Expression and Functions of Galectin-1 in Sensory and Motoneurons

A.D. Gaudet, J.D. Steeves, W. Tetzlaff and M.S. Ramer*

ICORD (International Collaboration on Repair Discoveries), Department of Zoology, 6270 University Blvd, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4

Abstract: Galectin-1 (Gal1) was the first identified member of the galectin family of -galactosidase-binding proteins. Gal1 has important roles in processes fundamental to growth and survival of an organism, including cell adhesion, cell proliferation and apoptosis, and is expressed in many tissues, including the nervous system. In the 1980s, research focused on the developmental regulation of Gal1 expression during neurogenesis. Gal1 was found to be expressed mainly in peripherally-projecting neurons beginning early in neurogenesis, and its expression is maintained at high levels in subpopulations of these neurons in the adult rodent. Although the expression pattern of Gal1 implied that it may be involved in axonal guidance or targeting of subsets of sensory and motoneurons, possible roles of Gal1 in the nervous system had not been confirmed until recently. Gal1 has since been shown to be required for the proper guidance of subsets of primary olfactory axons (to targets in the olfactory bulb) and of primary somatosensory axons (to targets in the superficial dorsal horn). In addition, Gal1 has been implicated in the regenerative response of axons following peripheral nerve injury. Gal1 has been shown to promote axonal regeneration through the activation of macrophages. Also, Gal1 may act within the injured neuron to enhance regrowth: the injury-induced regulation of Gal1 in numerous types of peripherally- and centrally-projecting neurons correlates positively with the regenerative potential of their axons. In this review, we discuss the expression pattern of Gal1 in sensory and motoneurons, and the potential roles of Gal1 in development, axonal regeneration and neuropathic pain.

Key Words: DRG, rhizotomy, axotomy, L-14.5, RL-14.5, axonal regeneration, neuropathic pain.

INTRODUCTION

The galectin family of proteins has the ability to bind galactosidase-containing carbohydrate structures, and it is through this specific interaction that galectins can mediate many essential processes within an organism, such as cell adhesion, cell proliferation, and apoptosis. Galectin-1 (Gal1) was the first galectin to be identified. The structure and function of Gal1 is dependent on its oxidation state: the reduced form of Gal1 is a homodimer of 14 kDa subunits and has lectin activity, whereas oxidized Gal1 is a monomer that lacks lectin activity and may instead act as a cytokine. The relative distribution of reduced and oxidized Gal1 in the nervous system has not been characterized, although oxidized Gal1 is thought to be more prevalent, based on the redox conditions in the extracellular environment. Previous studies have implicated Gal1 in the development of sub-classes of sensory neurons and their proper axonal connections, and in axonal regeneration and neuropathic pain following peripheral nerve injury. In this review, we will discuss the pattern of expression and the functions of Gal1 during neurogenesis and following nerve injury in sensory and motoneurons.

GALECTIN-1 IN PRIMARY SENSORY NEURON DEVELOPMENT

Expression Pattern of Galectin-1 During Sensory Neuron Development

Many lectins are developmentally regulated, and lectins are often found to be at highest concentrations in differenti-

differentiating tissue [1]. This generalization holds true for the regulation of Gal1 expression during sensory neuron development. Gal1 mRNA and protein are first detected in dorsal root ganglia (DRGs) of rats in early neurogenesis, at embryonic day (E) 13 [2-4]. At this stage, Gal1 immunoreactivity (-IR) is localized to sensory neuron somata within the DRG and in their central projections, some of which have grown up to the peripheral-central nervous system interface (the dorsal root entry zone, DREZ) [2, 4]. At E14, Gal1-IR is observed in growing intrasegmental dorsal root collateral axons in the dorsal root bifurcation zone[4], and is also found in afferents penetrating the dorsal horn at E16 [2]. By E18, Gal1 mRNA and protein are expressed in almost all sensory neurons, although small- and intermediate-diameter neurons, which were produced on E15 [5], have the highest Gal1-IR [3, 4]. At post-natal day (P) 0, Gal1 mRNA is detected at much higher levels in the DRG and spinal cord than in the brain, a pattern that is maintained in the adult animal [3]. Approximately, 30-40% of DRG neurons contained high Gal1 levels at P0 [4]. Within the spinal dorsal horn, sensory terminals in laminae I and II are intensely Gal1-IR, while those in laminae III-IV contain lower levels of Gal1. Other areas of the spinal cord that display Gal1-IR at P0 include sensory collaterals in the dorsal columns and motoneurons in the ventral horn [4].

Role of Galectin-1 in Sensory Neuron Development

Gal1 is localized to the nucleus and cytoplasm of DRG neurons, and is externalized by sensory neurons and Schwann cells via a non-classical pathway into the extracellular milieu [1, 6, 7]. Interestingly, early studies showed that neurons expressing Gal1 often co-express its specific lactoseries glycoconjugate [4, 8], and that embryonic DRG

*Address correspondence to this author at the ICORD, 6270 University Blvd, Vancouver, BC V6T 1Z4, Canada; Tel: +1-604-822-4956; Fax: +1-604-827-5169; E-mail: ramer@icord.org

neurons have the ability to bind Gal1 in dissociated cell culture [2]. Gal1-expressing neurons that also express its specific carbohydrate ligands are generally small-diameter and have central projections that terminate in the superficial laminae of the dorsal horn [4, 8]. Although Gal1 is expressed by nearly all DRG neurons, differential expression of its lactoseries epitope on these neurons suggests that this interaction could mediate the cross-linking of specific axonal subsets, possibly assisting in the formation of discrete nerve fascicles and terminal fields [3, 8]. Thus, the pattern of regulation of Gal1 in sensory neurons during neurogenesis suggests that the reduced form of this protein may act in concert with its complementary carbohydrate ligands to promote changes in cellular proliferation within the cell, or mediate axonal adhesion or targeting on the cell surface.

More recent studies have used the Gal1 null mutant (Gal1$^{-/-}$) mouse [9] to elucidate whether Gal1 has a role in the development of primary sensory neurons and their axonal connections. Puche *et al*. [10] studied axonal connectivity in the olfactory system of Gal1$^{-/-}$ mice, and found that a subset of primary sensory olfactory neurons failed to project to their correct targets in the caudal olfactory bulb in these mice. They also showed that Gal1 had neurite outgrowth-promoting activity when used as a substrate *in vitro*. This study was the first to implicate a lectin in the promotion of neurite outgrowth and in axonal pathfinding. Subsequent studies have demonstrated that the pattern of expression of Gal1 in the olfactory system is consistent with its proposed role in the targeting and maintenance of sensory axon connections in the mouse, rat and human [11-14].

Since Gal1 is important for development of the typical connectivity of the olfactory system, we were interested in whether Gal1 is involved in the proper development of DRG neurons and their central projections. While Gal1 is normally expressed in nearly all DRG neurons in the adult rat and mouse, Gal1-IR is especially intense in a sub-population of small-diameter somata that are implicated in transmission of nociceptive information [3, 4, 7, 15, 16]. These small-diameter, non-myelinated or thinly myelinated primary afferents comprise approximately 70% of all DRG neurons [17]. Using specific immunohistochemical markers (calcitonin gene-related peptide, CGRP; and isolectin B4, IB4), we examined the central terminal fields of small diameter, primary afferent fibres in the superficial laminae of the dorsal horn. We found that the central terminals of these small DRG neurons were significantly deeper in Gal1$^{-/-}$ mice relative to Gal1$^{+/+}$ mice [15]. Thus, axons projecting centrally from DRG neurons seem to overshoot their targets, as in the olfactory system. This suggests that these central projections are making aberrant connections in the dorsal horn.

In addition to having altered anatomy of nociceptive primary afferent terminals in the dorsal horn, Gal1$^{-/-}$ mice have a smaller proportion of nociceptive DRG neurons. We determined that Gal1$^{-/-}$ mice had a decreased proportion of a sub-population of small diameter cervical DRG neurons (IB4-binding), as well as an increased proportion of cells expressing markers for large diameter, mechanoreceptive and proprioceptive neurons (NF200-expressing) relative to Gal1$^{+/+}$ mice [15]. These results were supported by axonal counts in the Gal1$^{-/-}$ dorsal root: Gal1$^{-/-}$ mice had significantly

more myelinated axons in the dorsal root than did Gal1$^{+/+}$ mice, which is coincident with the increased proportion of large diameter somata within the DRG. Taken together, these data suggest that Gal1, which is normally expressed in a sub-population of small diameter DRG neurons, is required for the development of these neurons, or is required to specify a switch to this phenotype during development. Therefore, Gal1 is involved in the proper specification of DRG neuron phenotype during development, as well as in the targeting of central projections of nociceptive primary afferents.

The alterations in the anatomy of Gal1$^{-/-}$ DRG neurons corresponded with functional differences in the nociceptive responsiveness of Gal1$^{-/-}$ mice. Gal1$^{-/-}$ mice showed elevated thresholds in tests for responsiveness to noxious hot and cold, and also had fewer activated second order neurons in the dorsal horn following noxious temperature stimulation [15]. It was concluded that the presence of Gal1 during development is important for the specification of nociceptive DRG neurons, for the targeting of their central branches, and for the responsiveness to noxious stimuli.

In summary, although it is not essential for survival (Gal1$^{-/-}$ mice are viable), Gal1 has significant roles in the development of sensory neurons. Expression of Gal1 in the DRG begins in early neurogenesis and is maintained in nearly all DRG neurons throughout development. Gal1 can act as a substrate for neurite extension, and specific cell surface oligosaccharides expressed by sensory axons may bind Gal1 to facilitate growth and pathfinding during development. These findings are supported by the more recent studies on the altered neuroanatomy of the Gal1$^{-/-}$ mouse. Thus, Gal1 is involved in the targeting of subsets of sensory axons, and in the specification of a sub-population of DRG neurons during development.

GALECTIN-1 IN ADULT DORSAL ROOT GANG-LION NEURONS

Expression Pattern of Galectin-1 in Adult Primary Sensory Neurons

In the adult rat, Gal1 expression in the nervous system becomes localized mainly to neurons that project peripherally, although Gal1 is also found in some central tissues. Distribution of Gal1 mRNA and protein expression in sub-populations of DRG neurons during development, is essentially maintained in the adult. While Gal1 is expressed in nearly all adult DRG neurons, a sub-population of small diameter DRG neurons contains the highest levels of Gal1 mRNA and protein [3, 4, 7, 15, 16, 18-20]. High Gal1 mRNA levels were found in about 57% of cervical DRG neurons [16]. The proportion of cells with high Gal1-IR differs with the level of the neuraxis: approximately 48% of rat (and 68% of mouse) cervical DRG neurons stain intensely for Gal1 [15, 16], while between 20-26% of rat lumbar DRG neurons are Gal1-positive [7, 20]. Although the differential distribution of Gal1 may seem odd, variations in the rostro-caudal distribution of proteins in sensory ganglia at cranial and caudal levels have been described before. One example of this variation is the ATP receptor P2X3, which is expressed in a higher proportion of neurons in cervical DRGs than in lumbar DRGs [21]. Such differences are likely to reflect the

different peripheral projections of cervical and lumbar DRG neurons.

While the pattern of expression of Gal1 in the DRG has been characterized, its functions in the uninjured adult nervous system are not known. Given its role in development, it is possible that neuronal Gal1 is involved in the maintenance or plasticity of axonal connections in the adult. In addition, Gal1 has been implicated in axonal regeneration and neuropathic pain following nerve injury.

Potential Role of Galectin-1 in the Regenerative Response of Injured DRG Neurons

Injury to the peripheral projection of a sensory neuron leads to a robust regenerative response compared to axotomy of its central projection. This difference is attributable to the intrinsic response of the injured neuron itself, as well as to the extrinsic neuronal environment. Peripheral axotomy induces injured neurons to switch from 'transmission mode' to 'growth mode', a conversion that results in changes in the production of growth-associated proteins, cytoskeletal proteins, growth factor receptors, and neurotransmitters [22-26]. In contrast, axotomy of the dorsal root (dorsal rhizotomy) does not induce significant changes in the expression of many of these proteins within the injured neuron. In this section, we compile evidence that, taken together, suggests that Gal1 may act as a regeneration-associated gene within neurons following axonal injury, and will briefly consider the role of Gal1 in the environment extrinsic to the neuron.

Gal1 is regulated by peripheral nerve injury. Cameron and colleagues [27] were the first to demonstrate that density of Gal1-IR is increased in the central terminals of injured DRG neurons in the spinal dorsal horn in a model of peripheral neuropathy. In the uninjured rat spinal cord, Gal1-positive terminals are found mainly within lamina II, with diffuse staining in deeper laminae. Following chronic constriction injury, Gal1-IR is more dense in laminae I-III by 14 days and levels remain elevated for at least 70 days post-injury [27]. Similarly, Imbe *et al*.[20] found that Gal1-IR is increased in the medial portion of the superficial dorsal horn following sciatic nerve transection. In addition to studying the regulation of Gal1, Cameron *et al*. [27] found that the expression of growth-associated protein-43 (GAP-43), a protein implicated in axonal elongation and in the intrinsic regenerative response of injured neurons [28-30], is upregulated in laminae I and II of the dorsal horn from 14 to 70 days after peripheral nerve injury. When dorsal rhizotomy was performed two weeks after peripheral nerve ligation, the density of Gal1- and GAP-43-IR in the dorsal horn was significantly decreased [27]. Thus, peripheral nerve injury induces changes in the regulation of GAP-43 and Gal1 in DRG neurons that may affect the regenerative capacity of its axons.

More recently, our group has characterized changes in Gal1 expression in the somata of DRG neurons that result after axotomy of their peripheral and central projections. We found that Gal1 was present in an increased proportion of rat DRG neurons following spinal nerve, but not dorsal root axotomy (Table **1**) [16]. Gal1 mRNA, which was highly expressed in approximately 57% of uninjured DRG neurons, was highly expressed in a significantly larger proportion (approximately 80%) of DRG neurons at 7 and 14 days following spinal nerve lesion. In contrast, there was no significant change in the proportion of DRG neurons expressing Gal1 mRNA following dorsal rhizotomy. Likewise, intense Gal1-IR was observed in 48% of uninjured DRG neurons, and the proportion of DRG neurons with intense Gal1-IR increased to 65-70% of DRG neurons at 7 and 14 days post-peripheral axotomy. Dorsal rhizotomy did not induce significant changes in the expression of Gal1 protein. Peripheral nerve injury-induced upregulation of both Gal1 mRNA and protein was attributed to augmented Gal1 expression in large diameter, NF200-IR DRG neurons, which generally express low levels of Gal1 when not injured [16].

In addition to studying Gal1 expression following injury in DRG neurons, we examined Gal1 expression in the axonal environment. We confirmed that Gal1-IR is diminished significantly in the superficial layers of the dorsal horn following dorsal rhizotomy, a result that suggests that Gal1 is transported anterogradely in DRG neurons and may have roles in the maintenance or plasticity of axons in this area. Dorsal rhizotomy also induced changes in Gal1 mRNA expression within the spinal cord and the DREZ. Gal1 mRNA was upregulated significantly within degenerating sensory tracts in the dorsal horn and cuneate fasciculus at 7 and 14 days following dorsal rhizotomy [16]. Since the increase in Gal1 mRNA was confined to areas that contained axon and myelin debris, augmented Gal1 expression in cells endogenous to the spinal cord may have a role in the response of phagocytic cells to injury, like in the periphery [31]. Finally, dorsal rhizotomy also regulated Gal1 mRNA

Table 1.   Percent of Cervical DRG Neurons Positive for Galectin-1 (Gal1) mRNA and Protein Following Spinal Nerve Lesion and Dorsal Root Injury

	Uninjured	Spinal nerve lesion		Dorsal rhizotomy	
		7dpo	14dpo	7dpo	14dpo
Gal1 mRNA	57 %	*82 %	*76 %	64 %	60 %
Gal1-IR	48 %	*65 %	*71 %	53 %	49 %

Significant increases in the proportion of C7-C8 DRG somata highly expressing Gal1 mRNA and protein are observed at 7 and 14 days after peripheral axotomy relative to neurons in uninjured DRGs. In contrast, no significant changes in the percent of DRG neurons expressing Gal1 were found at 7 or 14 days following dorsal rhizotomy. * Indicates $p < 0.05$ compared to uninjured DRG. 'dpo', Days Post-Operative; '-IR', -Immunoreactivity. Data from McGraw *et al*. [16].

expression in cells within the DREZ [16]. Following dorsal rhizotomy, primary afferent fibres can regenerate up to, but not beyond the DREZ. Gal1 mRNA was increased significantly in the PNS part of the DREZ, in the dorsal root, at both 7 and 14 days following injury. It is expected that Schwann cells, which undergo proliferation and migration after nerve injury and are known to express Gal1, are responsible for the increased Gal1 mRNA in the dorsal root. In contrast, dorsal rhizotomy did not induce significant changes in the expression of Gal1 mRNA. Thus, Gal1 mRNA was upregulated in the peripheral portion of the DREZ, in which axons can regenerate, but remained at low levels in the CNS part of the DREZ, in which axonal regeneration is not supported.

There is growing evidence that Gal1 has an important role in axonal regeneration. Since the upregulation of Gal1 is positively correlated with the switch to growth mode in DRG neurons following peripheral axotomy, it is possible that this protein acts as a regeneration-associated gene intrinsically. In addition, Gal1 may have significant functions in the environment extrinsic to the injured neuron that affect its axon's ability to regenerate. The fact that Gal1 is differentially regulated in the PNS and CNS following axotomy, lends support to this idea; for instance, Horie *et al.* [31] found that Gal1 can induce macrophages to release an axonal regeneration-promoting factor (see related articles in this issue). Therefore, Gal1 may act both within the injured neuron and within its external milieu to promote axonal regeneration following nerve injury.

Potential Role of Galectin-1 in Neuropathic Pain

In addition to initiating axonal regrowth, peripheral nerve injury can cause severe, intractable pain, called neuropathic pain. There have been many mechanisms that have been proposed to underlie this condition, including spontaneous activity in injured neurons and altered function of intact neurons that interact with degenerating distal portions of injured neurons. Injury-induced changes in the neuronal expression of specific neuropeptides and their receptors, such as substance P and its receptor NK-1 [32-34], and in the expression of factors in the extracellular milieu, such as nerve growth factor [35, 36] and the pro-inflammatory cytokine interleukin-1 [37-39], have also been shown to have roles in pain in rodent models of peripheral neuropathy. It is possible that, in addition to aiding axonal elongation, Gal1 is involved in pain that results from nerve injury.

A recent study has implicated Gal1 in neuropathic pain. Imbe *et al.* [20] found that Gal1 was upregulated in the dorsal horn following peripheral nerve injury, and they were interested in whether Gal1 had an effect on the mechanical nociceptive threshold of rats. Using intrathecal administration of function-blocking anti-Gal1 antibodies, they demonstrated that Gal1 is involved in the potentiation of neuropathic pain in the dorsal horn. The increased mechanical thresholds of animals that received anti-Gal1 antibody treatment had an anatomical correlate: anti-Gal1 antibody treatment significantly attenuated the upregulation of NK-1 protein in the dorsal horn following peripheral axotomy. This result suggests that Gal1 may regulate NK-1 expression in dorsal horns indirectly, through activation of microglia

that subsequently release substances (e.g. excitatory transmitters, NO, growth factors) and induce upregulation of NK-1 [20]. Since substance P is the main excitatory neuropeptide in small diameter nociceptors that synapse in the superficial dorsal horn (an area also occupied by Gal1-IR afferents), it is therefore possible that injury-induced upregulation of NK-1 can result in increased pain. This is one mechanism by which Gal1 may act to cause pain following peripheral axotomy.

Our laboratory has studied nociceptive thresholds in Gal1$^{-/-}$ mice following peripheral nerve injury (unpublished results). We examined nociceptive thresholds for noxious heat, cold, and mechanical stimuli in the hindpaws of Gal1$^{-/-}$ and Gal1$^{+/+}$ mice at 3, 7, 14, 21, and 28 days after L5 spinal nerve ligation. As mentioned above, Gal1$^{-/-}$ mice have baseline differences in their responsiveness to noxious cold and heat. To correct for these disparities, we measured difference in scores between paws ipsilateral and contralateral to injury, in order to observe the increased sensitivity of the injured paw from an equal baseline between strains. Since Gal1 has been implicated in neuropathic pain, we expected that Gal1$^{-/-}$ mice would have a diminished responsiveness to noxious stimuli after peripheral nerve injury; however, Gal1$^{-/-}$ mice did not exhibit any significant differences in threshold between injured and uninjured paw relative to Gal1$^{+/+}$ mice (Fig. (**1**)). It would be interesting to test whether treatment of injured axons with Gal1 and anti-Gal1 antibody would affect the nociceptive thresholds of wild-type animals.

This review outlines the two studies that have investigated the role of Gal1 in neuropathic pain using behavioral testing. Given that Gal1 is upregulated in injured neurons and in their extrinsic environment following peripheral axotomy, it is quite possible that Gal1 has a significant role in neuropathic pain. Hopefully, future studies will elucidate whether this is the case.

GALECTIN-1 IN MOTONEURONS

Expression Pattern of Galectin-1 During Motoneuron Development and Adulthood

In addition to being expressed in sensory neurons, Gal1 is found in peripherally-projecting motoneurons of the spinal cord and brain. Like in DRG neurons, Gal1 mRNA is first observed in rat spinal motoneurons at E13, and is expressed at higher levels in these cells by E18 [3]. This high level of Gal1 mRNA expression is maintained in adult motoneurons. Within the brain, Gal1 transcripts were found in cranial nerve motoneurons, and at lower levels in projection neurons of the brainstem. Other areas of the brain do not express Gal1 [3].

As mentioned above, the developmental expression of Gal1 mRNA in motoneurons is maintained in the adult rat. In the adult brain, Gal1 mRNA is localized mainly in cranial nuclei of the brainstem, including the oculomotor, trigeminal, trochlear, abducens, facial, vestibular, vagus, and hypoglossal nuclei [40]. Other brainstem nuclei that were found to be positive for Gal1 mRNA included the red, reticular, and raphe nuclei [40]. Almost all other areas of the brain are devoid of Gal1 mRNA. As in the brainstem, motoneurons of the adult spinal cord contain high levels of Gal1 mRNA [3, 40, 41].
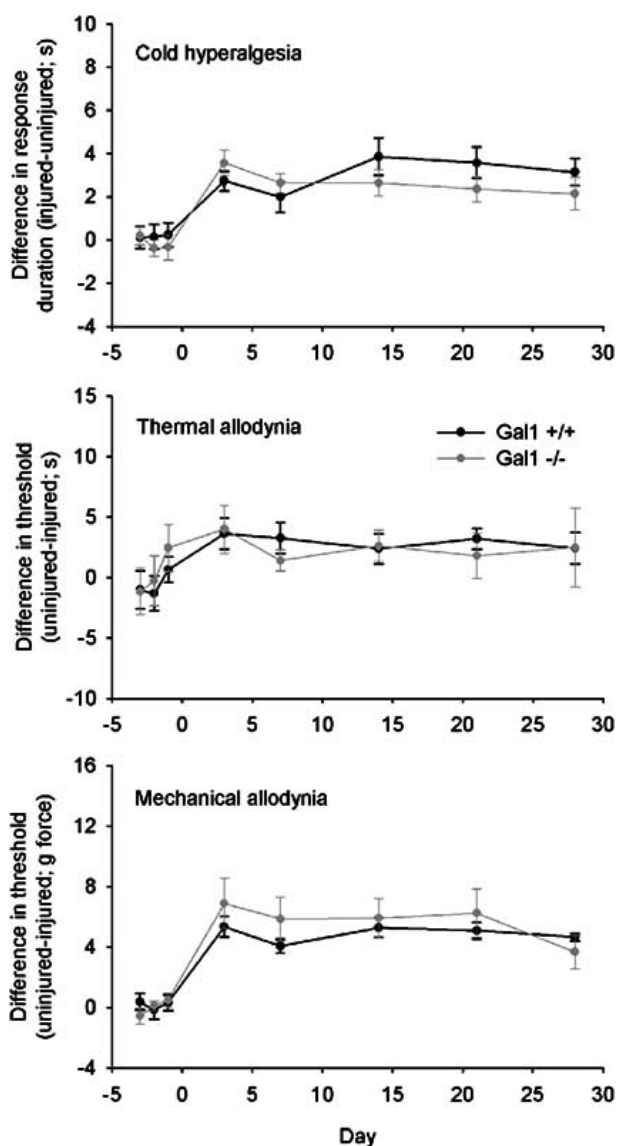
Fig. (1). Galectin-1 null mutant (Gal1$^{-/-}$) mice do not have significantly altered differences in thresholds for noxious stimuli in a model of neuropathic pain. Gal1$^{-/-}$ and Gal1$^{+/+}$ (129P3/J) mice underwent L5 spinal nerve ligation and were tested for cold hyperalgesia (acetone application, 5μl), thermal allodynia (plantar infrared laser), and mechanical allodynia (dynamic plantar aesthesiometer) three times pre-operatively and at 3, 7, 14, 21, and 28 days post-axotomy. Differences in ipsilateral and contralateral hindpaw scores were calculated for each mouse to account for baseline differences in the nociceptive thresholds of Gal1$^{-/-}$ and Gal1$^{+/+}$ mice. Although both mouse strains developed neuropathic pain states, there was no significant difference between groups. These results suggest that Gal1 is not required for the neuropathic pain states that often accompany peripheral nerve injury.

Role of Galectin-1 in the Regenerative Response of Injured Motoneurons

Gal1 has been shown to have a role in axonal regeneration following crush injury to the facial nerve. Nearly all axons that contribute to this nerve are derived from moto-

neurons, and functional reconnection can be assessed by recovery of vibrissae movement [42]. Following crush injury, Gal1 mRNA is upregulated in injured facial motoneurons by 6 hours [40], and increased Gal1 expression is observed until 7 days [43]. At 14 days post-axotomy, Gal1 levels are not significantly different from the contralateral nucleus. In contrast, the upregulation of Gal1 mRNA persists for at least 14 days following resection of the facial nerve [43]. We found that Gal1$^{+/+}$ mice recovered whisking functions (frequency and angle) by 11-12 days post-injury. Interestingly, mice lacking Gal1 recovered whisking function, significantly later than Gal1$^{+/+}$ mice [43]. Thus, the upregulation of Gal1 soon after injury, and its subsequent downregulation between 7-14 days later in neurons that are allowed to regenerate, suggest that Gal1 is involved in the outgrowth of injured axons. Gal1 mRNA may then be decreased upon target reinnervation.

To determine whether target-derived factors and/or trophic factors have a role in the regulation of Gal1, we injected colchicine, which arrests axonal transport, and glial cell line-derived neurotrophic factor, which is a trophic factor that is released following injury, into uninjured Gal1$^{+/+}$ mice. We found that animals treated with either factor displayed increased levels of Gal1 mRNA [43], implying that the axotomy-induced increase in Gal1 levels is due to both the loss of target-derived factors and to the upregulation of trophic factors and cytokines in the environment surrounding the injured axon.

In another recent study, we compared the injury-induced regulation of Gal1 in spinal motoneurons and rubrospinal neurons [41]. Peripheral axotomy resulted in a significant increase in the expression of Gal1 mRNA and protein in cervical spinal motoneurons at 7 and 14 days post-injury. Conversely, injury to axons projecting via the rubrospinal tract, which are contained in the CNS and do not normally regenerate, induced a significant downregulation of Gal1 mRNA in their somata within the red nucleus, at 7 and 14 days after injury (transection of the dorsolateral funiculus at C4). Given that Gal1 is downregulated in neurons of the red nucleus following CNS injury, we were interested in whether this protein is involved in the enhanced regenerative capacity of rubrospinal neurons that is observed following treatment with brain-derived neurotrophic factor (BDNF). Application of BDNF to the red nucleus has been shown to promote the regeneration of injured rubrospinal axons into peripheral nerve grafts, and results in increases in the expression of regeneration-associated genes in rubrospinal neurons [44, 45]. We found that BDNF treatment resulted in a significant increase in Gal1 mRNA in rubrospinal neurons compared to vehicle-treated animals at 7 days after injury [41]. Thus, the expression of Gal1 in rubrospinal and motoneurons is correlated with the regenerative potential of their axons into peripheral nerve environments.

CONCLUSION

Gal1 is a multi-functional protein that is developmentally regulated in the nervous system, and has roles in axonal regeneration and neuropathic pain following axotomy. During development, the reduced form of Gal1 may interact with its carbohydrate ligand on elongating axons to promote

connection with proper targets. The expression pattern of Gal1 in rat neurogenesis is consistent with its potential role in axonal pathfinding and targeting. In the adult rat, the upregulation of Gal1 in DRG and motoneurons following peripheral axotomy suggests that it may act as a regeneration-associated gene, and its increased expression in cells in peripheral nerves supports the reported role of oxidized Gal1 in promoting axonal regeneration indirectly. Finally, the differential regulation of Gal1 in rubrospinal neurons following injury to their axons, and following injury in combination with BDNF treatment, lends strong support to the hypothesis that Gal1 acts to promote axonal regeneration intrinsically. Thus, the regulation of Gal1 during sensory and motoneuron development and following nerve injury suggests that this protein may be a useful therapeutic agent for enhancing axonal regeneration in the injured PNS and CNS.

ACKNOWLEDGEMENTS

ABBREVIATIONS

BDNF	=	Brain-derived neurotrophic factor
CGRP	=	Calcitonin gene-related peptide
CNS	=	Central nervous system
dpo	=	Days post-operative
DRG	=	Dorsal root ganglion
E-	=	Embryonic day
Gal1	=	Galectin-1
GAP-43	=	Growth-associated protein (43 kDa)
IB4	=	Isolectin B4
-IR	=	Immunoreactivity
NF200	=	Heavy neurofilament (200 kDa)
P-	=	Postnatal day
PNS	=	Peripheral nervous system

REFERENCES

[1] Barondes, S.H.; Cerra, R. F.; Cooper, D. N.; Haywood-Reid, P. L. and Roberson, M. M. (1984) *Biol. Cell.*, **51**(2), 165-172.

[2] Dodd, J. and Jessell, T. M. (1986) *J. Exp. Biol.*, **124** 225-238.

[3] Hynes, M.A.; Gitt, M.; Barondes, S. H.; Jessell, T. M. and Buck, L. B. (1990) *J. Neurosci.*, **10**(3), 1004-1013.

[4] Regan, L.J.; Dodd, J.; Barondes, S. H. and Jessell, T. M. (1986) *Proc. Natl. Acad. Sci. U. S. A.*, **83**(7), 2248-2252.

[5] Altman, J. and Bayer, S. A. (1984) *Adv. Anat. Embryol. Cell Biol.*, **85** 1-164.

[6] Cooper, D.N. and Barondes, S. H. (1990) *J. Cell Biol.*, **110**(5), 1681-1691.

[7] Sango, K.; Tokashiki, A.; Ajiki, K.; Horie, M.; Kawano, H.; Watabe, K.; Horie, H. and Kadoya, T. (2004) *Eur. J. Neurosci.*, **19**(1), 55-64.

[8] Dodd, J. and Jessell, T. M. (1985) *J. Neurosci.*, **5**(12), 3278-3294.

[9] Poirier, F. and Robertson, E. J. (1993) *Development*, **119**(4), 1229-1236.

[10] Puche, A.C.; Poirier, F.; Hair, M.; Bartlett, P. F. and Key, B. (10-10-1996) *Dev. Biol.*, **179**(1), 274-287.

[11] Crandall, J.E.; Dibble, C.; Butler, D.; Pays, L.; Ahmad, N.; Kostek, C.; Puschel, A. W. and Schwarting, G. A. (2000) *J. Neurobiol.*, **45**(4), 195-206.

[12] Heilmann, S.; Hummel, T.; Margolis, F. L.; Kasper, M. and Witt, M. (2000) *Histochem. Cell Biol.*, **113**(3), 241-245.

[13] St John, J.A. and Key, B. (11-18-1999) *Brain Res. Dev. Brain Res.*, **117**(2), 171-178.

[14] Tenne-Brown, J.; Puche, A. C. and Key, B. (1998) *Int. J. Dev. Biol.*, **42**(6), 791-799.

[15] McGraw, J.; Gaudet, A. D.; Oschipok, L.; Steeves, J. D.; Poirier, F.; Tetzlaff, W. and Ramer, M. S. (2005) *Pain*. In press.

[16] McGraw, J.; Gaudet, A. D.; Oschipok, L.; Kadoya, T.; Horie, H.; Steeves, J. D.; Tetzlaff, W. and Ramer, M. S. (2005) *Exp. Neurol.* Submitted.

[17] Snider, W.D. and McMahon, S. B. (1998) *Neuron*, **20**(4), 629-632.

[18] Fukaya, K.; Hasegawa, M.; Mashitani, T.; Kadoya, T.; Horie, H.; Hayashi, Y.; Fujisawa, H.; Tachibana, O.; Kida, S. and Yamashita, J. (2003) *J. Neuropathol. Exp. Neurol.*, **62**(2), 162-172.

[19] Horie, H.; Inagaki, Y.; Sohma, Y.; Nozawa, R.; Okawa, K.; Hasegawa, M.; Muramatsu, N.; Kawano, H.; Horie, M.; Koyama, H.; Sakai, I.; Takeshita, K.; Kowada, Y.; Takano, M. and Kadoya, T. (11-15-1999) *J. Neurosci.*, **19**(22), 9964-9974.

[20] Imbe, H.; Okamoto, K.; Kadoya, T.; Horie, H. and Senba, E. (12-12-2003) *Brain Res.*, **993**(1-2), 72-83.

[21] Ramer, M.S.; Bradbury, E. J. and McMahon, S. B. (2001) *J. Neurochem.*, **77**(3), 864-875.

[22] Donnerer, J. (2003) *Pharmacology*, **67**(4), 169-181.

[23] Fenrich, K. and Gordon, T. (2004) *Can. J. Neurol. Sci.*, **31**(2), 142-156.

[24] Fu, S.Y. and Gordon, T. (1997) *Mol. Neurobiol.*, **14**(1-2), 67-116.

[25] Snider, W.D.; Zhou, F. Q.; Zhong, J. and Markus, A. (2002) *Neuron*, **35**(1), 13-16.

[26] Bisby, M.A. and Tetzlaff, W. (1992) *Mol. Neurobiol.*, **6**(2-3), 107-123.

[27] Cameron, A.A.; Cliffer, K. D.; Dougherty, P. M.; Garrison, C. J.; Willis, W. D. and Carlton, S. M. (1997) *J. Comp Neurol.*, **379**(3), 428-442.

[28] Bomze, H.M.; Bulsara, K. R.; Iskandar, B. J.; Caroni, P. and Skene, J. H. (2001) *Nat. Neurosci.*, **4**(1), 38-43.

[29] Strittmatter, S.M.; Igarashi, M. and Fishman, M. C. (1994) *J. Neurosci.*, **14**(9), 5503-5513.

[30] Igarashi, M.; Li, W. W.; Sudo, Y. and Fishman, M. C. (1995) *J. Neurosci.*, **15**(8), 5660-5667.

[31] Horie, H.; Kadoya, T.; Hikawa, N.; Sango, K.; Inoue, H.; Takeshita, K.; Asawa, R.; Hiroi, T.; Sato, M.; Yoshioka, T. and Ishikawa, Y. (2004) *J. Neurosci.*, **24**(8), 1873-1880.

[32] Abbadie, C.; Brown, J. L.; Mantyh, P. W. and Basbaum, A. I. (1996) *Neuroscience*, **70**(1), 201-209.

[33] Malmberg, A.B. and Basbaum, A. I. (1998) *Pain*, **76**(1-2), 215-222.

[34] Noguchi, K.; Kawai, Y.; Fukuoka, T.; Senba, E. and Miki, K. (1995) *J. Neurosci.*, **15**(11), 7633-7643.

[35] Ramer, M.S.; Kawaja, M. D.; Henderson, J. T.; Roder, J. C. and Bisby, M. A. (7-17-1998) *Neurosci. Lett.*, **251**(1), 53-56.

[36] Ro, L.S.; Chen, S. T.; Tang, L. M. and Jacobs, J. M. (1999) *Pain*, **79**(2-3), 265-274.

[37] Sung, C.S.; Wen, Z. H.; Chang, W. K.; Ho, S. T.; Tsai, S. K.; Chang, Y. C. and Wong, C. S. (2004) *Brain Res.*, **1015**(1-2), 145-153.

[38] Sweitzer, S.; Martin, D. and DeLeo, J. A. (2001) *Neuroscience*, **103**(2), 529-539.

[39] Sweitzer, S.M.; Colburn, R. W.; Rutkowski, M. and DeLeo, J. A. (1999) *Brain Res.*, **829**(1-2), 209-221.

[40] Akazawa, C.; Nakamura, Y.; Sango, K.; Horie, H. and Kohsaka, S. (2004) *Neuroscience*, **125**(1), 171-178.

[41] McGraw, J.; Oschipok, L. W.; Liu, J.; Hiebert, G. W.; Mak, C. F. W.; Horie, H.; Kadoya, T.; Steeves, J. D.; Ramer, M. S. and Tetzlaff, W. (2004) *Neuroscience*, **128**(4), 713-719.

[42] Isokawa-Akesson, M. and Komisaruk, B. R. (1987) *Exp. Brain Res.*, **65**(2), 385-398.

[43]   McGraw, J.; McPhail, L. T.; Oschipok, L. W.; Horie, H.; Poirier, F.; Steeves, J. D.; Ramer, M. S. and Tetzlaff, W. (2004) *Eur. J. Neurosci.*, **20**(11), 2872-2880.

[44]   Kobayashi, N.R.; Fan, D. P.; Giehl, K. M.; Bedard, A. M.; Wiegand, S. J. and Tetzlaff, W. (1997) *J. Neurosci.*, **17**(24), 9583-9595.

[45]   Kwon, B.K.; Liu, J.; Messerer, C.; Kobayashi, N. R.; McGraw, J.; Oschipok, L. and Tetzlaff, W. (2002) *Proc. Natl. Acad. Sci. USA*, **99**(5), 3246-3251.