Published in final edited form as:

Exp Neurol. 2012 February ; 233(2): 866–870. doi:10.1016/j.expneurol.2011.12.017.

Neural Cell Adhesion Molecule and its Polysialic Acid Moiety Exhibit Opposing and Linked Effects on Neuropathic Hyperalgesia

Abderrahman El Maarouf^a, Yuri Kolesnikov^b, Gavril Pasternak^b, and Urs Rutishauser^a

^aDepartments of Cell Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY, 10021, USA

^bMolecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY, 10021, USA

Abstract

Spinal lamina II, where nociceptive C-fibers terminate, expresses high amounts of the polysialylated form of neural cell adhesion molecule (PSA-NCAM). While enzymatic removal of the PSA moiety from NCAM did not affect normal sensitivity to thermal stimuli, it exacerbated nerve injury-induced neuropathic hyperalgesia. The genetic removal of the NCAM core protein also did not alter thermal sensitivity. However in the presence of a peripheral nerve injury, NCAM null-mutants exhibited a complete suppression of thermal hyperalgesia. This strong NCAM mutant phenotype appears to involve the long form of NCAM's cytoplasmic domain, in that it is duplicated by selective genetic deletion of the NCAM-180 isoform. PSA appears therefore to provide a mechanism for modulation of chronic sensory overload, by means of attenuation of the activity of the NCAM-180 isoform, which reduces nociceptive transmission.

Keywords

Nociception; thermal hyperalgesia; polysialic acid; NCAM; chronic pain

Introduction

Polysialic acid (PSA) is a long, negatively-charged glycopolymer that modulates cell-cell interactions mediated by its carrier protein, the neural cell adhesion molecule (NCAM), as well as by other adhesion molecules, such as cadherins, L1 and integrins (Rutishauser, 2008). High levels of PSA-NCAM are expressed in lamina II of the spinal dorsal horn where nociceptive C-fibers terminate (El Maarouf, et al., 2005). We have previously demonstrated

© 2011 Elsevier Inc. All rights reserved.

Correspondence: A. El Maarouf: a-el-maarouf@ski.mskcc.org, or U. Rutishauser: u-rutishauser@mskcc.org, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY, 10021, USA, Ph: 212-639-8343; Fax: 212.794.6236.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

that PSA in spinal lamina II serves to alleviate nerve lesion-induced neuropathic hyperalgesia (El Maarouf, et al., 2005), and our goal here is to further understand the mechanisms controlled by PSA that are involved in development of hyperalgesia.

The NCAM core protein is involved in synapse formation, stability and plasticity (for review, see (Garrow and El-Husseini, 2006)). Its cytoplasmic splicing isoforms are variably expressed among different synapses as well as between pre- and post-synaptic elements of the same synapse (Schuster, et al., 2001). Both NCAM and/or its PSA moiety can modulate plasticity at some but not all synapses, and in the hippocampus can have effects on long-term potentiation (LTP) (Gascon, et al., 2007, Garrow and El-Husseini, 2006, Polo-Parada, et al., 2004, Rafuse, et al., 2000). NCAM synaptic function can have significant behavioral implications, ranging from memory processes (Cremer, et al., 1994, Gascon, et al., 2007) to strenuous locomotor tasks (Polo-Parada, et al., 2004). Invertebrate homologues of NCAM (apCAM and fasciclin II) are also directly involved in morphological rearrangement of synapses (Martin and Kandel, 1996, Schuster, et al., 1996).

Given these roles in synapse structure and physiology, the NCAM core protein is well suited to be a participant in neuropathic hyperalgesia. The present study tests the hypothesis that if an increase in NCAM interactions following PSA removal is responsible for the observed increase in hyperalgesia, then the elimination of the NCAM protein should produce the opposite effect. Indeed, in contrast to the fact that removal of PSA, while leaving NCAM intact, resulted in increased hyperalgesia, deletion of the entire glycoprotein in NCAM mutant mice completely eliminated hyperalgesia. More specifically, the selective elimination of the NCAM-180 isoform is sufficient to reproduce this effect. It appears therefore that by attenuating NCAM-180 interactions, PSA provides a mechanism that reduces chronic stressful overload.

Materials and methods

Sciatic chronic constriction injury (CCI)

There are three major NCAM splicing isoforms, NCAM-120, NCAM-140 and NCAM-180 (Fig. 1A), and the present study used NCAM-null mutants (lacking all NCAM isoforms) (Cremer, et al., 1994), NCAM-180 mutants (lacking the NCAM-180 isoform only) (Tomasiewicz, et al., 1993) and wildtype littermate mice (C57Bl/6). Adult males were anesthetized (pentobarbital 50 mg/Kg; Sigma), and their left sciatic nerve was exposed. Two loose 7-0 ligatures (Ethicon, NJ) were placed around the nerve at mid-thigh about 1.5 mm from one another (El Maarouf, et al., 2005). The muscle and skin layers were then closed and the animals returned to husbandry after recovery from anesthesia. Animal care and experimentation were carried out according to institutional and NIH guidelines.

EndoN Injection

For enzymatic removal of PSA from NCAM, animals were injected intrathecally by 1 μ l of endoN (200 U/ μ l) (El Maarouf, et al., 2005). Control animals received an equal volume of vehicle solution (PBS). Animals that underwent CCI received endoN or vehicle at the time of surgery.

Assessment of hyperalgesia

Induction of neuropathic pain by CCI was evaluated by assessment of thermal hyperalgesia using the paw withdrawal test (Plantar analgesia meter, model 400 stimulator, IITC, Inc., CA). Mice were placed on a glass floor (32°C) and habituated to the test environment for ~45 min. The radiant heat source (14% active intensity) was focused onto the plantar surface of the hindpaw and the latency of limb withdrawal was recorded (Hargreaves, et al., 1988). For each test, the mean of 2 readings was considered as tolerance latency and 20 seconds as cutoff latency. Testing included 6 groups of mice with CCI [WT Vehicle (n = 9), WT EndoN (n = 10), WT (n = 8), NCAM-null (n = 8), NCAM-180-/- (n = 8) and NCAM-180-/- EndoN (n = 10)], as well as 3 groups of unoperated animals [WT Vehicle (n = 9), WT EndoN (n = 9) and NCAM-180-/-EndoN (n = 5)]. A one-way ANOVA was used for statistical comparison of measurements in the absence of CCI. Presurgical values were used as a baseline reference for post-CCI measurements, and animals were retested under the same conditions for 3 weeks after surgery. An ANOVA test for repeated measures and Dunnett's posthoc test were used for statistical comparison of post-CCI data.

PSA-NCAM immunostaining

Three additional unligated adult males from each group were anesthetized and fixed by transcardial perfusion with 25 ml of a 7.4 pH phosphate buffer saline solution followed by 25 ml of a 4% paraformaldehyde, 0.1 M, 7.4 pH phosphate buffer solution. The lumbar spinal segments (4-6) were post-fixed overnight at 4°C in the same fixative, and 50 µm vibratome transverse sections were prepared and immunostained for PSA (5A5 mouse monoclonal IgM antibody, 1:2000 and Cy3-goat anti-mouse IgM secondary antibody, 1:800) (El Maarouf, et al., 2005). Immunostaining was imaged using a Zeiss 510 confocal microscope.

Results

Thermal hyperalgesia is moderated by NCAM polysialylation in lamina II

PSA expression—As expected, PSA immunostaining of lumbar spinal cross-sections in wildtype animals showed high expression levels in the superficial layers of the dorsal horn; and intrathecal injection of endoN (200 units) completely abolished NCAM polysialylation in the spinal cord (Fig. 1A).

Thermal hyperalgesia—Our initial PSA removal studies using the hotplate test (El Maarouf, et al., 2005) were confirmed in C57Bl/6 mice using the plantar analgesia meter. The sciatic CCI procedure alone (vehicle-treated; n = 9) was found to cause significant ($p < 0.01$) shortenings in withdrawal latencies (Fig. 1C). Although PSA removal from NCAM by endoN did not alter the withdrawal latency of unoperated animals (15.3 ± 0.9 s before and 14.4 ± 1.3 s one week after injection; n = 9) (Fig. 1B), the same treatment (endoN-treated; n = 10) significantly exacerbated CCI-induced hyperalgesia (Fig. 1C, $p < 0.01$). These findings confirm that expression of PSA in the spinal cord helps to decrease hyperalgesia and keep it at levels that could be otherwise more severe.

NCAM-null mutation abolishes thermal hyperalgesia

To investigate the possible role of the NCAM polypeptide in the action of PSA removal on neuropathic hyperalgesia, the behavioral analysis was repeated using the NCAM null mutant (lacking all NCAM isoforms as well as the PSA moiety) and wildtype littermate mice carrying a sciatic CCI. During pre-CCI testings, the mean baseline latencies for hindpaw withdrawal were 14.2 ± 1.2 s for wildtype ($n = 8$) and 16.5 ± 0.8 s for NCAM-null mutant animals ($n = 8$). These values were not statistically different, indicating a normal sensitivity of NCAM-deficient animals to thermal stimuli (Fig. 1D).

As anticipated, CCI caused a significant ($p < 0.01$) drop in withdrawal latency in wildtype animals, by comparison to pre-surgical baseline (Fig. 1E). By contrast, measurements in NCAM-deficient mice revealed a complete absence of thermal hyperalgesia, with latencies being identical to baseline values for 3 weeks after CCI (Fig. 1E). It appears therefore that although NCAM is not essential for sensitivity to thermal stimuli, it plays an instrumental role in the establishment of thermal hyperalgesia following nerve injury.

Selective deletion of NCAM-180 isoform is sufficient to abolish thermal hyperalgesia

There are three major NCAM splicing isoforms, NCAM-120, NCAM-140 and NCAM-180. They share the same extracellular domains, but differ with respect to the length of their cytoplasmic tail (lipid-linked, short, long respectively) (Fig. 1A). Mice lacking all isoforms (NCAM-null) have been generated. While mice with selective ablation of NCAM-120 or 140 are not available, it has been possible to selectively target the long cytoplasmic domain of the 180 variant to generate mice that specifically lack NCAM-180, but still retain the other isoforms (Tomasiewicz, et al., 1993). In the physiological evaluation of the 180 mutants, pre-CCI latencies of paw withdrawal were similar to those of wildtype controls (Fig. 1D) (14.2 ± 1.2 s for wildtype ($n = 8$) and 15.01 ± 0.9 s for NCAM-180^{-/-} ($n = 8$)). Strikingly, post-CCI tests revealed that the absence of the 180 isoform alone is sufficient to reproduce the lack of thermal hyperalgesia observed in animals lacking all NCAM isoforms (Fig. 1E). This suggests an essential role of the NCAM-180 cytoplasmic domain in the development of this phenotype.

As shown in Figure 1A, PSA is expressed in the *substantia gelatinosa* of NCAM-180^{-/-}, thus the physiological evaluation was repeated in another group of 180 mutants that were pre-treated with endoN. Again, PSA removal did not change baseline latencies in the absence of CCI (Fig. 1D) (15.8 ± 1.1 before and 16.1 ± 1.2 s 1 week after endoN injection; $n = 5$). Interestingly, post-CCI results ($n = 10$) were identical to those obtained with NCAM-180^{-/-} without endoN, in that no hyperalgesia was measured during 3 weeks after CCI (Fig. 1E).

Discussion

The present study indicates that, as with PSA removal, NCAM deficiency does not detectably affect sensitivity to thermal stimuli in the absence of a nerve lesion. On the other hand, while PSA cleavage from NCAM in wildtypes shortens latencies to thermal stimulation in the CCI neuropathic model, loss of NCAM blocks hyperalgesia, and this

effect appears to be specific to the NCAM-180 isoform. In fact, in the absence of this isoform, the outcome of CCI is no longer sensitive to the presence or absence of PSA. That is, the action of PSA on the other isoforms does not seem to affect hyperalgesia.

This dramatic difference between the role of the core protein and that of its carbohydrate moiety is consistent with the fact that PSA can serve as an inhibitor of NCAM's function in cell-cell interactions. It is also reasonable that the genetic manipulation has a greater effect on NCAM function than that of the PSA-induced inhibition, as this glycosylation results in a reduction in but not an elimination of cell-cell interactions (Fujimoto, et al., 2001, Johnson, et al., 2004, Johnson, et al., 2005). However, as the genetic mutation eliminates NCAM from all tissues, its effect on hyperalgesia could involve additional neuronal structures or processes. NCAM deletion can be associated with increased anxiety (Conboy, et al., 2010), but as pain and anxiety usually are positively correlated (Dougher, 1979), such effects of emotion on hyperalgesia are unlikely. It is possible that a germline NCAM mutation could produce developmental changes that might affect the adult nociceptive system. However, it has been reported that interactions of glial cell line-derived neurotrophic factor (GDNF) receptor with the NCAM extracellular domain can directly attenuate hyperalgesia (Boucher, et al., 2000, Sakai, et al., 2008, Sakai and Suzuki, 2008), possibly by interfering with NCAM homophilic binding (Paratcha, et al., 2003).

All three major NCAM isoforms are expressed in the dorsal part of lumbar spinal cord in adult wildtype mice, and both the 140 and 180 can be polysialylated (Supplement 1). As reported earlier (Polo-Parada, et al., 2004), compensation mechanisms seem to upregulate PSA-NCAM-140 following deletion of the 180 isoform (Supplement 1), which would explain the similarity of immunostaining between this mutant and wildtypes. Specific NCAM-120 or 140 mutants are not available to test their respective role in hyperalgesia. Nevertheless, the fact that the selective 180 deletion is sufficient to eliminate hyperalgesia, and the presence of 120 and 140, with or without PSA, does not affect the CCI outcome, should exclude their involvement. The possibility exists that the different isoforms have different cellular localizations, and it will be important to examine their ultrastructural distribution in the dorsal horn with respect to elements involved in nociception.

A role for the NCAM polypeptide has been observed at the neuromuscular junction. As in the nociceptive system, null mutation of NCAM does not appear to alter the normal function of this synapse, but transmission failure is detected under the conditions produced by high-frequency repetitive stimulation or strenuous locomotion (Polo-Parada, et al., 2004, Rafuse, et al., 2000). The similarity in the findings in both the nociceptive and neuromuscular transmission is suggestive of a direct function for NCAM at the synapse, at least under conditions of overstimulation. Furthermore, reproduction of both phenotypes after selective deletion of the 180 isoform points to a function for its long cytoplasmic tail in the response to repetitive stimulation of these peripheral projections (Polo-Parada, et al., 2004, Polo-Parada, et al., 2005).

At the neuromuscular junction, a highly conserved C-terminal domain sequence (KENESKA) of NCAM-180 has the ability to sustain the fast vesicular replenishment necessary for synaptic transmission under over-stimulating conditions (Polo-Parada, et al.,

2005). Furthermore, RhoA-binding kinase- α (ROK- α) preferentially binds to this isoform (Buttner, et al., 2003). RhoA is also involved in thermal hyperalgesia (Inoue, et al., 2004, Ohsawa, et al., 2011), and it would therefore be interesting to examine the role of NCAM-180 interactions with the KENESKA peptide or ROK- α at nociceptive synapses in the spinal dorsal horn. Possible relationships between the NCAM-180 mechanism and other molecules involved in hyperalgesia, e.g., the tyrosine kinase Fyn (Abe, et al., 2005) and p38 mitogen-activated protein kinase (p38 MAPK) (Svensson, et al., 2003), deserve attention as well.

By virtue of its large polyanionic charge, PSA occupies enormous hydrated volumes that increase the distance between opposing membranes (Rutishauser, 2008). Thus it is also possible that PSA expression could widen the synaptic cleft and thereby affect neurotransmission. In any case, there is direct evidence that PSA can facilitate the withdrawal of non-peptidergic C-terminals from their targets in spinal lamina II that occurs with nerve lesion-induced neuropathic pain, and which in turn could alleviate hyperalgesia (Bailey and Ribeiro-da-Silva, 2006; El Maarouf, et al., 2005). Accordingly, we have found a strong correlation between levels of staining of these terminals in lamina II and hyperalgesia in NCAM-null and NCAM-180-/- as well (Supplement 2). This result is in agreement with the increased withdrawal of regenerating neuromuscular junctions (Chipman, et al., 2010) and instability of mossy fiber terminals in NCAM-null animals (De Paola, et al., 2003), as well as with the role of invertebrate homologues of NCAM (apCAM and fasciclin II) in morphological rearrangement of synapses (Martin and Kandel, 1996, Schuster, et al., 1996).

Taken together, the available information suggests that NCAM and PSA play a pivotal and opposing role in those aspects of activity that are associated with rapid or chronic neurotransmission. On one hand, the cytoplasmic domain of NCAM-180 appears to be essential for establishment or maintenance of repetitive release of neurotransmitter. On the other hand, PSA acts at the cell surface in what appears to be a protective mechanism (El Maarouf, et al., 2005, Rutishauser, 2008) to allow a reduction in excessively strong input to the CNS. Given the fact that PSA attenuates NCAM action (both extra- and intracellularly), their combination would seem particularly potent in managing this type of neural input. Of course, this model presently combines results from very different physiological contexts (tetanic neuromuscular firing and chronic nociceptive activity), and it will be important to establish whether both mechanisms exist and operate in the nociceptive system.

Conclusions

It appears in view of the present findings that by modulating the function of NCAM-180, PSA provides a mechanism to reduce sensory overload. Finally, because of the complete alleviation of CCI-induced hyperalgesia via perturbation of a specific protein domain, it may be valuable to explore these mechanisms in terms of novel approaches for treatment of neuropathic pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Abe T, Matsumura S, Katano T, Mabuchi T, Takagi K, Xu L, Yamamoto A, Hattori K, Yagi T, Watanabe M, Nakazawa T, Yamamoto T, Mishina M, Nakai Y, Ito S. Fyn kinase-mediated phosphorylation of NMDA receptor NR2B subunit at Tyr1472 is essential for maintenance of neuropathic pain. *Eur J Neurosci*. 2005; 22:1445–1454. [PubMed: 16190898]
- Bailey AL, Ribeiro-da-Silva A. Transient loss of terminals from non-peptidergic nociceptive fibers in the substantia gelatinosa of spinal cord following chronic constriction injury of the sciatic nerve. *Neuroscience*. 2006; 138:675–690. [PubMed: 16413131]
- Boucher TJ, Okuse K, Bennett DL, Munson JB, Wood JN, McMahon SB. Potent analgesic effects of GDNF in neuropathic pain states. *Science*. 2000; 290:124–127. [PubMed: 11021795]
- Buttner B, Kannicht C, Reutter W, Horstkorte R. The neural cell adhesion molecule is associated with major components of the cytoskeleton. *Biochem Biophys Res Commun*. 2003; 310:967–971. [PubMed: 14550299]
- Chipman PH, Franz CK, Nelson A, Schachner M, Rafuse VF. Neural cell adhesion molecule is required for stability of reinnervated neuromuscular junctions. *Eur J Neurosci*. 2010; 31:238–249. [PubMed: 20074227]
- Conboy L, Bisaz R, Markram K, Sandi C. Role of NCAM in emotion and learning. *Adv Exp Med Biol*. 2010; 663:271–296. [PubMed: 20017029]
- Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kraemer P, Scheff S, et al. Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature*. 1994; 367:455–459. [PubMed: 8107803]
- De Paola V, Arber S, Caroni P. AMPA receptors regulate dynamic equilibrium of presynaptic terminals in mature hippocampal networks. *Nat Neurosci*. 2003; 6:491–500. [PubMed: 12692557]
- Dougher MJ. Sensory decision theory analysis of the effects of anxiety and experimental instructions on pain. *J Abnorm Psychol*. 1979; 88:137–144. [PubMed: 447896]
- El Maarouf A, Kolesnikov Y, Pasternak G, Rutishauser U. Polysialic acid-induced plasticity reduces neuropathic insult to the central nervous system. *Proc Natl Acad Sci U S A*. 2005; 102:11516–11520. [PubMed: 16055555]
- Fujimoto I, Bruses JL, Rutishauser U. Regulation of cell adhesion by polysialic acid. Effects on cadherin, immunoglobulin cell adhesion molecule, and integrin function and independence from neural cell adhesion molecule binding or signaling activity. *J Biol Chem*. 2001; 276:31745–31751. [PubMed: 11425861]
- Gascon E, Vutskits L, Kiss JZ. Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. *Brain Res Rev*. 2007; 56:101–118. [PubMed: 17658613]
- Gerrow K, El-Husseini A. Cell adhesion molecules at the synapse. *Front Biosci*. 2006; 11:2400–2419. [PubMed: 16720322]
- Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain*. 1988; 32:77–88. [PubMed: 3340425]
- Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J, Ueda H. Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat Med*. 2004; 10:712–718. [PubMed: 15195086]
- Johnson CP, Fujimoto I, Perrin-Tricaud C, Rutishauser U, Leckband D. Mechanism of homophilic adhesion by the neural cell adhesion molecule: use of multiple domains and flexibility. *Proc Natl Acad Sci U S A*. 2004; 101:6963–6968. [PubMed: 15118102]
- Johnson CP, Fujimoto I, Rutishauser U, Leckband DE. Direct evidence that neural cell adhesion molecule (NCAM) polysialylation increases intermembrane repulsion and abrogates adhesion. *J Biol Chem*. 2005; 280:137–145. [PubMed: 15504723]
- Martin KC, Kandel ER. Cell adhesion molecules, CREB, and the formation of new synaptic connections. *Neuron*. 1996; 17:567–570. [PubMed: 8893013]
- Ohsawa M, Aasato M, Hayashi SS, Kamei J. RhoA/Rho kinase pathway contributes to the pathogenesis of thermal hyperalgesia in diabetic mice. *Pain*. 2011; 152:114–122. [PubMed: 20980102]
- Paratcha G, Ledda F, Ibanez CF. The neural cell adhesion molecule NCAM is an alternative signaling receptor for GDNF family ligands. *Cell*. 2003; 113:867–879. [PubMed: 12837245]

- Polo-Parada L, Bose CM, Plattner F, Landmesser LT. Distinct roles of different neural cell adhesion molecule (NCAM) isoforms in synaptic maturation revealed by analysis of NCAM 180 kDa isoform-deficient mice. *J Neurosci.* 2004; 24:1852–1864. [PubMed: 14985425]
- Polo-Parada L, Plattner F, Bose C, Landmesser LT. NCAM 180 acting via a conserved C-terminal domain and MLCK is essential for effective transmission with repetitive stimulation. *Neuron.* 2005; 46:917–931. [PubMed: 15953420]
- Rafuse VF, Polo-Parada L, Landmesser LT. Structural and functional alterations of neuromuscular junctions in NCAM-deficient mice. *J Neurosci.* 2000; 20:6529–6539. [PubMed: 10964958]
- Rutishauser U. Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. *Nat Rev Neurosci.* 2008; 9:26–35. [PubMed: 18059411]
- Sakai A, Asada M, Seno N, Suzuki H. Involvement of neural cell adhesion molecule signaling in glial cell line-derived neurotrophic factor-induced analgesia in a rat model of neuropathic pain. *Pain.* 2008; 137:378–388. [PubMed: 17967506]
- Sakai A, Suzuki H. NCAM as a target for GDNF-induced analgesia in neuropathic pain. *J Nippon Med Sch.* 2008; 75:136–137. [PubMed: 18648169]
- Schuster CM, Davis GW, Fetter RD, Goodman CS. Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity. *Neuron.* 1996; 17:655–667. [PubMed: 8893023]
- Schuster T, Krug M, Stalder M, Hackel N, Gerardy-Schahn R, Schachner M. Immunoelectron microscopic localization of the neural recognition molecules L1, NCAM, and its isoform NCAM180, the NCAM-associated polysialic acid, beta1 integrin and the extracellular matrix molecule tenascin-R in synapses of the adult rat hippocampus. *J Neurobiol.* 2001; 49:142–158. [PubMed: 11598921]
- Svensson CI, Hua XY, Protter AA, Powell HC, Yaksh TL. Spinal p38 MAP kinase is necessary for NMDA-induced spinal PGE(2) release and thermal hyperalgesia. *Neuroreport.* 2003; 14:1153–1157. [PubMed: 12821799]
- Tomasiewicz H, Ono K, Yee D, Thompson C, Goridis C, Rutishauser U, Magnuson T. Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system. *Neuron.* 1993; 11:1163–1174. [PubMed: 8274281]

Abbreviations

PSA	polysialic acid
NCAM	neural cell adhesion molecule
CCI	chronic constriction injury

Figure 1. PSA removal from NCAM exacerbates thermal hyperalgesia, whereas lack of all NCAM isoforms or of the 180 variant alone abolishes it

A. There are three major NCAM isoforms, 120, 140 and 180 KD. The three variants have different size cytoplasmic domains, and all three can be polysialylated. *Top*. Drawings of the 3 NCAM isoforms in the presence of PSA in the *wildtype* and without PSA after *endoN* treatment. No PSA-NCAM is present in the *NCAM-null* animal, while the NCAM-120 and 140 as well as PSA are present in *NCAM-180-/-* (Ellipse: extracellular Ig-like domains; Square: fibronectin III domain). *Bottom*. Patterns of PSA expression in cross-sections of lumbar spinal superficial laminae. In the *wildtype*, high levels of PSA immunostaining are observed in superficial laminae, including lamina II. Following *in vivo* intrathecal injection of *endoN*, PSA is removed from NCAM and immunostaining is absent. Similarly, PSA is absent from *NCAM-null* samples, however PSA immunostaining is still present in *NCAM-180-/-* spinal cords. (n = 3 for each group; dotted lines indicate lamina II; Scale bar: 200 μm).

B. In the absence of CCI, removal of PSA from NCAM does not detectably change the thermal sensitivity, in that latencies of hindpaw withdrawal are the same in the presence (WT Vehicle) or absence of PSA (WT EndoN). **C.** Sciatic CCI alone (WT Vehicle) lowers the threshold of sensitivity to thermal stimuli, and removal of PSA by endoN (WT EndoN) intensifies this hyperalgesia ($p < 0.01$). **D.** Total loss of NCAM or selective deletion of NCAM-180 does not detectably alter thermal sensitivity in the absence of CCI; hindpaw withdrawal latencies were the same in the presence (WT) or absence of NCAM (NCAM-null), as well as when only the 180 isoform was deleted (NCAM-180^{-/-}). PSA removal does not change thermal sensitivity in NCAM-180 mutants (NCAM-180^{-/-}EndoN). **E.** Sciatic CCI in wildtype (WT) animals significantly ($p < 0.01$) shortened the latency of paw withdrawal. This hyperalgesia did not occur in NCAM-null mutant (NCAM-null) animals following the same sciatic CCI; their latencies were identical to baseline values (BL) for the 3-week study period. Interestingly, selective loss of NCAM-180 was sufficient to eliminate thermal hyperalgesia; latencies of NCAM-180 mutants after CCI remained similar to baseline (BL) in the presence (NCAM-180^{-/-}) and absence (NCAM-180^{-/-}EndoN) of PSA. There was no significant difference between NCAM-null, NCAM-180^{-/-} and NCAM-180^{-/-}EndoN during the 3 weeks of the study. Error bars: SEM.