994; Curtis et al., 1994; Sun et al., 1994; Murphy et al., 1995; Sun and Zigmond, 1996a; Nagamoto-Combs et al., 1999; Cao et al., 2006; Wu et al., 2007). Evidence for the involvement of these cytokines in the conditioning lesion response first came from experiments with LIF ^{-/-} and LIF ^{+/+} mice. Two weeks after a unilateral sciatic nerve transection, L4 and L5 DRG were dissociated and placed in cell culture for 18 h, and neurite outgrowth on laminin was compared to that from naive neurons. While a conditioning lesion effect was observed in neurons from both genotypes, the length of the longest neurite of the LIF ^{-/-} neurons was only 59% of that

in LIF $+/+$ neurons (Cafferty et al., 2001). Addition of exogenous LIF to the LIF $-/-$ neurons restored the conditioned lesion effect to its normal magnitude.

Subsequent experiments with IL-6 $-/-$ and IL-6 $+/+$ mice suggest that this cytokine is also involved in the conditioning lesion effect. Again, a conditioning lesion effect on laminin was found in mice of both genotypes; however, the length of the longest neurite from the knockout mice was only 37% of that seen in the wild type animals (Cafferty et al., 2004). As with LIF, addition of IL-6 to the IL-6 $-/-$ neurons restored the normal conditioning lesion effect. Cao et al. (2006) reported that addition of exogenous IL-6 stimulated neurite outgrowth on MAG; nevertheless, they found no difference between wild type and IL-6 $-/-$ mice in the growth capacity of sensory neurons either in the presence or absence of MAG.

Further study of the role of gp130 cytokines has involved the use of the JAK2 inhibitor AG490. AG490 inhibited the increased outgrowth of DRG neurons on laminin after a sciatic nerve lesion (Liu and Snider, 2001; Qiu et al., 2005), and the growth of the central process of these neurons in vivo (Qiu et al., 2005). On the other hand, Cao et al. (2006) reported that the inhibitor produced no inhibition of the cAMP-stimulated outgrowth of these neurons on MAG.

gp130 cytokines are required for the conditioning lesion effect in sympathetic neurons

Based on their respective results on sensory neurons, Cao et al. (2006) concluded that IL-6 was sufficient but not necessary to mimic the conditioning lesion effect on axonal growth, while Cafferty et al. (2004) concluded that IL-6 played a necessary role in this effect. Given that our own studies on sympathetic neurons failed to find an effect of LIF on the conditioning lesion effect (Shoemaker et al., 2005) and given that we have found that, in addition to LIF, IL-6, IL-11, and oncostatin M are induced in the SCG after axotomy (Habecker et al., 2009), we decided to take an approach that deleted the effects of this whole family of cytokines. Evidence that signaling by these cytokines is absent in the conditional knockout animals that we used is the failure of STAT3 phosphorylation to occur in SCG neurons after axotomy (Fig. 1). In our studies both in explant and cell culture, the effect of a unilateral conditioning lesion on neurite outgrowth was completely blocked in the ipsilateral ganglion of the knockout animals. In these same animals, growth in the control contralateral ganglion was normal. Thus, our data are consistent with the hypothesis that gp130 cytokine action plays a necessary role in the conditioning lesion effect in sympathetic neurons.

As already noted, gp130 cytokines lead to the phosphorylation and activation of STAT proteins, which translocate to the nucleus, bind to a cytokine response element and trigger changes in gene expression (Taga, 1997). Two questions that need to be addressed concerning the cytokine regulation of the conditioning lesion effect are what are the relevant cellular target(s) of these cytokines and activation of which gene(s) downstream from the cytokines mediate this effect. Evidence indicates that Schwann cells and sensory and sympathetic neurons all express gp130 (Marz et al., 1996; Mizuno et al., 1997; Dowsing et al., 1999; Gardiner et al., 2002; O'Brien and Nathanson, 2007). In glial cells, one gene that is known to be sensitive to gp130 cytokines is the neurofilament protein glial fibrillary acidic protein (GFAP; Yoshida et al., 1993). Recently, it was reported that the early increase in GFAP seen in Schwann cells after axotomy is diminished in IL-6 $-/-$ mice (Lee et al., 2009). Whether GFAP expression would alter the conditioning lesion effect, however, is not clear (e.g., Triolo et al., 2006; Desclaux et al., 2009).

One neuronal gene known to be stimulated in sensory and sympathetic neurons after injury and widely thought to be involved in axonal regeneration is growth associated protein (GAP) 43 (Bisby, 1988; van der Zee, 1989; Fu and Gordon, 1997; Hou et al., 1998). Interestingly, Cafferty et al. (2004) found that the increase in GAP43 expression that occurs in DRGs from wild type animals one week after a sciatic nerve lesion is not seen in IL6 $-/-$ animals. Another

protein whose expression is increased dramatically after axotomy of sensory and sympathetic neurons is the neuropeptide galanin (Zigmond et al., 1996). The axotomy induced increase in galanin in the SCG was substantially reduced in LIF $-/-$ mice and was completely abolished in gp130^{DBHcre} animals (Rao et al., 1993; Sun and Zigmond, 1996b; Habecker et al., 2009). Similarly, in sensory neurons, the axotomy induced increase in galanin expression was inhibited in both LIF $-/-$ and IL-6 $-/-$ mice (Corness et al., 1996; Sun and Zigmond, 1996a; Murphy et al., 1999). It is highly likely that part of the cytokine promotion of the conditioning lesion effect is mediated by galanin, as the conditioning lesion in sensory neurons is depressed in galanin knockout animals (Sachs et al., 2007).

The gene for the transcription factor ATF3 has been shown to be activated in peripheral neurons in response to axotomy (Tsuji et al., 2000; Hyatt Sachs et al., 2007). Overexpression of ATF3 in neonatal sympathetic ganglion explants via a viral vector was found to increase neurite outgrowth (Nakagomi et al., 2003). Recently, it was proposed that this transcription factor mediates the increased intrinsic growth state of sensory neurons leading to increased neurite outgrowth on permissive substrates (Seijffers et al., 2007). Our previous quantitative data (Habecker et al., 2009) and the results presented here indicate that gp130 cytokines play only a minor role in the induction of ATF3. Furthermore, they demonstrate that the increase in neurite outgrowth produced by a conditioning lesion can be blocked in spite of a dramatic increase in ATF3 expression. Thus, an increase in ATF3 expression is not sufficient for triggering the conditioning lesion effect in sympathetic neurons, rather some increase in gene expression down stream of gp130 cytokine signaling is required for this effect to occur.

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Fig. 1.

Immunostaining for phosphoSTAT3 in SCG explants from wild type (A,B) and gp130^{DBHcre} mice (C,D) without (A,C) and with (B,D) a conditioning lesion. The internal and external carotid nerves were transected unilaterally and seven days later the ipsilateral and contralateral SCG were placed in explant culture. After 48 h, the ganglia were sectioned and immunostained for phosphoSTAT3. Immunopositive neurons were present in the wild type ganglia both contralateral (A) and ipsilateral (B) to the conditioning lesion; however, no positive neurons were found in the contralateral (C) or the ipsilateral (D) mutant ganglia. The scale bar represents 50 μ m.

Fig. 2.

A,B. Quantitation of neurite outgrowth from explants of SCG following unilateral axotomy in wild type (C57) and gp130^{DBH^{cre}} (gp130) mice. Seven days after unilateral axotomy, the ipsilateral (Ax) and contralateral (Sh) SCG were placed in Matrigel and maintained in explant culture. Twenty four (A) and forty eight (B) hours later, the explants were photographed under phase contrast microscopy, and the lengths of the 20 longest neurites were determined. The means and S.E.M. of the lengths of the longest neurites are shown for 9 ganglia at 24 h and for 6–9 ganglia at 48 h. *, represents a difference from the control value of the same genotype at $p < 0.003$ (analyzed by one-way ANOVA followed by Tukey's post test). C–F. Phase contrast microscopy of SCG placed in explant culture for 48 h. Ganglia from wild type (C,E) and

gp130^{DBHcre} mice (D,F) placed in culture 7 days after a sham operation (C,D) or after axotomy (E,F). Arrows point to individual neurites. The scale bar represents 100 μ m.

Fig. 3.

Neurite outgrowth from dissociated SCG neurons maintained in cell culture. Seven days after unilateral axotomy of neurons in the SCG of both wild type (A,C) and gp130^{DBHcre} (B,D) mice, the contralateral (A,B) and ipsilateral (C,D) ganglia were dissociated, and the cells maintained in culture. After twenty four hours, the cultures were immunostained for β III tubulin and photographed. The neurons selected for this micrograph represent the means of the length of the longest neurite obtained for each experimental group. The scale bar represents 50 μ m. E. Quantitation of neurite outgrowth from SCG neurons of wild type and gp130^{DBHcre} mice maintained in cell culture following a conditioning lesion. Following immunostaining for β III tubulin, the length of the longest neurite was determined for each neuron that had a process at

least 1.5 times the diameter of the cell body. *, represents a difference from the control value of the same genotype at $p < 0.02$ (analyzed by one-way ANOVA followed by Tukey's post test).

Fig. 4. ATF3 expression in neurons in the SCG in wild type (A,C) and gp130^{DBH^{cre}} mice (B,D) seven days after a conditioning lesion either prior to (A,B) or after 48 h in explant culture (C,D). The scale bar represents 50 μ m.