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Sciatic Nerve Transection Triggers Release and Intercellular Transfer of a Genetically Expressed Macromolecular Tracer in Dorsal Root Ganglia

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Abstract

We recently developed a genetic transneuronal tracing approach that allows for the study of circuits that are altered by nerve injury. We generated transgenic (ZW-X) mice in which expression of a transneuronal tracer, wheat germ agglutinin (WGA), is induced in primary sensory neurons, but only after transection of their peripheral axon. By following the transneuronal transport of the tracer into the central nervous system (CNS) we can label the circuits that are engaged by the WGA-expressing damaged neurons. Here we used the ZW-X mouse line to analyze dorsal root ganglia (DRG) for intraganglionic connections between injured sensory neurons and their neighboring “intact” neurons. Because neuropeptide Y (NPY) expression is strongly induced in DRG neurons after peripheral axotomy, we crossed the ZW-X mouse line with a mouse that expresses Cre recombinase under the influence of the NPY promoter. As expected, sciatic nerve transection triggered WGA expression in NPY-positive DRG neurons, most of which are of large diameter. As expected, double labeling for ATF-3, a marker of cell bodies with damaged axons, showed that the tracer predominated in injured (i.e., axotomized) neurons. However, we also found the WGA tracer in DRG cell bodies of uninjured sensory neurons. Importantly, in the absence of nerve injury there was no intraganglionic transfer of WGA. Our results demonstrate that intraganglionic, cell-to-cell communication, via transfer of large molecules, occurs between the cell bodies of injured and neighboring noninjured primary afferent neurons.

INDEXING TERMS

wheat germ agglutinin; pain; dorsal root ganglion; axotomy; tracing; neuron-glia communication

Because synapses are absent in the dorsal root ganglion (DRG), sensory neurons are considered anatomically isolated from one another (Lieberman, 1976). Several studies have shown, however, that repetitive stimulation of DRG neurons results in transient depolarization of “neighboring” (nonstimulated) neurons within the same ganglion (Devor and Wall, 1990). This phenomenon, also referred as “cross-excitation,” increases in the

setting of peripheral nerve injury (Amir and Devor, 2000; Xu and Zhao, 2003). Other studies reported that small molecules are released locally within sensory ganglia. For example, direct application of KCl, veratridine, or capsaicin to DRG neurons or peripheral nerve stimulation evokes the release of both substance P (SP) and ATP within trigeminal ganglia (Neubert et al., 2000; Matsuka et al., 2001). Taken together, these observations suggest that there is somatic release of molecules from DRG neurons. Whether release of molecules has a functional significance, via an action of specific receptors expressed by DRG neurons, remains to be determined. Of particular interest is the possibility that local release of neurotransmitters after nerve injury enhances the spontaneous activity of damaged sensory neurons (Kajander et al., 1992).

Here we used a genetic tracing system to determine whether or not there is intraganglionic communication between injured and intact sensory neurons and whether this transfer is of large proteins. We targeted expression of the transneuronal tracer wheat germ agglutinin (WGA) to injured sensory neurons (first-order neuron), using our recently described ZW-X line, and analyzed transneuronal transfer of the WGA to neighboring DRG neurons (which do not express the tracer). In these studies we crossed the ZW-X mouse (Braz and Basbaum, 2009; see Results) with a BAC transgenic mouse that expresses Cre recombinase under the control of the neuropeptide Y (NPY) promoter (DeFalco et al., 2001). In double transgenic NPY-ZW-X mice, Cre-mediated excision of the floxed lacZ cDNA (required for WGA expression) only occurs in NPY-expressing neurons. As NPY is strongly induced in a subset of sensory neurons after nerve injury (Wakizaka et al., 1991; Noguchi et al., 1993; Zhang et al., 1993), it was possible to induce expression of the tracer in a restricted number of damaged DRG neurons. In this study we report that there is transfer of WGA from the cell bodies of DRG neurons with injured axons to neighboring cell bodies that have intact peripheral axons. These observations demonstrate intercellular transfer of large molecules between neighboring neurons, but only in the setting of nerve injury.

MATERIALS AND METHODS

Mouse lines

All experiments were reviewed and approved by the Institutional Care and Animal Use Committee at the University of California San Francisco. We generated double transgenic NPY-ZW-X mice in which transneuronal anterograde transport of the tracer WGA can be triggered by nerve injury of sensory neurons that express NPY. These mice were generated by crossing our ZW-X line (Braz and Basbaum, 2009) with mice that express the Cre recombinase in NPY positive neurons (DeFalco et al., 2001; gift from Dr. Friedman). To generate Per-ZW mice, we crossed Peripherin-Cre mice (Jackson Laboratories, Bar Harbor, ME) (Zhou et al., 2002) with the ZW mice (Braz et al., 2002, 2005; Braz and Basbaum, 2008).

Nerve injury

Four-week-old male Per-ZW and NPY-ZW-X mice were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg) / xylazine (8 mg/kg). For axotomy, an incision was made in the lateral left hindleg at the level of the mid-thigh. The sciatic nerve was exposed, cut, and ≈ 2 mm of distal nerve was removed. The overlying muscle and skin were sutured and the animals were allowed to recover before returned to their home cage.

Antibodies

Rabbit anti-WGA (1:50,000, Sigma, St. Louis, MO; Cat. No. T4144), goat anti-WGA (1:500, Vector Laboratories, Burlingame, CA; Cat. No. AS2024), rabbit anti-NPY (1:2,000, generous gift from Dr. J.M. Allen, London, UK), chicken anti- β -galactosidase (1:6,000,

Abcam, Cambridge, MA; Cat. No. ab9361), mouse anti-NF200 (1:10,000, Sigma, Cat. No. N0142), rabbit anti-ATF3 (1:1,000, Santa Cruz Biotechnologies, Santa Cruz, CA; Cat. No. sc-188).

Antibody characterization

Table 1 lists the antibody characteristics, some of which are also included in the manufacturer's information sheets.

Anti-WGA antibodies were raised in rabbit or goat using purified WGA as the immunogen. Our own studies have demonstrated that neither anti-WGA antibodies show immunostaining in wildtype mice (i.e., in mice that do not express the WGA transgene).

Anti-NPY antibodies were raised in a rabbit given natural porcine NPY conjugated to bovine serum albumin (BSA). The antiserum is directed to the N-terminal region (1–19) of NPY (Allen et al., 1984). The pattern of NPY-immunoreactivity that we observed with this antibody is comparable to those reported in many other studies of the peripheral nerve injury-induced distribution of NPY in the rodent DRG (Wakizaka et al., 1991; Noguchi et al., 1993; Zhang et al., 1993).

Anti-ATF3 antibodies were raised against a peptide derived from the C-terminus of human ATF3 (131–181). In western blots of HeLa cells, the anti-ATF3 antibody detects a single band at ≈ 27 kD. Our own experiments show that in the absence of nerve injury there is no ATF3 immunoreactivity in the DRG.

Anti-NF200 antibodies were raised against the C-terminus of enzymatically dephosphorylated pig neurofilament H-subunit. When tested by immunoblotting of rat spinal cord extract, the antibody specifically recognizes the neurofilament of molecular weight 200 kD. The pattern of NF200-immunoreactivity that we observed with this antibody is very comparable to those reported in many other studies of the distribution of NF200 in the mouse (Hubert et al., 2008; Jaafari et al., 2008).

Anti- β -galactosidase antibodies were produced by hyperimmunizing rabbits with the full-length (β -gal enzyme from *Escherichia coli*). Our studies have established that there is no (β -gal immunoreactivity in wildtype mice (i.e., in mice that do not express the lacZ transgene).

Immunohistochemistry

To localize the transgene and transported WGA, we anesthetized Per-ZW and NPY-ZW-X mice 1 week after axotomy with Avertin (250 mg/kg) and then perfused them transcardially with 10 mL of saline (0.9% NaCl) followed by 30 mL of 3.7% formaldehyde in phosphate buffer (PB) 0.1 M, pH 7.4, at room temperature (RT). Tissues were dissected out, postfixed in the same solution for 3 hours, and cryoprotected in 30% sucrose phosphate-buffered saline (PBS) overnight at 4°C. Fourteen- μ m cryostat sections (DRG) were preincubated for 30 minutes at RT in PBS containing 0.5% Triton X-100, 10% BSA, and 10% normal goat serum (NGS) and then immunostained overnight at RT in PBS containing 0.5% Triton X-100, 1% BSA, 1% NGS (NPBST) and the primary antibodies. After washing in NPBST, sections were incubated for 1 hour with secondary antibodies (Alexa 488- or 546-conjugated IgG; 1:700), rinsed in NPBST, mounted in Fluoromount-G (Southern Biotechnology, Birmingham, AL), and coverslipped. Sections were viewed with a Nikon Eclipse fluorescence microscope and images were collected with a Spot Camera. Brightness and contrast were adjusted using Adobe Photoshop, v. 6.0 (San Jose, CA).

Cell counting

Serial sections of entire L4–5 DRGs from three animals were cut (14 μ m) and placed on Superfrost microslides (Fisherbrand, Pittsburgh, PA). To ensure that all neurons were sampled, we mounted, immunostained, and eventually counted patterns in every fourth section of the ganglia. With this approach, at least 6–7 sections per DRG per animal were counted, which, depending on the marker studied, correspond to 600–1,000 neurons sampled per DRG. All counts were corrected for cell size using the Abercrombie formula ($T/(T+h)$) where T is the thickness of the section and h the average size of 25 labeled cell nuclei of each type. We established the following correction factors: 0.69 for WGA singled-labeled cells; 0.78 for WGA+ β -galactosidase+ double-labeled cells; 0.76 for WGA+NPY+ double-labeled cells; and 0.84 for WGA+NF200+ double-labeled cells. To calculate the percentage of double-labeled neurons (marker vs. WGA) we divided the corrected number of double-labeled neurons by the corrected number of single WGA-labeled neurons and multiplied the result by 100. Values are given as mean $\pm$ standard deviation (STD).

Electron microscopy

For electron microscopy we perfused ZW-X mice through the aorta with 4% formaldehyde, 0.5% glutaraldehyde in 0.1M PB, pH 7.4, 1 week after sciatic nerve injury. The L4 and L5 DRGs were dissected and fixed for 3 additional hours, rinsed with PB, and embedded in 4% agar. Sixty- μ m vibratome sections were cut, then permeabilized with 50% ethanol for 60 minutes. The glutaraldehyde influence on epitope recognition by the antibodies was blocked with PB containing 0.1M glycine for 30 minutes. Nonspecific sites were blocked with 10% NGS and 2% BSA in PB. Samples were incubated with rabbit anti-WGA (Sigma) 1:50,000 with 10% NGS, 0.05% Thimerosol in PB for 84 hours at 20°C. After a rinse with PB the samples were immersed in Vector biotinylated goat antirabbit 1:200 (Vector Laboratories) with 10% NGS in PB overnight rinsed with PB then immersed in Vector ABC 1:200 with 2% BSA in 0.1M PB overnight. Following rinses with PB the tissue was reacted with DAB GOD (diaminobenzidine) (NH₄Cl d-glucose N-ammonium sulfate), rinsed with DH₂O 30 seconds, then postfixed with 1% osmium tetroxide for 60 minutes, dehydrated in ethanol, infiltrated with propylene oxide and Eponate 812 epoxy resin, embedded between two pieces of ACLAR material, and polymerized at 60°C. Tissue was selected and remounted on blank epoxy blocks for ultrathin sectioning. Sections were stained with 2% uranyl acetate and Reynolds lead citrate then examined and photographed in a JEOL 100CXII at 80 kV.

RESULTS

Peripheral axotomy induces expression of WGA in DRG of ZW-X mice

As for the original ZW mouse (Braz et al., 2002, 2005; Braz and Basbaum, 2008), the ZW-X mouse is a transgenic mouse that expresses the transneuronal tracer WGA in neurons after Cre recombination. Presumably because of a random insertion of the transgene in the mouse genome, expression of the tracer in the ZW-X mouse only occurs in sensory neurons whose peripheral axons have been damaged (Fig. 1A–C; see also Braz and Basbaum, 2009). To induce the expression of WGA in large myelinated primary afferent neurons, we crossed the ZW-X mouse with NPY-Cre mice (DeFalco et al., 2001) in which the Cre recombinase is expressed in NPY-positive sensory neurons. Under normal conditions, NPY is rarely expressed (if at all) in DRG neurons. This is illustrated in Figure 1D, which shows that in double transgenic NPY-ZW-X mice there is no detectable expression of NPY in the absence of injury (in five animals we recorded no more than two immunopositive neurons per DRG). Consistent with this observation, we found that WGA is not expressed prior to injury (Fig. 1F). In distinct contrast and consistent with several reports, we found that NPY expression is strongly upregulated in DRG neurons ipsilateral to the sciatic nerve transection (Fig. 1E). We observed NPY upregulation 1 week after the nerve injury (the first timepoint of

observation) and the NPY was still detectable 8 weeks postinjury (data not shown). As previously reported, immunoreactive NPY predominated in medium and large-diameter neurons, but several small sensory neurons were also labeled.

Figure 1G illustrates that 1 week after sciatic nerve transection there is a pronounced induction of WGA expression in DRG neurons, ipsilateral to the injury. The great majority of WGA-immunoreactive neurons immunostained for NPY ($85.3 \pm 1.7\%$; Fig. 2A–C) and for a marker of DRG neurons with myelinated axons (viz., NF200; $>90\%$), and were of medium to large-diameter ($>25 \mu\text{m}$; Fig. 2D–F). To confirm that the WGA expression was indeed triggered by the nerve injury, we double-labeled for WGA and for activating transcription factor 3 (ATF-3), which marks axotomized neurons (Tsujino et al., 2000). As shown in Figure 2G–I, the great majority of WGA-immunoreactive sensory neurons costained for ATF-3 ($87.4 \pm 1.9\%$), confirming that the WGA induction was related to the Cre-mediated recombination event that occurred in axotomized neurons.

Intraganglionic transfer of WGA from injured to intact sensory neurons

In all animals studied ($n = 5$), however, we also detected a significant number of WGA-positive DRG neurons that were neither NPY- nor ATF-3-positive ($13.8 \pm 2.3\%$ and $14.4 \pm 2.5\%$, respectively; arrows Fig. 2C,I). The presence of WGA in uninjured sensory neurons indicates that the tracer must have been released at the level of the DRG, presumably by injured (ATF3- and NPY-positive) DRG neurons and taken up by neighboring “intact” (ATF3- and NPY-negative) neurons.

To rule out the possibility that the WGA had resulted from a Cre recombination event in intact sensory neurons, (i.e., that there was a leak in the Cre expression), we double-labeled the DRG for β -gal and WGA. As WGA synthesis only occurs in neurons in which the lacZ cDNA is deleted by Cre excision, the presence of WGA in a neuron that also contains β -gal provides very strong evidence that the WGA derived from transport from a neighboring neuron. Figure 3 clearly shows that some of the WGA-immunoreactive neurons costained for β -gal ($13.4 \pm 4.9\%$, arrows). As neurons in which a Cre excision event occurred cannot concurrently express lacZ and WGA (because of the lacZ stop codon, see Fig. 1), this latter result confirmed that there must have been trans-neuronal transport of the tracer within the DRG.

Intraganglionic transfer of WGA from injured sensory neurons to surrounding satellite cells

The intercellular transfer of the WGA was not restricted to neurons. Nonneuronal cells of the DRG also contained the WGA tracer after axotomy. This is illustrated in Figure 4, showing, at both light and electron microscopy levels, the presence of WGA-positive satellite cells in the DRG ipsilateral to the sciatic nerve transection. As there is no evidence that these cells express NPY after (or even before) injury, the existence of WGA-positive satellite cells in the injured DRG is another illustration of intraganglionic release and local endocytosis of WGA after sciatic nerve transection.

Intraganglionic transfer of WGA is nerve injury-dependent

To determine whether the intraganglionic transfer of WGA required nerve injury, we repeated the same analysis in transgenic mice that express the WGA in the absence of nerve injury. In these experiments we crossed ZW mice with mice in which Cre is expressed in peripherin-positive DRG neurons (Per-Cre). Because peripherin is expressed in a large population of DRG neurons during development (Zhou et al., 2002), under normal conditions, i.e., in the absence of nerve injury, double transgenic Per-ZW mice express the WGA in a mixed population of large and small-diameter DRG neurons (Braz and Basbaum,

2009). Double labeling of the DRG of these Per-ZW mice with antibodies against β -gal and WGA demonstrated that only in neurons in which a Cre-recombination event had occurred (i.e., β -gal negative) was the WGA expressed (Fig. 5A–C; $0/212 \pm 63$ β -gal+/WGA+ neurons per DRG, per animal, $n = 3$). In distinct contrast, following peripheral nerve injury (axotomy), $8.8 \pm 0.6\%$ of WGA+ Δ PI Γ neurons costained for β -gal (Fig. 5D–F; $21 \pm 3/242 \pm 51$ β -gal+/WGA+ neurons per DRG, per animal, $n = 3$), indicating that the nerve injury had induced the intraganglionic transfer of the tracer.

DISCUSSION

We recently demonstrated that nerve injury-induced expression of the WGA transneuronal tracer in sensory neurons is a simple and effective genetic approach to study central nervous system (CNS) circuits engaged by sensory neurons whose peripheral axons have been damaged (Braz and Basbaum, 2009). Here we demonstrate that induction of WGA in axotomized, myelinated sensory neurons results in labeling of neighboring intact sensory neurons and surrounding satellite cells within the same ganglion, indicating that intraganglionic communication exists among injured and noninjured cells.

Nerve injury-induced intraganglionic transfer of WGA

Previous studies described ephaptic interactions between neurons, in which currents generated by a specific neuron can alter the excitability of neighboring neurons (Devor and Wall, 1990). In addition to these ephaptic interactions, the present results demonstrate that chemical, cell-to-cell communication also occurs within the ganglion. Thus, up to 15% of noninjured (β -gal-positive, but ATF3- and NPY-negative) DRG neurons immunostained for WGA, clearly indicating that the tracer was transneuronally transferred from injured (ATF3- and NPY-positive) to neighboring “intact” sensory neurons. Importantly, we readily detected WGA labeling in intact DRG neurons at least 2 weeks after the nerve injury. As 2 weeks is more than sufficient time for any Cre recombination to have occurred and for the β -gal protein to be metabolized and disappear, we are confident with our conclusion that there is indeed intercellular transfer of WGA from injured to noninjured sensory neurons. On the other hand, we only detected intercellular transfer of the tracer when the tracer expression was triggered by nerve injury. To the extent that WGA is representative of large molecule transfer in the DRG, this form of cell-to-cell communication is likely very limited in the intact DRG. We recognize, of course, that because of the detection threshold of immunolabeling, very low level release may have been missed. An interesting possibility is that because the transport of WGA is enhanced by synaptic activity (Jankowska, 1985; Valtschanoff et al., 1992), the axotomy may have facilitated detection of the intraganglionic release and transfer of the WGA from DRG cell bodies with injured peripheral axons.

To our knowledge, this is the first direct in vivo demonstration that a peripheral nerve injury triggers (or at least dramatically increases) somatic release of a macromolecule and its local endocytosis by DRG cell bodies (neurons and satellite cells). Our results suggest that this intraganglionic dialog contributes to the pathophysiological consequences of injury, for example, thermal and mechanical hypersensitivity. Indeed, our findings may provide an anatomical correlate of the in vitro electrophysiological studies of Liu et al. (2000), who found that peripheral axotomy increases subthreshold membrane potential oscillations and ectopic discharges in DRG neurons. Importantly, because we detected transneuronal transfer of WGA 2 weeks after the nerve injury (data not shown) it appears that the transfer machinery is engaged and sustained over long periods of time and may contribute to long-term DRG dysfunction.

In fact, numerous in vitro and in vivo studies (Neubert et al., 2000; Matsuka et al., 2001; Scanlin et al., 2008) have demonstrated the release of small molecules from DRG neurons (see Shinder and Devor, 1994, for a comprehensive list). Moreover, many DRG neurons express receptors for these small molecules (peptides, amino acids, etc.). These observations provide the basis for the claim that individual neurons of the DRG do not “function as independent sensory communication elements” (Amir and Devor, 1996). Our results provide further support for that perspective.

Interestingly, in a recent study we found that peripheral administration of a variety of noxious chemical stimuli (e.g., capsaicin and mustard oil) induced expression of ATF3 (a marker of nerve injury), in lumbar DRG neurons but not only in neurons that expressed TRPV1 and TRPA1 (the receptor targets of capsaicin and mustard oil, respectively; Braz and Basbaum, 2010). Conceivably, this “indirect” expression of ATF3 in non-TRP-expressing neurons occurred secondary to the intraganglionic communication between injured and uninjured DRG neurons. Whether this involved chemical or electrical communication and whether small or large molecules are involved remains to be determined.

Crosstalk between injured and intact sensory neurons

There is considerable evidence that peripheral nerve injuries alter the properties of uninjured sensory neurons, both within and outside the ganglion that contain the cell bodies of the injured axons. For example, damage to axons of neurons located in lumbar L5 and L6 DRGs in the spinal nerve ligation model (Kim and Chung, 1992) leads to phenotypic changes in neurons located in the “spared,” i.e., uninjured, lumbar L4 ganglion (Fukuoka et al., 2001, 2002). Moreover, many electrophysiological studies have described cross-excitation in DRGs and implicated this process in the development of neuropathic pain-relevant sensory abnormalities (LaMotte et al., 1996; Amir and Devor, 2000; Liu et al., 2003; Ma et al., 2003). Here we found that signals (i.e., proteins) released from injured cell bodies can influence uninjured neurons within the same ganglion. Taken together, these results emphasize that the consequences of injury to primary afferent fibers is manifest not only as changes in activity of the injured afferents, but also of uninjured, neighboring axons (Ma et al., 2003). Of particular interest is the possibility that the macromolecular transfer is topographically organized, allowing crosstalk among functionally distinct populations of DRG neurons or whether the transfer is another form of volume conduction.

Intraganglionic neuronal-glial communication

Recently, Thalakoti et al. (2007) reported that inflammation enhanced the transfer of a dye (True Blue) from neuronal cell bodies to the surrounding glia in the trigeminal ganglia (TG), and concluded that neuron–glia signaling exists in the TG, probably via gap junctions. These authors suggested that such signaling (of small molecules) could contribute to the sensitization of pain transmission neurons (nociceptors) within the TG, and that this may be a pathophysiological contributor to migraine. In another study, Zhang et al. (2007) also provided strong evidence for neuronal–glial communication by showing that the somatic vesicular release of ATP from cultured whole DRG ganglia activated P2 $\times$ 7 receptors in satellite cells, which in turn increased the excitability of DRG neurons by locally releasing other molecules. Consistent with these findings, we also found that there was intraganglionic transfer of WGA to satellite cells in the DRG ipsilateral to the nerve injury. Thus, neuronal–glial transfer of macromolecules can also occur. However, as the transfer of WGA involves a much larger molecule, it precludes the passage of the tracer via gap junctions.

Tracing of neural circuits following nerve injury

Given that a peripheral nerve injury triggers the transneuronal transfer of WGA from injured to neighboring intact DRG neurons, the WGA labeling pattern revealed in the spinal cord (where DRG neurons terminate) may, in fact, reflect the projections that arise from both the injured (WGA-expressing first-order neurons) and intact DRG neurons. This would certainly complicate the interpretation of transneuronal tracing studies. For several reasons, however, we believe that this is not the case. First, the number of WGA-labeled intact DRG neurons (after intraganglionic transfer) is relatively small (up to 15%, 2 weeks after the injury) which, given the fact that the detection of transneuronally transferred WGA is concentration-dependent, would only account for a very small amount of the spinal cord labeling. Second, because only the first-order neuron expresses the WGA, there is dilution of the tracer at each stage of transneuronal transport. Thus, the likelihood of detecting spinal cord transneuronal transfer of WGA from intact DRG neurons (which in this case would correspond to third-order neurons in the spinal cord) is very small. Furthermore, because the transneuronal transfer of the tracer is also time-dependent, we agree that an analysis of the spinal cord labeling patterns at earlier timepoints should reflect a more accurate picture of projections from noninjured DRG neurons. In fact, we previously reported that the expression of the tracer in the ZW-X mouse is initiated as early as 1 day postinjury and its transneuronal transfer can be detected as soon as 3 days postinjury (Braz and Basbaum, 2009).

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LITERATURE CITED

- Allen JM, Yeats JC, Adrian TE, Bloom SR. Radioimmunoassay of neuropeptide Y (NPY). *Regul Pept.* 1984; 8:61–70. [PubMed: 6546997]
- Amir R, Devor M. Chemically mediated cross-excitation in rat dorsal root ganglia. *J Neurosci.* 1996; 16:4733–4741. [PubMed: 8764660]
- Amir R, Devor M. Functional cross-excitation between A- and C-neurons in dorsal root ganglia. *Neuroscience.* 2000; 95:189–195. [PubMed: 10619475]
- Braz JM, Basbaum AI. A genetically expressed transneuronal tracer reveals direct and indirect serotonergic descending control circuits. *J Comp Neurol.* 2008; 507:1990–2003. [PubMed: 18273889]
- Braz JM, Basbaum AI. Triggering genetically-expressed transneuronal tracers by peripheral axotomy reveals convergent and segregated sensory neuron-spinal cord connectivity. *Neuroscience.* 2009; 163:1220–1232. [PubMed: 19647044]
- Braz JM, Basbaum AI. Differential ATF3 expression in dorsal root ganglion neurons reveals the profile of primary afferents engaged by diverse noxious chemical stimuli. *Pain.* 2010; 150:290–301. [PubMed: 20605331]
- Braz JM, Rico B, Basbaum AI. Transneuronal tracing of diverse CNS circuits by Cre-mediated induction of wheat germ agglutinin in transgenic mice. *Proc Natl Acad Sci U S A.* 2002; 99:15148–15153. [PubMed: 12391304]
- Braz JM, Nassar MA, Wood JN, Basbaum AI. Parallel “pain” pathways arise from subpopulations of primary afferent nociceptor. *Neuron.* 2005; 47:787–793. [PubMed: 16157274]
- DeFalco J, Tomishima M, Liu H, Zhao C, Cai X, Marth JD, Enquist L, Friedman JM. Virus-assisted mapping of neural inputs to a feeding center in the hypothalamus. *Science.* 2001; 291:2608–2613. [PubMed: 11283374]
- Devor M, Wall PD. Cross-excitation in dorsal root ganglia of nerve-injured and intact rats. *J Neurophysiol.* 1990; 64:1733–1746. [PubMed: 2074461]

- Fukuoka T, Kondo E, Dai Y, Hashimoto N, Noguchi K. Brain-derived neurotrophic factor increases in the uninjured dorsal root ganglion neurons in selective spinal nerve ligation model. *J Neurosci*. 2001; 21:4891–4900. [PubMed: 11425916]
- Fukuoka T, Tokunaga A, Tachibana T, Dai Y, Yamanaka H, Noguchi K. VR1, but not P2X(3), increases in the spared L4 DRG in rats with L5 spinal nerve ligation. *Pain*. 2002; 99:111–120. [PubMed: 12237189]
- Hubert T, Bourane S, Venteo S, Mechaly I, Puech S, Valmier J, Carroll P, Fichard-Carroll A. Fibroblast growth factor homologous factor 1 (FHF1) is expressed in a subpopulation of calcitonin gene-related peptide-positive nociceptive neurons in the murine dorsal root ganglia. *J Comp Neurol*. 2008; 507:1588–1601. [PubMed: 18220257]
- Jaafari N, Khomitch-Baud A, Gilhodes JC, Hua G, Jule Y. Qualitative and quantitative analysis of tachykinin NK2 receptors in chemically defined human colonic neuronal pathways. *J Comp Neurol*. 2008; 507:1542–1558. [PubMed: 18219665]
- Jankowska E. Further indications for enhancement of retrograde transneuronal transport of WGA-HRP by synaptic activity. *Brain Res*. 1985; 341:403–408. [PubMed: 4041803]
- Kajander KC, Wakisaka S, Bennett GJ. Spontaneous discharge originates in the dorsal root ganglion at the onset of a painful peripheral neuropathy in the rat. *Neurosci Lett*. 1992; 138:225–228. [PubMed: 1319012]
- Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain*. 1992; 50:355–363. [PubMed: 1333581]
- Lieberman, AR. Sensory ganglia. In: Landon, DN., editor. *The peripheral nerve*. London: Chapman and Hall; 1976. p. 188–127.
- Liu CN, Michaelis M, Amir R, Devor M. Spinal nerve injury enhances subthreshold membrane oscillations in DRG neurons: relation to neuropathic pain. *J Neurophysiol*. 2000; 84:205–215. [PubMed: 10899197]
- Ma C, Shu Y, Chen Y, Yao H, Greenquist KW, White FA, LaMotte RH. Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons. *J Neurophysiol*. 2003; 89:1588–1602. [PubMed: 12612024]
- Matsuka Y, Neubert JK, Maidment NT, Spigelman I. Concurrent release of ATP and substance P within guinea pig trigeminal ganglia in vivo. *Brain Res*. 2001; 915:248–255. [PubMed: 11595216]
- Neubert JK, Maidment NT, Matsuka Y, Adelson DW, Kruger L, Spigelman I. Inflammation-induced changes in primary afferent-evoked release of substance P within trigeminal ganglia in vivo. *Brain Res*. 2000; 871:181–191. [PubMed: 10899285]
- Noguchi K, De Leon M, Nahin RL, Senba E, Ruda MA. Quantification of axotomy-induced alteration of neuropeptide mRNAs in dorsal root ganglion neurons with special reference to neuropeptide Y mRNA and the effects of neonatal capsaicin treatment. *J Neurosci Res*. 1993; 35:54–66. [PubMed: 7685398]
- Scanlin HL, Carroll EA, Jenkins VK, Balkowiec A. Endomorphin-2 is released from newborn rat primary sensory neurons in a frequency- and calcium-dependent manner. *Eur J Neurosci*. 2008; 27:2629–2642. [PubMed: 18513316]
- Shinder V, Devor M. Structural basis of neuron-to-neuron cross excitation in dorsal root ganglia. *J Neurocyt*. 1994; 23:515–531.
- Thalakoti S, Patil VV, Damodaram S, Vause CV, Langford LE, Freeman SE, Durham PL. Neuron-glia signaling in trigeminal ganglion: implications for migraine pathology. *Headache*. 2007; 7:1008–1023. [PubMed: 17635592]
- Tsujino H, Kondo E, Fukuoka T, Dai Y, Tokunaga A, Miki K, Yonenobu K, Ochi T, Noguchi K. Activating transcription factor 3 (ATF3) induction by axotomy in sensory and motoneurons: a novel neuronal marker of nerve injury. *Mol Cell Neurosci*. 2000; 15:170–182. [PubMed: 10673325]
- Valtschanoff JG, Weinberg RJ, Rustioni A. Peripheral injury and anterograde transport of wheat germ agglutinin-horse radish peroxidase to the spinal cord. *Neuroscience*. 1992; 50:685–696. [PubMed: 1279465]

- Wakisaka S, Kajander KC, Bennett GJ. Increased neuropeptide Y (NPY)-like immunoreactivity in rat sensory neurons following peripheral axotomy. *Neurosci Lett*. 1991; 124:200–203. [PubMed: 1712437]
- Xu GY, Zhao ZQ. Cross-inhibition of mechanoreceptive inputs in dorsal root ganglia of peripheral inflammatory cats. *Brain Res*. 2003; 970:188–194. [PubMed: 12706260]
- Zhang X, Meister B, Elde R, Verge VM, Hökfelt T. Large calibre primary afferent neurons projecting to the gracile nucleus express neuropeptide Y after sciatic nerve lesions: an immunohistochemical and in situ hybridization study in rats. *Eur J Neurosci*. 1993; 5:1510–1519. [PubMed: 7506974]
- Zhang X, Chen Y, Wang C, Huang LY. Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. *Proc Natl Acad Sci U S A*. 2007; 104:9864–9869. [PubMed: 17525149]
- Zhou L, Nepote V, Rowley DL, Levacher B, Zvara A, Santha M, Mi QS, Simonneau M, Donovan MM. Murine peripherin gene sequences direct Cre recombinase expression to peripheral neurons in transgenic mice. *FEBS Lett*. 2002; 523:68–72. [PubMed: 12123806]

Figure 1.

Axotomy induced expression of the ZW transgene in NPY-ZW-X transgenic mice. **A:** ZW-X transgenic mice carry a DNA construct in which both the LacZ (β -galactosidase-neomycin resistance fusion protein) and WGA cDNAs are under the control of the ubiquitous cytomegalovirus enhanced, chicken β -actin promoter (C β A). Under normal conditions, only the *LacZ* gene is expressed. After Cre recombination the loxP-flanked *LacZ* is excised, and the WGA is transcribed. **B:** Due to random insertion of the ZW transgene in the genome of ZW-X mice, under normal conditions sensory neurons do not express the lacZ cDNA. **C:** In contrast, after nerve injury (in this example, sciatic nerve transection), there is a very strong

induction of lacZ, ipsilateral to the axotomy. **D:** In a similar vein, under normal conditions, neuropeptide Y (NPY) is not expressed in DRG neurons. **E:** However, nerve injury induces expression of NPY in large numbers of sensory neurons, most of which are of medium-to-large diameter. **F:** In NPY-ZW-X double transgenic animals, in the absence of peripheral nerve injury, there is no expression of the transneuronal tracer WGA in sensory neurons. **G:** However, nerve injury induces strong expression of WGA in large number of sensory neurons of NPY-ZW-X mice. Scale bar = 100 μ m for B,D,F; 200 μ m for A,C,E.

Figure 2.

WGA induction in myelinated sensory neurons is Cre-mediated (in NPY-ZW-X mice). After axotomy, sensory neurons that express WGA (**A,D,G**) also express NPY (**B,C**), the neurofilament 200 marker of cell bodies with myelinated axons (NF200; **E,F**) and ATF3 (**H,I**). This illustrates that axotomy-induced expression of Cre recombinase in NPY-positive DRG neurons led to excision of the floxed lacZ cDNA and expression of WGA in myelinated (NF200-positive), injured (ATF3-positive) sensory neurons. Note, however, that some WGA-immunoreactive neurons did not express NPY or ATF3 (arrows in **C,I**, respectively), indicating that the WGA did not arise from Cre-mediated excision in these neurons but rather was transneuronally transferred from injured (WGA+/NPY+/ATF3+) to uninjured (WGA+/NPY-/ATF3-) sensory neurons (see also Fig. 3). Insets contain high magnifications of WGA/marker double-labeled cells. Scale bar = 100 μ m for **A-C**; 200 μ m for **D-I**.

Figure 3.

Intraganglionic transfer of WGA from injured to uninjured sensory neurons. Because DRG neurons cannot express simultaneously the lacZ and WGA cDNAs (see plasmid construct in Fig. 1), the presence of both WGA (red) and β -gal (green) in DRG neurons (arrows) indicates that the WGA must have been transneuronally transferred from injured (ATF3- and NPY-positive, but β -gal-negative) to uninjured (β -gal-positive and ATF3-negative) neurons, establishing intraganglionic transneuronal transfer of WGA. Insets contain high magnifications of WGA/marker double labeled cells. Scale bar = 100 μ m.

Figure 4.

Intraganglionic transfer of WGA from injured sensory neurons to surrounding satellite cells. The presence of WGA (red) in satellite cells (arrows in **A,B**) that surround sensory neurons further demonstrates that there is intraganglionic communication between the cell bodies of injured sensory neurons and nonneuronal cells following nerve injury. Nuclei are stained blue (DAPI). These results were confirmed at the electron microscopic level. **C,D**: A neuron that is WGA-immunoreactive lies adjacent to a WGA-immunoreactive satellite cell. The rectangle in C is magnified in D and highlights the satellite cell (outlined in black) and DAB immunoreaction product (arrows). Note also that an adjacent neuron (asterisk in C) is unlabeled. Scale bar = 100 μm for A; 20 μm for B; 6.2 μm for C.

Figure 5.

In the absence of nerve injury, there is no intraganglionic transfer of WGA. **A–C:** In Per-ZW mice, under normal conditions (WT), the WGA tracer (red) is expressed in a wide variety of sensory neurons, none of which express β-gal (green). **D–F:** In contrast, after peripheral nerve injury (transection of the sciatic nerve; SNC), we observed a small number of WGA+ neurons that contained β-gal (arrows and insets), reflecting thus intraganglionic transfer of WGA. Scale bar = 100 μm.

TABLE 1

Primary Antibodies

Antigen	Immunogen	Manufacturing Details	Dilution
WGA	Purified WGA	Sigma, rabbit, catalogue #T4144	1:50,000
WGA	Purified WGA	Vector Lab. Inc., goat, catalogue #AS2024	1:500
NPY	Porcine NPY (1–19)	Gift from Dr. JM Allen (London, UK)	1:2,000
B-galactosidase	Full length enzyme	Abcam, chicken, catalogue #ab9361	1:6,000
NF200	C-term. pig NF H-subunit	Sigma, mouse, catalogue #N0142	1:10,000
ATF3	Human ATF3 (131–181)	Santa Cruz, rabbit, catalogue #sc-188	1:1,000