

Primary Motor Neurons Fail To Up-Regulate Voltage-Gated Sodium Channel Na_v1.3/Brain Type III Following Axotomy Resulting From Spinal Cord Injury

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Epilepsy occurs in a small proportion of patients with spinal cord injury (SCI), but whether it is due to concomitant traumatic head injury or to changes in cortical motor neurons secondary to axotomy within the spinal cord is not known. Na_v1.3/brain type III sodium channel expression is up-regulated following peripheral axotomy of dorsal root ganglion (DRG) and facial motor neurons, but, to date, Na_v1.3 expression has not been examined in upper (cortical) motor neurons following axotomy associated with SCI. In the present study, we examine Na_v1.3 expression in upper motor neurons within rat primary motor cortex following midthoracic (T9) dorsal column transection, which severs the axons of those cells. Axotomized pyramidal cells were identified by retrograde transport of fluorogold. Immunolabeled cells were confined to layer V of the primary motor cortex and exhibited low levels of Na_v1.3 staining. After axotomy, no significant changes were detected in Na_v1.3 density or distribution in injured or uninjured cells, compared with control brains, in contrast to up-regulation of Na_v1.3 in ipsilateral DRG neurons after sciatic nerve transection. These results do not preclude a role for voltage-gated sodium channels in post-SCI epilepsy but suggest that up-regulated expression of Na_v1.3 channel is not involved.

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Voltage-gated sodium channels are responsible for the generation and propagation of action potentials in neurons, and various subtypes of these channels are preferentially expressed at different stages of development in the nervous system. In the rat brain, at least four different subtypes are widely expressed to varying degrees (see Goldin et al., 2000; Novakovic et al., 2001), including the tetrodotoxin (TTX)-sensitive (TTX-S) Na_v1.3/brain type III sodium channel. Na_v1.3 mRNA is expressed at relatively high levels in embryonic dorsal root ganglion (DRG) neurons but is barely detectable by postnatal day

30 (Waxman et al., 1994; Felts et al., 1997). In the adult rat brain, only low levels of Na_v1.3 are detectable (Beckh et al., 1989; Brysch et al., 1991; Furuyama et al., 1993).

After injury, however, changes in channel deployment and distribution can occur in adult neurons. For example, up-regulated expression of Na_v1.3 mRNA and protein is observed in DRG neurons of adult rats following axotomy of their peripheral projections (Waxman et al., 1994; Dib-Hajj et al., 1996; Black et al., 1999) and after tight spinal nerve ligation (Kim et al., 2001). Facial nerve transection also results in increased Na_v1.3 mRNA in secondary lower motor neurons within the facial nucleus (Iwahashi et al., 1994). This reconfiguration of sodium channel subtypes likely contributes to the development of abnormal spontaneous discharges (Devor et al., 1992; Matzner and Devor, 1992). Indeed, axotomy of adult DRG neurons results in the up-regulation of a TTX-S current associated with Na_v1.3 that exhibits rapid repriming, which allows firing at higher-than-normal frequencies (Cummins and Waxman, 1997; Cummins et al., 2001).

The relative contribution of Na_v1.3 to neuronal excitability has not been studied in other forms of nervous system trauma. After brain (Bush et al., 1999; Graber et al., 1999) and spinal cord (Topka et al., 1991) injury (SCI), cortical neurons can become hyperexcitable, raising the possibility that sodium channel dysregulation underlies changes in their firing properties. As the major projection neurons of the cerebral cortex, pyramidal cells, including pyramidal neurons of the primary motor cortex (M1), send

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axon collaterals to local cortical regions (Aroniadou and Keller, 1993; Deuchars et al., 1994; Landry et al., 1994) that play an important role in the collective electrical activity of cortical neuron ensembles and in the establishment and spread of seizure activity during epilepsy (Franceschetti et al., 1995; Prince and Jacobs, 1998; Bush et al., 1999; Hablitz and DeFazio, 2000; Kharazia and Prince, 2001). Given that sodium channels have been implicated in certain forms of epilepsy (Bartolomei et al., 1997; Escayg et al., 2000; Whitaker et al., 2001; for review see Moulard et al., 2001) and that antiepileptic drugs target sodium channels (for reviews see Ragsdale and Avoli, 1998; White, 1999), we tested the hypothesis that expression of Na_v1.3 channels would be up-regulated in M1 pyramidal cells following injury to their axons within the spinal cord.

MATERIALS AND METHODS

Animal Care

Experiments were carried out in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and all animal protocols were approved by the Yale University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (150–175 g) were used for this study. Animals were housed under a 12 hr light-dark cycle in a pathogen-free area, with free access to water and food, at the VA Healthcare System (West Haven, CT).

Sciatic Nerve Axotomy and Dorsal Column Lesions

For sciatic nerve axotomy, adult male Sprague-Dawley rats ($n = 6$) were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (5 mg/kg) intraperitoneally. The right sciatic nerve was exposed at the midhigh level, tightly ligated with 4-0 silk suture near the sciatic notch proximal to the pyriform ligament, transected distal to the ligature, and the proximal stump was placed in a silicon cuff to prevent regeneration (Waxman et al. 1994). The cuff contained the active ingredient in fluorogold, hydroxystilbamine methanesulfonate (4% wt/vol in dH₂O; Molecular Probes, Eugene, OR), for retrograde labeling of axotomized DRG neurons. The intact contralateral sciatic nerves served as controls. The overlying muscles and skin were closed in layers with 4-0 silk sutures and skin staples, respectively, and the animal was allowed to recover.

Selective lesioning of the dorsal column (DC), which contains the descending axons of upper (cortical) motor neurons in M1, was performed in different animals ($n = 6$). Six additional animals underwent laminectomy only and served as controls. After induction of deep anesthesia and a T9 laminectomy, the area between the dorsal root entry zones was transected with ophthalmic microscissors, producing a complete bilateral DC lesion (Neumann and Woolf, 1999). Care was taken not to injure adjacent blood vessels. Gelfoam (Pharmacia and Upjohn, Kalamazoo, MI) impregnated with 5 μ l hydroxystilbamine methanesulfonate (4% in dH₂O) was carefully placed into the lesion cavity to label axotomized corticospinal tract (CST) neurons retrogradely, and the surgical site was closed. This surgery resulted in paraparesis in all animal subjected to the procedure but did not prevent locomotion or impair eating, drinking, or

elimination; weight loss was only transient. The extent of the lesion laterally and in a dorsoventral direction down to the central canal was confirmed at the end of the study by histology.

Seven to eleven days after sciatic nerve injury or DC transection surgery, rats were sacrificed with an overdose of ketamine/xylazine and perfused with 0.9% saline, followed by 4% paraformaldehyde in 0.14 M Sorensen's phosphate buffer (pH 7.4). Brain and L4–L6 DRG tissue was collected after 30 min of fixation. Tissue cryopreserved in 30% sucrose was used for immunocytochemical studies.

Antibody Characterization

A site-directed polyclonal antibody (16153#3) was generated against a polypeptide sequence [(C)HLEGNHRADGDRFP; 511–524] within the intracellular loop connecting domains I and II of the rat Na_v1.3 sodium channel. The peptide was conjugated with KLH and injected into rabbits (Zymed, South San Francisco, CA). The antiserum was purified on a peptide affinity column prior to use. Antibody specificity was established by immunostaining Na_v1.3-transfected human embryonic kidney (HEK) cells; preadsorption of the antibody with cognate peptide eliminated Na_v1.3 immunolabeling.

Immunocytochemistry

Thin cryosections (12 μ m) of L4–L6 DRG and brain tissue from perfused rats were mounted on Fischer Superfrost/Plus glass slides and run in parallel for detection of Na_v1.3 protein. Slides were incubated sequentially at room temperature in 1) blocking solution consisting of phosphate-buffered saline (PBS) containing 5% normal goat serum, 2% bovine serum albumin (BSA), 0.1% Triton X-100, and 0.02% sodium azide, 30 min; 2) primary Na_v1.3 antibody (16153#3, 1:2,000), overnight in blocking solution; 3) PBS, six times for 5 min each; 4) goat anti-rabbit IgG-Cy3 (1:3,000; Amersham Pharmacia Biotech, Piscataway, NJ) in blocking solution, 2 hr; and 5) PBS, six times for 5 min each. Slides were mounted with Aqua-Polymount (Polysciences, Warrington, PA). Control experiments included incubation without primary or secondary antibodies, which yielded only background levels of signal.

Quantitative Analysis

A Nikon Eclipse E800 light microscope was used for sample observation using 20 \times objective lenses. Fluorescent images were digitally captured with a Dage-MTI DC330 camera. Quantitative microdensitometry was performed (see Black et al. 1997; Fjell et al. 1999b) using IPLab Spectrum software (Scanalytics, Fairfax, VA). Fluorescent signal intensity was obtained by manually outlining individual cells ($n = 20$ –40 cells per DRG or brain per animal) and using IPLab integrated densitometry functions to calculate the mean signal intensity for the selected area. In DRG tissue, cells were sampled only when the nucleus was visible within the plane of section. Background levels of signal were subtracted, and control and experimental conditions were evaluated in identical manners. Images were arranged with Adobe Photoshop 5.5. Superimposition of cortical layers with specific brain regions was performed from anatomical landmarks using stereological atlas images (Paxinos and Watson, 1998). Statistical comparisons of control and experimental groups were performed with a two-sample *t*-test using an alpha level of

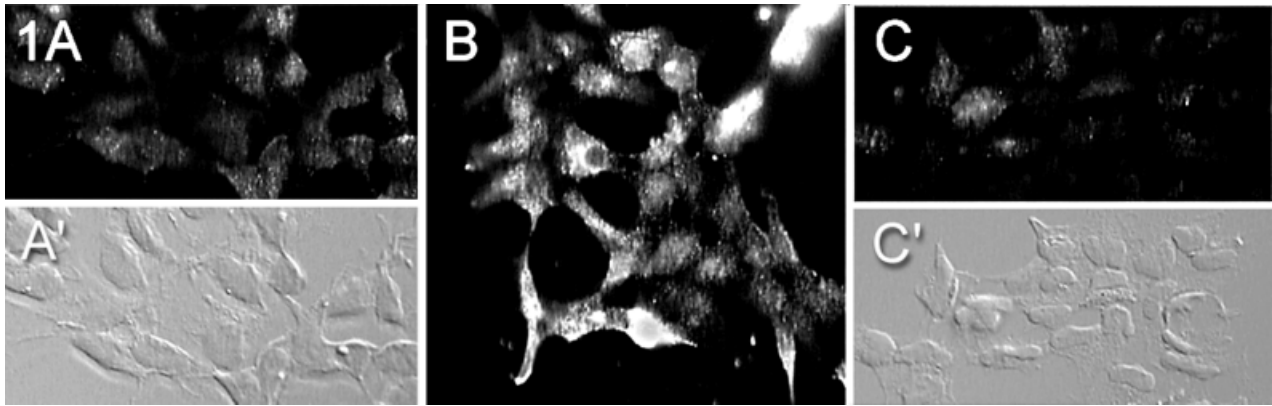


Fig. 1. Antibody 16153#3 immunolabeling of sodium channel $\text{Na}_v1.3$ -transfected HEK cells. Non-transfected HEK cells exhibit only background levels of immunofluorescence (A), whereas HEK cells stably transfected with $\text{Na}_v1.3$ exhibit robust immunolabeling with antibody 16153#3 (B). Preadsorption of antibody 16153#3 with cognate peptide eliminated immunostaining in stably transfected HEK cells (C). A' and C' show differential interference contrast images of fluorescent images shown in A and C, respectively.

significance of 0.05 and were plotted using Jandel Sigmaplot 2001 software (version 7.0). All data are expressed as mean \pm standard deviation (SD).

RESULTS

Antibody Characterization

To establish the specificity of antibody 16153#3 against sodium channel $\text{Na}_v1.3$, HEK cells stably transfected with $\text{Na}_v1.3$ were immunoreacted with the antibody. Nontransfected HEK cells exhibited only background levels of immunofluorescence with antibody 16153#3; in contrast, $\text{Na}_v1.3$ -transfected HEK cells displayed robust immunolabeling with the antibody (Fig. 1A,B). Preadsorption of antibody 16153#3 with 200-fold molar excess of cognate peptide eliminated immunolabeling of the transfected cells (Fig. 1C). Moreover, cryosections of adult rat cerebellum and spinal cord reacted with antibody 16153#3 did not display immunofluorescence above background levels (data not shown).

Immunocytochemistry and Quantification

Bilateral transection of the spinal cord DC at T9 results in robust retrograde labeling of the cell bodies of these axons within the primary motor cortex (M1) from Bregma -0.3 through -1.3 at 7–11 days following axotomy (Fig. 2A). Fluorogold-positive backfilled cells demonstrated morphologies consistent with their classification of pyramidal cells, characterized as pyramid-shaped cell bodies that give rise to a singular apical dendrite and smaller basal dendrites (Fig. 2B), and were confined to the inner pyramidal layer (layer V) of M1 (Fig. 2C).

Representative images documenting $\text{Na}_v1.3$ staining in control and axotomized DRG, and control and brain from spinally injured animals are shown in Figure 3. In DRG neurons ipsilateral to the sciatic nerve axotomy (Fig. 3A) but not in the contralateral DRG (Fig. 3B), increased

levels of $\text{Na}_v1.3$ immunoreactivity are observed 7 days after sciatic nerve ligation and transection. Extremely weak $\text{Na}_v1.3$ immunoreactivity is observed within all cortical layers in control brains, and it appears to be expressed in all cell types at very low levels (Fig. 3C). In brains examined 7–11 days after midthoracic transection SCI, $\text{Na}_v1.3$ immunoreactivity is relatively unchanged within the M1 region of the cortex (Fig. 3D). In injured animals, neither density nor distribution of $\text{Na}_v1.3$ -positive neurons appears to be different compared with uninjured controls.

Quantification reveals that, after sciatic nerve lesion, $\text{Na}_v1.3$ staining intensity is significantly ($P < 0.01$) increased in the ipsilateral DRG neurons (61.22 ± 13.93 arbitrary units) compared with neurons within the uninjured contralateral side (19.68 ± 6.702 units; Fig. 4A). In contrast, no significant changes ($P = 0.496$) are observed in $\text{Na}_v1.3$ staining intensity in the brain following midthoracic DC axotomy in either nonbackfilled or fluorogold-positive backfilled cells (30.21 ± 14.66 and 29.18 ± 15.10 units, respectively) compared with control animals (28.93 ± 9.065 units; Fig. 4B).

DISCUSSION

In the present study, we examined changes in $\text{Na}_v1.3$ expression in upper (cortical) motor neurons following midthoracic DC transection as occurs following SCI. We present data showing that $\text{Na}_v1.3$ protein is not up-regulated in these axotomized cells at 7–11 days after injury; time points at which changes are observed to peak in DRG cells (Dib-Hajj et al., 1996; Black et al., 1999).

The very low level of expression of $\text{Na}_v1.3$ protein in our study agrees with the weak expression of $\text{Na}_v1.3$ mRNA reported in the cerebral cortex by in situ hybridization (Brysch et al., 1991; Furuyama et al., 1993). In these studies, $\text{Na}_v1.3$ detection is confined to neither

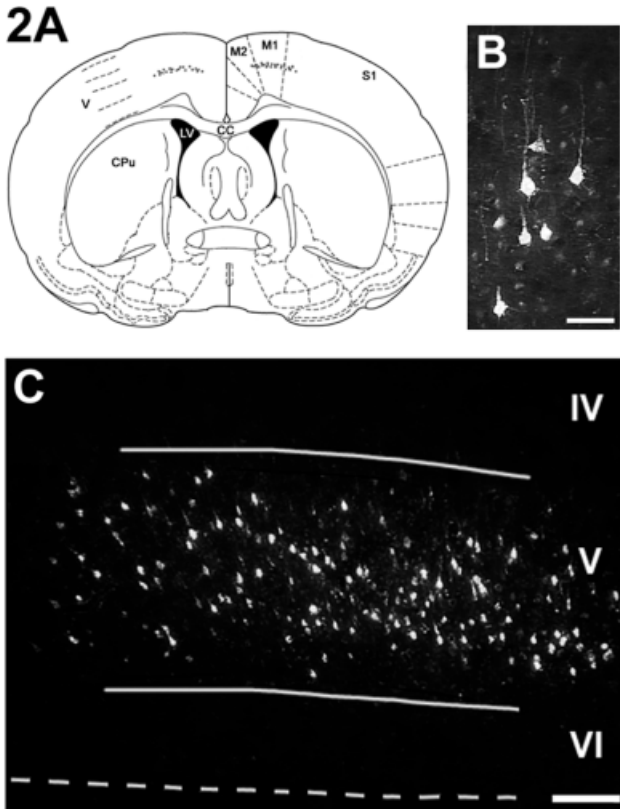


Fig. 2. Schematic representation of a coronal atlas section (Paxinos and Watson, 1997) of rodent brain corresponding to Bregma -0.3 illustrating the location of fluorogold-positive backfilled neurons 7–11 days following dorsal column transection (A). High magnification shows a large apical dendrite emerging dorsally from pyramidal cells in this region of typical morphology (B). Cells are clustered and confined to layer V (V) of the primary motor cortex (M1), shown in relation to adjacent structures, such as the secondary motor cortex (M2), primary somatosensory cortex (S1), caudate putamen (Cpu), lateral ventricle (LV), and corpus callosum (CC). Photodocumentation demonstrates robust retrograde labeling of pyramidal neurons in layer V (C). Scale bars = 20 μ m in B; 100 μ m in C.

particular cortical layers nor particular cell types and is observed in layers II–IV, although a subcellular localization is as yet unknown. It can be inferred that pyramidal cells in layer V express very low levels of Na_v1.3 based on the unique morphological features of this cell class, which include an apical dendrite that extends toward the cortical surface, oblique dendrites that extend laterally from the shaft, and basal dendrites that exit the lower aspects of the cell. Some cells that appear to be nonpyramidal also display weak Na_v1.3 immunolabeling. In the human central nervous system, Na_v1.3 has recently been studied. The structure of Na_v1.3 and its distribution in the human brain resembles that in the rat brain, although levels of expression are seemingly more abundant in adult human brain compared with rat brain (Chen et al., 2000; Whitaker et al., 2000). One report suggests that Na_v1.3 protein is

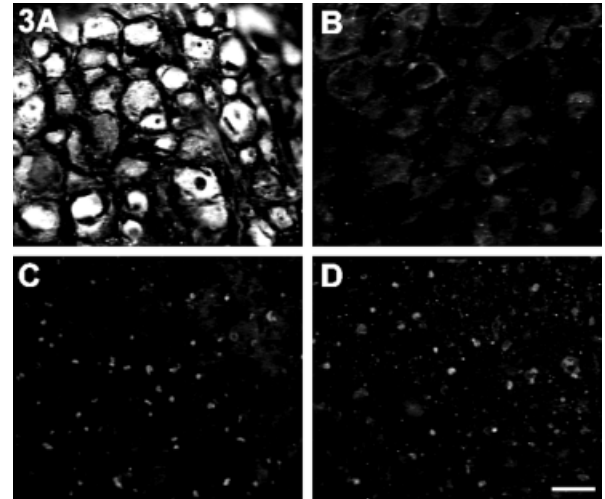


Fig. 3. Photomicrographs of Na_v1.3 immunoreactivity in L4–L6 DRGs of the ipsilateral (A) and contralateral (B) sides 7 days following sciatic nerve ligation, and transection and of coronal sections through M1 motor cortex in control animals (C) and 7–11 days following T9 dorsal column transection SCI (D). After sciatic nerve injury, signal intensity is increased in the ipsilateral DRG, whereas, on the contralateral side, very little signal is detectable. A low level of Na_v1.3 immunostaining is observed in both control and injured brains within the M1 region of the cortex, which is relatively unchanged after DC lesion. Scale bar = 50 μ m.

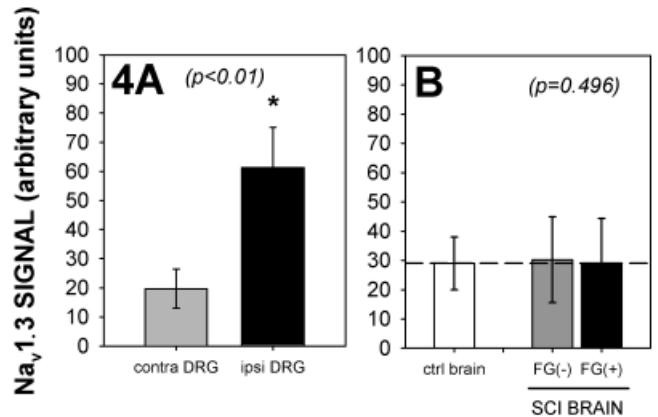


Fig. 4. Quantification of mean \pm SD Na_v1.3 signal intensity 7 days following sciatic nerve ligation and transection (A) and 7–11 days following T9 dorsal column transection SCI (B). In the ipsilateral L4–L6 DRG, Na_v1.3 staining is significantly ($*P < 0.01$) increased compared with the uninjured contralateral side ($n = 6$ animals; 20–40 cells per DRG per animal). In contrast, after DC lesion, there were no significant changes ($P = 0.496$) in Na_v1.3 staining density observed in M1 motor cortex, in either backfilled [FG(+)] or nonbackfilled [FG(-)] cells, relative to control ($n = 6$ animals per group; 20–40 cells per brain per animal).

up-regulated in injured human nerves but not in injured TrkA-positive DRG cells (Coward et al., 2001).

The failure of axotomized upper motor neurons to up-regulate Na_v1.3 contrasts with the increased Na_v1.3

mRNA and protein expression that occur in DRG neurons following both axotomy (Waxman et al., 1994; Dib-Hajj et al., 1996; Black et al., 1999) and ligation (Kim et al., 2001) of the sciatic nerve and in facial motor neurons after transection of their axons within the facial nerve (Iwahashi et al., 1994).

The lack of Na_v1.3 up-regulation in primary motor neurons following axotomy, in comparison with DRG and facial nucleus neurons, may reflect differing effects of the injuries on the availability of neurotrophic factor(s). In particular, nerve growth factor (NGF) has been shown to modulate sodium channel expression. After peripheral nerve injury, levels of NGF (Korsching and Thoenen, 1985; Nagata et al., 1987; Zhou et al., 1994) and its high-affinity TrkA receptor (Li et al., 2001) are decreased in DRG neurons, presumably because of interrupted peripheral transport. Importantly, a link between NGF and sodium channels has been demonstrated in a variety of *in vitro* (Rudy et al., 1987; Mandel et al., 1988; Kalman et al., 1990; Omri and Meiri, 1990; Aguayo and White, 1992; Lesser and Lo, 1995; Black et al., 1997; Oyelese et al., 1997) and *in vivo* (Dib-Hajj et al., 1998; Fjell et al., 1999a) models. Moreover, it has been shown that exogenously added NGF prevents the up-regulation of Na_v1.3 in an *in vitro* model of axotomy (Black et al., 1997).

In the brain, NGF and TrkA receptors are found in the cortex (Korsching et al., 1985; Pioro and Cuello, 1990; Nishio et al., 1994; Pitts and Miller, 1995), and NGF stimulates growth of pyramidal cells as well as prevents atrophy after injury (Kolb et al., 1997a,b). However, unlike the case in the DRG, NGF levels remain unchanged in the cortex after injury. After midthoracic contusion SCI, which typically transects the CST, Liebl et al. (2001) report no significant changes in TrkA, TrkB, or TrkC mRNA expression in the motor cortex. In support of these results, others show that hypoglossal motor neurons do not up-regulate TrkA after axonal transection (Tuszynski et al., 1996). Assuming the relative importance of NGF/TrkA in modulation of Na_v1.3 expression, it might be predicted that, because no detectable changes take place in either cortical NGF or TrkA after CST transection, it is unlikely that these neurons will undergo changes in Na_v1.3 deployment at least by this mechanism.

Alternatively, the lack of up-regulation of Na_v1.3 after axotomy of primary motor neurons may be due to the trophic-dependence hypothesis, known as the "proximity effect" (Elliott et al., 1997), which suggests that, if target-derived neurotrophic supply is interrupted from distant spinal sources, cortical motor neurons may continue to receive supplementary (or even stronger) paracrine support from one of many remaining collaterals still connected to the soma. Indeed, pyramidal neurons located in layer V are in extensive contact with cells in cortical layers I–VI (Aroniadou and Keller, 1993; Tseng et al., 1993; Landry et al., 1994; Kaneko et al., 2000; Pasikova et al., 2001), so it is conceivable that factors from supraspinal sources contribute to, or are required for, proper cell function (Schutte et al., 2000). Support for this suggestion

is provided by data showing that many adult rodent CST neurons survive axotomy at spinal levels (McBride et al., 1989), whereas more proximal transections performed at the level of the internal capsule result in greater CST cell death (Giehl and Tetzlaff, 1996; Bonatz et al., 2000).

In conclusion, the results presented here do not preclude a role for voltage-gated sodium channels in the development of epilepsy following SCI but suggest that up-regulated expression of Na_v1.3 is not involved. Future experiments will be necessary to investigate the role of spinal cord lesions in expression of voltage-gated sodium channels and effects on cortical neuronal excitability in this context.

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REFERENCES

- Aguayo LG, White G. 1992. Effects of nerve growth factor on TTX- and capsaicin-sensitivity in adult rat sensory neurons. *Brain Res* 570:61–67.
- Aroniadou VA, Keller A. 1993. The patterns and synaptic properties of horizontal intracortical connections in the rat motor cortex. *J Neurophysiol* 70:1553–1569.
- Bartolomei F, Gastaldi M, Massacrier A, Planells R, Nicolas S, Cau P. 1997. Changes in the mRNAs encoding subtypes I, II and III sodium channel alpha subunits following kainate-induced seizures in rat brain. *J Neurocytol* 26:667–678.
- Beckh S, Noda M, Lubbert H, Numa S. 1989. Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development. *EMBO J* 8:3611–3616.
- Black JA, Langworthy K, Hinson AW, Dib-Hajj SD, Waxman SG. 1997. NGF has opposing effects on Na⁺ channel III and SNS gene expression in spinal sensory neurons. *NeuroReport* 8:2331–2335.
- Black JA, Cummins TR, Plumpton C, Chen YH, Hormuzdiar W, Clare JJ, Waxman SG. 1999. Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. *J Neurophysiol* 82:2776–2785.
- Bonatz H, Rohrig S, Mestres P, Meyer M, Giehl KM. 2000. An axotomy model for the induction of death of rat and mouse corticospinal neurons *in vivo*. *J Neurosci Methods* 100:105–115.
- Brysch W, Creutzfeldt OD, Luno K, Schlingensiepen R, Schlingensiepen KH. 1991. Regional and temporal expression of sodium channel messenger RNAs in the rat brain during development. *Exp Brain Res* 86:562–567.
- Bush PC, Prince DA, Miller KD. 1999. Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. *J Neurophysiol* 82:1748–1758.
- Chen YH, Dale TJ, Romanos MA, Whitaker WR, Xie XM, Clare JJ. 2000. Cloning, distribution and functional analysis of the type III sodium channel from human brain. *Eur J Neurosci* 12:4281–4289.
- Coward K, Aitken A, Powell A, Plumpton C, Birch R, Tate S, Bountra C, Anand P. 2001. Plasticity of TTX-sensitive sodium channels PN1 and brain III in injured human nerves. *NeuroReport* 12:495–500.
- Cummins TR, Waxman SG. 1997. Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. *J Neurosci* 17:3503–3514.

- Cummins TR, Aglieco F, Renganathan M, Herzog RI, Dib-Hajj SD, Waxman SG. 2001. Na_v1.3 sodium channels: rapid repriming and slow closed-state inactivation display quantitative differences after expression in a mammalian cell line and in spinal sensory neurons. *J Neurosci* 21:5952–5961.
- Deuchars J, West DC, Thomson AM. 1994. Relationships between morphology and physiology of pyramid-pyramid single axon connections in rat neocortex in vitro. *J Physiol* 478:423–435.
- Devor M, Wall PD, Catalan N. 1992. Systemic lidocaine silences ectopic neuroma and DRG discharge without blocking nerve conduction. *Pain* 48:261–268.
- Dib-Hajj S, Black JA, Felts P, Waxman SG. 1996. Down-regulation of transcripts for Na channel alpha-SNS in spinal sensory neurons following axotomy. *Proc Natl Acad Sci USA* 93:14950–14954.
- Dib-Hajj SD, Black JA, Cummins TR, Kenney AM, Kocsis JD, Waxman SG. 1998. Rescue of alpha-SNS sodium channel expression in small dorsal root ganglion neurons after axotomy by nerve growth factor in vivo. *J Neurophysiol* 79:2668–2676.
- Elliott EJ, Parks DA, Fishman PS. 1997. Effect of proximal axotomy on GAP-43 expression in cortical neurons in the mouse. *Brain Res* 755:221–228.
- Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. 2000. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS + 2. *Nat Genet* 24:343–345.
- Felts PA, Yokoyama S, Dib-Hajj S, Black JA, Waxman SG. 1997. Sodium channel alpha-subunit mRNAs I, II, III, NaG, Na6 and hNE (PN1): different expression patterns in developing rat nervous system. *Brain Res Mol Brain Res* 45:71–82.
- Fjell J, Cummins TR, Davis BM, Albers KM, Fried K, Waxman SG, Black JA. 1999a. Sodium channel expression in NGF-overexpressing transgenic mice. *J Neurosci Res* 57:39–47.
- Fjell J, Cummins TR, Fried K, Black JA, Waxman SG. 1999b. In vivo NGF deprivation reduces SNS expression and TTX-R sodium currents in IB4-negative DRG neurons. *J Neurophysiol* 81:803–810.
- Franceschetti S, Guatteo E, Panzica F, Sancini G, Wanke E, Avanzini G. 1995. Ionic mechanisms underlying burst firing in pyramidal neurons: intracellular study in rat sensorimotor cortex. *Brain Res* 696:127–139.
- Furuyama T, Morita Y, Inagaki S, Takagi H. 1993. Distribution of I, II and III subtypes of voltage-sensitive Na⁺ channel mRNA in the rat brain. *Brain Res Mol Brain Res* 17:169–173.
- Giehl KM, Tetzlaff W. 1996. BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. *Eur J Neurosci* 8:1167–1175.
- Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. 2000. Nomenclature of voltage-gated sodium channels. *Neuron* 28:365–368.
- Graber KD, Prince DA. 1999. Tetrodotoxin prevents posttraumatic epileptogenesis in rats. *Ann Neurol* 46:234–242.
- Hablitz JJ, DeFazio RA. 2000. Altered receptor subunit expression in rat neocortical malformations. *Epilepsia* 41:82–85.
- Iwahashi Y, Furuyama T, Inagaki S, Morita Y, Takagi H. 1994. Distinct regulation of sodium channel types I, II and III following nerve transection. *Brain Res Mol Brain Res* 22:341–345.
- Kalman D, Wong B, Horvai AE, Cline MJ, O'Lague PH. 1990. Nerve growth factor acts through cAMP-dependent protein kinase to increase the number of sodium channels in PC12 cells. *Neuron* 4:355–366.
- Kaneko T, Cho R, Li Y, Nomura S, Mizuno N. 2000. Predominant information transfer from layer III pyramidal neurons to corticospinal neurons. *J Comp Neurol* 423:52–65.
- Kharazia VN, Prince DA. 2001. Changes of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors in layer V of epileptogenic, chronically isolated rat neocortex. *Neuroscience* 102:23–34.
- Kim CH, Oh Y, Chung JM, Chung K. 2001. The changes in expression of three subtypes of TTX sensitive sodium channels in sensory neurons after spinal nerve ligation. *Brain Res Mol Brain Res* 95:153–161.
- Kolb B, Cote S, Ribeiro-da-Silva A, Cuello AC. 1997a. Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. *Neuroscience* 76:1139–1151.
- Kolb B, Gorny G, Cote S, Ribeiro-da-Silva A, Cuello AC. 1997b. Nerve growth factor stimulates growth of cortical pyramidal neurons in young adult rats. *Brain Res* 751:289–294.
- Korsching S, Thoenen H. 1985. Nerve growth factor supply for sensory neurons: site of origin and competition with the sympathetic nervous system. *Neurosci Lett* 54:201–205.
- Korsching S, Auburger G, Heumann R, Scott J, Thoenen H. 1985. Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J* 4:1389–1393.
- Landry P, Wilson CJ, Kitai ST. 1984. Morphological and electrophysiological characteristics of pyramidal tract neurons in the rat. *Exp Brain Res* 57:177–190.
- Lesser SS, Lo DC. 1995. Regulation of voltage-gated ion channels by NGF and ciliary neurotrophic factor in SK-N-SH neuroblastoma cells. *J Neurosci* 15:253–261.
- Li L, Deng YS, Zhou XF. 2000. Downregulation of TrkA expression in primary sensory neurons after unilateral lumbar spinal nerve transection and some rescuing effects of nerve growth factor infusion. *Neurosci Res* 38:183–191.
- Liebl DJ, Huang W, Young W, Parada LF. 2001. Regulation of Trk receptors following contusion of the rat spinal cord. *Exp Neurol* 167:15–26.
- Mandel G, Cooperman SS, Maue RA, Goodman RH, Brehm P. 1988. Selective induction of brain type II Na⁺ channels by nerve growth factor. *Proc Natl Acad Sci USA* 85:924–928.
- Matzner O, Devor M. 1992. Na⁺ conductance and the threshold for repetitive neuronal firing. *Brain Res* 597:92–98.
- McBride RL, Feringa ER, Garver MK, Williams JK Jr. 1989. Prelabeled red nucleus and sensorimotor cortex neurons of the rat survive 10 and 20 weeks after spinal cord transection. *J Neuropathol Exp Neurol* 48:568–576.
- Moulard B, Picard F, le Hellard S, Agulhon C, Weiland S, Favre I, Bertrand S, Malafosse A, Bertrand D. 2001. Ion channel variation causes epilepsies. *Brain Res Brain Res Rev* 36:275–284.
- Nagata Y, Ando M, Takahama K, Iwata M, Hori S, Kato K. 1987. Retrograde transport of endogenous nerve growth factor in superior cervical ganglion of adult rats. *J Neurochem* 49:296–302.
- Neumann S, Woolf CJ. 1999. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* 23:83–91.
- Nishio T, Furukawa S, Akiguchi I, Oka N, Ohnishi K, Tomimoto H, Nakamura S, Kimura J. 1994. Cellular localization of nerve growth factor-like immunoreactivity in adult rat brain: quantitative and immunohistochemical study. *Neuroscience* 60:67–84.
- Novakovic SD, Eglén RM, Hunter JC. 2001. Regulation of Na⁺ channel distribution in the nervous system. *Trends Neurosci* 24:473–478.
- Omri G, Meiri H. 1990. Characterization of sodium currents in mammalian sensory neurons cultured in serum-free defined medium with and without nerve growth factor. *J Membrane Biol* 115:13–29.
- Oyelese AA, Rizzo MA, Waxman SG, Kocsis JD. 1997. Differential effects of NGF and BDNF on axotomy-induced changes in GABA(A)-receptor-mediated conductance and sodium currents in cutaneous afferent neurons. *J Neurophysiol* 78:31–42.

- Pasikova NV, Marchenko VG, Kositsyn NS. 2001. Structural bases of intracortical processes underlying the synchronization of epileptic potentials in the sensorimotor areas of the neocortex in rats. *Neurosci Behav Physiol* 31:497–501.
- Paxinos G, Watson C. 1998. *The rat brain in stereotaxic coordinates*, 4th ed. San Diego: Academic Press.
- Pioro EP, Cuello AC. 1990. Distribution of nerve growth factor receptor-like immunoreactivity in the adult rat central nervous system. Effect of colchicine and correlation with the cholinergic system. *Neuroscience* 34:57–87.
- Pitts AF, Miller MW. 1995. Expression of nerve growth factor, p75, and trk in the somatosensory and motor cortices of mature rats: evidence for local trophic support circuits. *Somatosens Mot Res* 12: 329–342.
- Prince DA, Jacobs K. 1998. Inhibitory function in two models of chronic epileptogenesis. *Epilepsy Res* 32:83–92.
- Ragsdale DS, Avoli M. 1998. Sodium channels as molecular targets for antiepileptic drugs. *Brain Res Brain Res Rev* 26:16–28.
- Rudy B, Kirschenbaum B, Rukenstein A, Greene LA. 1987. Nerve growth factor increases the number of functional Na channels and induces TTX-resistant Na channels in PC12 pheochromocytoma cells. *J Neurosci* 7:1613–1625.
- Schutte A, Yan Q, Mestres P, Giehl KM. 2000. The endogenous survival promotion of axotomized rat corticospinal neurons by brain-derived neurotrophic factor is mediated via paracrine, rather than autocrine mechanisms. *Neurosci Lett* 290:185–188.
- Topka H, Cohen LG, Cole RA, Hallett M. 1991. Reorganization of corticospinal pathways following spinal cord injury. *Neurology* 41:1276–1283.
- Tseng GF, Prince DA. 1993. Heterogeneity of rat corticospinal neurons. *J Comp Neurol* 335:92–108.
- Tuszynski MH, Mafong E, Meyer S. 1996. Central infusions of brain-derived neurotrophic factor and neurotrophin-4/5, but not nerve growth factor and neurotrophin-3, prevent loss of the cholinergic phenotype in injured adult motor neurons. *Neuroscience* 71:761–771.
- Waxman SG, Kocsis JD, Black JA. 1994. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *J Neurophysiol* 72:466–470.
- Whitaker WR, Clare JJ, Powell AJ, Chen YH, Faull RL, Emson PC. 2000. Distribution of voltage-gated sodium channel alpha-subunit and beta-subunit mRNAs in human hippocampal formation, cortex, and cerebellum. *J Comp Neurol* 422:123–319.
- Whitaker WR, Faull RL, Dragunow M, Mee EW, Emson PC, Clare JJ. 2001. Changes in the mRNAs encoding voltage-gated sodium channel types II and III in human epileptic hippocampus. *Neuroscience* 106:275–285.
- White HS. 1999. Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. *Epilepsia* 5:2–10.
- Zhou XF, Zettler C, Rush RA. 1994. An improved procedure for the immunohistochemical localization of nerve growth factor-like immunoreactivity. *J Neurosci Methods* 54:95–102.