

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of April 29, 2010):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/321/5889/702>

Supporting Online Material can be found at:

<http://www.sciencemag.org/cgi/content/full/321/5889/702/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/321/5889/702#related-content>

This article **cites 23 articles**, 9 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/321/5889/702#otherarticles>

This article has been **cited by** 34 article(s) on the ISI Web of Science.

This article has been **cited by** 10 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/cgi/content/full/321/5889/702#otherarticles>

This article appears in the following **subject collections**:

Neuroscience

<http://www.sciencemag.org/cgi/collection/neuroscience>

Our data show that iPS-Hep and iPS-Stm cells are different from iPS-fibroblasts in three properties. First, iPS-Hep and iPS-Stm cells contribute to adult chimeras even with the selection for *Fbx15*. Second, no increased tumorigenicity was observed in chimera mice derived from iPS-Hep and iPS-Stm cells evaluated up to 30 weeks. These two properties are similar to iPS-fibroblast cells that we recently generated without Myc retroviruses (11). This suggests that Myc plays a smaller role in the generation of iPS-Hep and iPS-Stm cells than its role in iPS-fibroblast cells. To test this possibility, we individually omitted each of the four factors to see the effect on the generation of iPS cells from hepatocytes. When we omitted Oct3/4, Sox2, or Klf4, no iPS cell colonies emerged (fig. S12A). By contrast, the omission of Myc decreased the colony numbers only 20 to 40% from those obtained by the four factors (fig. S12, A and B). This is in contrast to the generation of iPS-fibroblasts, in which the colony numbers decreased more than 90% upon the Myc omission (11), supporting the minor role of Myc in the generation of iPS cells from hepatocytes.

The third difference between iPS-Hep and iPS-Stm cells and iPS-fibroblasts is that the two former iPS have fewer RIS than did the latter. Retroviral expression levels of the four factors were higher in hepatocytes than in fibroblasts (fig. S13). This may explain, at least in part, the fewer RIS in iPS-Hep cells. In addition, it has been shown that ES cells have characteristics of the epithelium, such as tight intercellular contact and surface expression of E-cadherin (13). We confirmed that the expressions of E-cadherin and β -catenin in hepatocytes were higher than those in fibroblasts and equivalent to those in ES cells (fig. S13). This similarity may also contribute to the fewer RIS in iPS-Hep and iPS-Stm cells. Further studies are required to determine the precise molecular mechanisms underlying the substantial differences between iPS-Hep and iPS-Stm cells versus iPS-fibroblasts.

To examine the origin of iPS-Hep cells, we conducted a genetic lineage tracing experiment (Fig. 4A). The Nanog-reporter mice, in which GFP and the puromycin resistance gene was knocked into *Nanog* for selection of iPS cells, were first crossed with mice expressing a loxP-CAT-loxP- β -gal (where CAT is chloramphenicol acetyltransferase) cassette from the constitutively active promoter (14) and then crossed with mice expressing the Cre recombinase driven by the *albumin* promoter (15). In the triple transgenic mice, β -gal activity is turned on upon the activation of the *albumin* gene and continues even when *albumin* is turned off. Primary hepatocytes were isolated, and iPS cells were generated by the four factors. Fourteen days after the transfection, puromycin selection was initiated. By 30 days after the transfection, >100 GFP-positive colonies were obtained (Fig. 4B, left and center). Most of them were also positive for β -gal (Fig. 4B, right), indicating that iPS-Hep

cells were derived from hepatocytes or other *albumin*-expressing cells but not from undifferentiated cells that do not express albumin. A few GFP-positive and β -gal-negative colonies were observed, which may have arisen from albumin-negative cells that coexist in primary hepatocyte cultures, or they may simply reflect incomplete excision by Cre.

This study demonstrates that the four transcription factors successfully reprogrammed somatic cells that had differentiated into a stage in which the *albumin* promoter is turned on. In addition, we showed that generation of iPS-Hep and iPS-Stm cells do not require retroviral integration into specific sites. This finding suggests that it might be possible to generate iPS cells with gene transfer methods free from an integration mechanism that may result in tumorigenicity after transplantation to patients (16).

Note added in proof: After publication of this manuscript online in *Science Express*, we became aware of the need for corrections, which are now published in the Letters section of this issue (see Corrections and Clarifications on page 641 and the revised SOM at www.sciencemag.org/cgi/content/full/1154884/DC2). In addition, after publication of the manuscript in *Science Express*, one chimera mouse derived from iPS-Hep cells developed an obvious tumor, and two other chimeras and one F1 mouse derived from iPS Hep/Stm cells showed areas of abnormal tissues that may represent tumors or be due to an inflammation response.

References and Notes

1. K. Okita, T. Ichisaka, S. Yamanaka, *Nature* **448**, 313 (2007).
2. K. Takahashi, S. Yamanaka, *Cell* **126**, 663 (2006).
3. K. Takahashi *et al.*, *Cell* **131**, 861 (2007).

4. J. Yu *et al.*, *Science* **318**, 1917 (2007); published online 19 November 2007 (10.1126/science.1151526).
5. I. H. Park *et al.*, *Nature* **451**, 141 (2008).
6. S. Yamanaka, *Cell Stem Cell* **1**, 39 (2007).
7. Y. Tokuzawa *et al.*, *Mol. Cell Biol.* **23**, 2699 (2003).
8. H. Niwa, K. Yamamura, J. Miyazaki, *Gene* **108**, 193 (1991).
9. A. Meissner, M. Wernig, R. Jaenisch, *Nat. Biotechnol.* **25**, 1177 (2007).
10. R. Blelloch, M. Venere, J. Yen, M. Ramalho-Santos, *Cell Stem Cell* **1**, 245 (2007).
11. M. Nakagawa *et al.*, *Nat. Biotechnol.* **26**, 101 (2008).
12. X. Yang *et al.*, *Nat. Genet.* **39**, 295 (2007).
13. H. L. Spencer *et al.*, *Mol. Biol. Cell* **18**, 2838 (2007).
14. K. Sakai, J. Miyazaki, *Biochem. Biophys. Res. Commun.* **237**, 318 (1997).
15. C. Postic *et al.*, *J. Biol. Chem.* **274**, 305 (1999).
16. A. W. Nienhuis, C. E. Dunbar, B. P. Sorrentino, *Mol. Ther.* **13**, 1031 (2006).
17. S. Yamanaka, K. S. Poksay, K. S. Arnold, T. L. Innerarity, *Genes Dev.* **11**, 321 (1997).
18. We thank M. Koyanagi and K. Tanabe for scientific discussion; M. Narita, H. Miyachi, and S. Kitano for technical assistance; R. Kato and R. Iyama for administrative assistance; J. Miyazaki for CAG-CAT-Z mice; T. Kitamura for Plat-E cells and pMXs retroviral vectors; and R. Farese for Rf8 ES cells. This study was supported in part by a grant from the Program for Promotion of Fundamental Studies in Health Sciences of National Institute of Biomedical Innovation, a grant from the Leading Project of Ministry of Education, Culture, Sports, Science, and Technology (MEXT), a grant from Uehara Memorial Foundation, and Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS) and MEXT (to S.Y.). T. A. and K.O. are JSPS research fellows. K.Y. is an Inoue fellow.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1154884/DC1

Materials and Methods

Figs. S1 to S13

Tables S1 and S2

7 January 2008; accepted 7 February 2008

Published online 14 February 2008;

10.1126/science.1154884

Include this information when citing this paper.

The Cell and Molecular Basis of Mechanical, Cold, and Inflammatory Pain

Bjarke Abrahamsen,¹ Jing Zhao,¹ Curtis O. Asante,² Cruz Miguel Cendan,¹ Steve Marsh,² Juan Pedro Martinez-Barbera,³ Mohammed A. Nassar,¹ Anthony H. Dickenson,² John N. Wood^{1*}

Peripheral pain pathways are activated by a range of stimuli. We used diphtheria toxin to kill all mouse postmitotic sensory neurons expressing the sodium channel $Na_v1.8$. Mice showed normal motor activity and low-threshold mechanical and acute noxious heat responses but did not respond to noxious mechanical pressure or cold. They also showed a loss of enhanced pain responses and spontaneous pain behavior upon treatment with inflammatory insults. In contrast, nerve injury led to heightened pain sensitivity to thermal and mechanical stimuli indistinguishable from that seen with normal littermates. Pain behavior correlates well with central input from sensory neurons measured electrophysiologically in vivo. These data demonstrate that $Na_v1.8$ -expressing neurons are essential for mechanical, cold, and inflammatory pain but not for neuropathic pain or heat sensing.

Pain requires input from specialized peripheral sensory neurons, as the loss of these cells results in a pain-free phenotype (1–3). There is evidence that there are modality-

specific sets of nociceptors hard-wired into the central nervous system. Heat and mechanical damage-evoked behavior show no genetic linkage, and these stimuli lead to different patterns

of *c-fos* expression in the dorsal horn of the spinal cord. Recordings from the thalamus show that neurons activated by peripheral application of heat are different from those that are activated by noxious pressure (4). However, electrophysiological analysis of skin-nerve preparations have revealed damage-sensing neurons that respond to heat and mechanical insults (5). In culture, many small-diameter sensory neurons respond to both mechanical stimuli and activation by capsaicin or thermal stimuli (6). On the basis of these *in vitro* studies, it has been assumed that most nociceptive neurons are polymodal.

Attempts to ascribe pain modalities and behavior to individual sensory neuron receptors have been problematic, probably because of the existence of multiple damage-sensing mol-

ecules. A good example is the capsaicin receptor TRPV1, which is activated by high temperatures but does not seem to be essential for acute heat sensing *in vivo* (7, 8).

We used a genetic approach to identify neurons that convey essential information sufficient to activate central pathways and elicit a sensation of pain and a behavioral response *in vivo* (9). A thoroughly characterized $Na_v1.8$ knock-in Cre-expressing mouse that shows normal pain behavior was used to excise a floxed stop upstream of a globally expressed diphtheria toxin A (DTA)-subunit gene (10, 11). By crossing heterozygous Cre mice with homozygous toxin-expressing floxed mice, equal numbers of control and experimental toxin-expressing (DTA) mice were generated (fig. S1). The DTA chain kills the $Na_v1.8$ Cre-expressing neurons (11) and has no toxic effects when released into the extracellular space. The numbers of cells staining with antibodies to neurofilament N200 protein (a marker of A fiber associated sensory neurons) was slightly reduced in DTA mice (13%). Most nociceptive sensory neurons express periph-

erin (12), and the majority (>85%) of peripheral-positive neurons were killed (Fig. 1 and fig. S2). Counterstaining with isolectin-B4 and antibodies to calcitonin gene-related peptide (CGRP) (Fig. 1), showed that almost no detectable isolectin B4 (IB4)-positive neurons were retained, whereas ~12% of CGRP-positive neurons were spared. The remaining CGRP-positive neurons were heterogeneous, expressing substance P (36%) and TRPV1 (66%) (fig. S2). In the spinal cord, a lamina I-associated subset of CGRP-expressing terminals was present in the DTA mouse, but all the detectable IB4-positive neurons were absent (Fig. 1 and fig. S2).

We examined the behavior of the mice (10, 13). Having shown that motor coordination was normal we examined acute pain thresholds for thermal and mechanical stimuli. Thermal threshold responses measured with a Hargreaves apparatus showed small altered threshold levels similar to those found in $Na_v1.7$ and $Na_v1.8$ knockouts (fig. S3), whereas behavior on a hot plate at a number of noxious temperatures (Fig. 2) showed no significant deficits between the DTA and control mice.

¹Molecular Nociception Group, University College London (UCL), Gower Street, London, WC1E 6BT, UK. ²Department of Pharmacology, UCL, Gower Street, London, WC1E 6BT, UK. ³Neural Development Unit, UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK.
*To whom correspondence should be addressed. E-mail: j.wood@ucl.ac.uk

Fig. 1. Deletion of nociceptors using diphtheria toxin. (A and B) Cross section of L4 DRGs stained with anti-peripherin (green) and anti-neurofilament heavy chain antibodies (red). Peripherin-labeled neurons are reduced by >85% in the DTA mouse (B) compared with the littermate controls (A). (C and D) Central termination of sensory neurons. Cross section of the lumbar spinal cord stained with CGRP (red) and IB4 (green). In lamina I/II, some CGRP-positive terminals were identified in the DTA mouse (D), whereas all detectable IB4-positive terminals were deleted compared with the littermate controls (C). (E and F) Expression of CGRP, IB4, and substance P binding by subsets of sensory neurons. Effectively complete ablation of IB4 binding is seen in the DTA mouse (E). Also in the DTA mouse, 12% of control neurons express CGRP (F), a peptide found in TrkA-positive nociceptors.

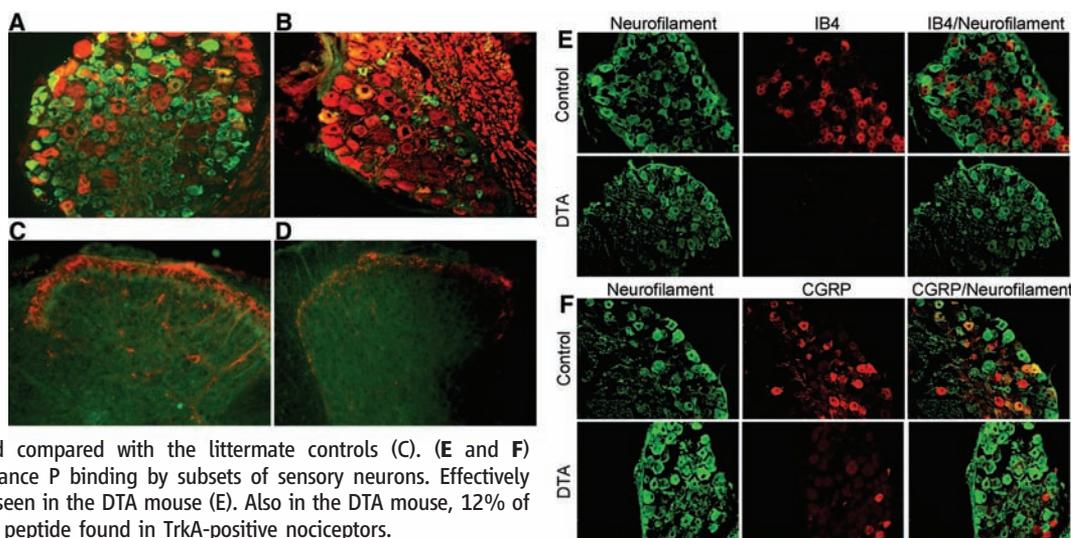
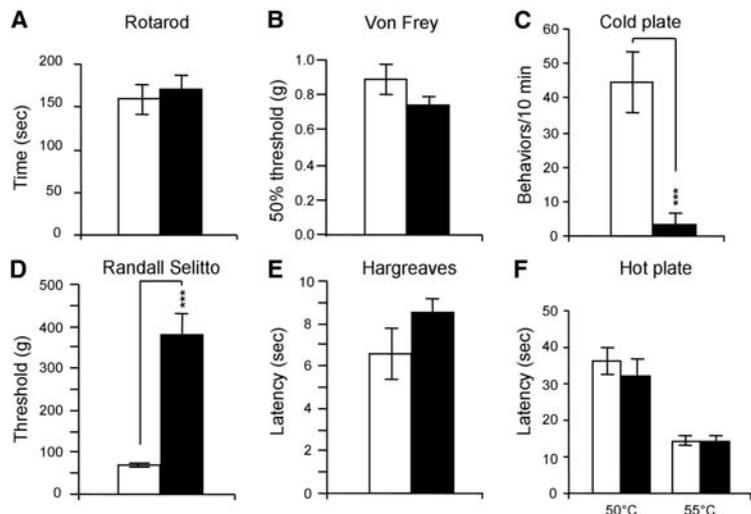


Fig. 2. Acute pain behavior in littermate controls (white bars) and DTA mice (black bars). (A) Motor function; time spent on the rotarod (littermate controls: $n = 9$ mice, 158 ± 4.55 s; DTA: $n = 14$, 171 ± 7.18 s). No difference was observed ($P > 0.01$). (B) Von Frey; mechanical thresholds were measured using von Frey filaments (littermate controls: $n = 13$; DTA: $n = 14$). No difference was observed ($P > 0.01$). (C) Cold behavior (littermate controls: $n = 7$, 44.43 ± 8.71 behaviors; DTA: $n = 7$, 3.57 ± 2.82 behaviors). An almost complete resistance to cold was observed ($***P < 0.0005$). (D) Randall-Selitto apparatus (littermate controls: $n = 8$, 69.48 ± 5.00 g; DTA: $n = 13$, 381.67 ± 48.65 g). A strong resistance to noxious mechanical stimulation was observed in DTA mice ($***P < 0.0005$). (E) Hargreaves; noxious thermal stimulation (littermate controls: $n = 7$, 6.55 ± 1.21 s; DTA: $n = 6$, 8.48 ± 0.71 s). No difference was observed at the $P > 0.01$ level. (F) Hot plate; no differences were observed at either 50°C ($P > 0.01$) (littermate controls: $n = 7$, 31.93 ± 5.06 s; DTA: $n = 13$, 36.35 ± 3.66 s) or 55°C ($P > 0.01$) (littermate controls: $n = 15$, 14.26 ± 1.75 s; DTA: $n = 10$, 14.42 ± 1.43 s). In all panels, error bars indicate SE.

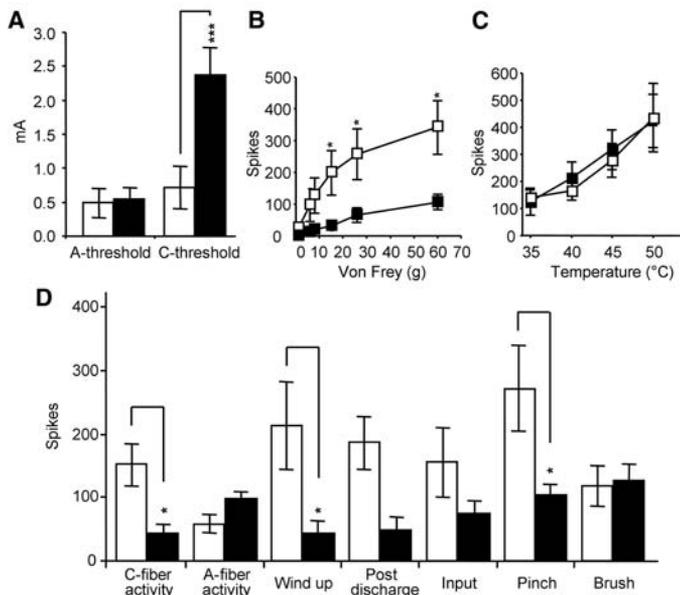


Thus, noxious heat perception is hardly affected by the loss of $Na_v1.8$ -expressing neurons. Given the importance of CGRP in setting heat thresholds (14), the CGRP-expressing sensory neurons that remain in the DTA mice may be involved in heat sensing. In contrast to the normal levels of heat perception, there was no response to cold in the

DTA mouse. $Na_v1.8$ has recently been shown to be the only voltage-gated sodium channel that remains functional in cold temperatures (15). Cooling behavior mediated mainly through activation of TRPM8 was normal, however (fig. S4). Responses to low-threshold mechanical stimuli applied with von Frey hairs were also normal in the

DTA mice. In contrast, behavioral responses to noxious mechanosensation were absent in the DTA mouse (Fig. 2). Extracellular recording from wide dynamic range (WDR) neurons in the spinal cord deep laminae in response to short duration thermal and mechanical input showed deficits in punctuate noxious mechanical ($P > 0.05$) but not thermal coding consistent with the acute pain behavioral data (16) (Fig. 3).

Fig. 3. In vivo electrophysiology of WDR neurons in DTA mice. (A) Increased C fiber firing thresholds of WDR neurons in DTA mice (black) ($n = 9$) and their littermate controls (white) ($n = 8$). (B) Evoked responses to von Frey filaments showed a mechanical deficit in DTA mice. (C) Evoked responses to thermal stimuli remained unchanged in DTA mice. (D) Responses to transcutaneous electrical stimulation of the receptive field. C fiber (but not A fiber) thresholds of activation, wind up, and post discharge were all significantly decreased in DTA mice (16). Evoked responses from noxious pinch were significantly decreased in DTA mice ($P > 0.05$); responses from non-noxious brush remained unchanged. Asterisks denote significant differences when compared with littermate controls ($P < 0.05$, Student's t test, unpaired). Error bars indicate SE.



We next examined inflammatory and neuropathic pain behavior in the DTA and littermate control mice (Fig. 4). There was an almost complete suppression of the second phase of the formalin response, which has been ascribed to activation of TRPA1 (17). The thermal hyperalgesia associated with Freund's complete adjuvant (FCA) injections was also lost. Foot-lifting after FCA injection [a measure of spontaneous pain (18)] was absent in the DTA mice. $Nav1.8$ -expressing neurons thus play an essential role in thermal and mechanical hyperalgesia and spontaneous pain after an inflammatory insult such as FCA. Carageenan- and nerve growth factor-evoked hyperalgesia were also absent in the DTA mice (fig. S5).

The cells responsible for inflammatory thermal hyperalgesia are thus different from those that detect acute noxious heat. Nociceptors that are part of the $Na_v1.8$ -expressing population may play a role in thermosensation only after becoming activated in inflammatory conditions and thus lowering pain thresholds. Inflammatory mediators are able to sensitize the $Na_v1.8$ -expressing neurons lost in the DTA mice (19).

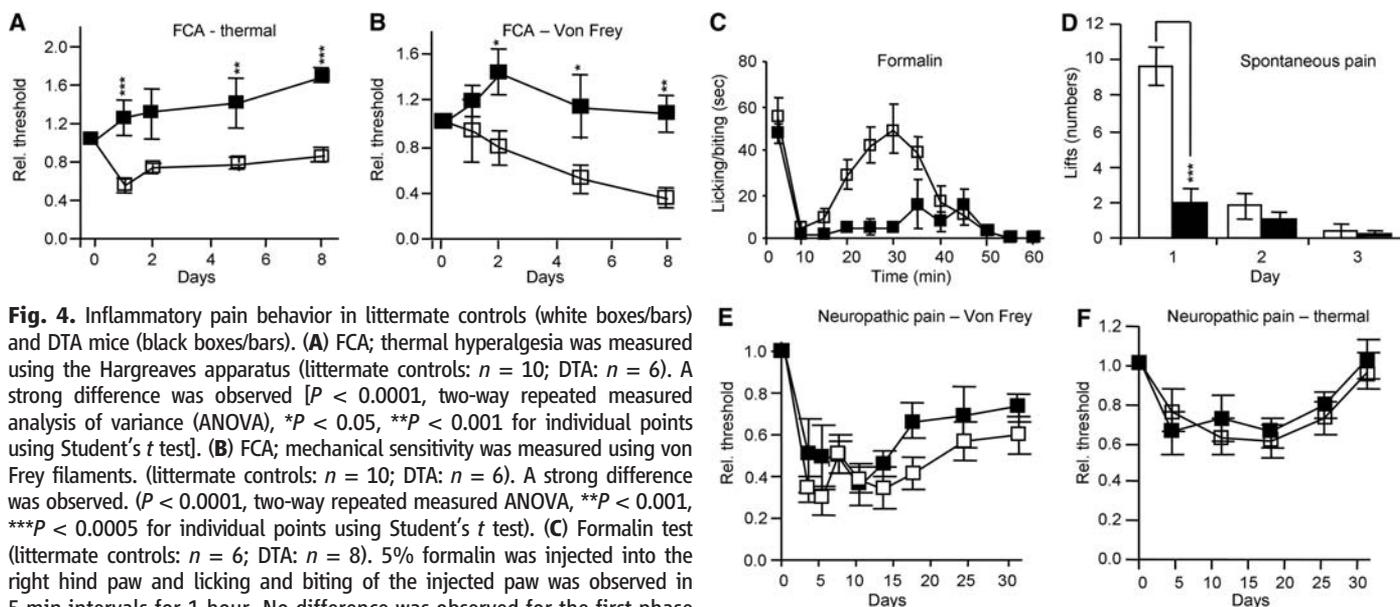


Fig. 4. Inflammatory pain behavior in littermate controls (white boxes/bars) and DTA mice (black boxes/bars). (A) FCA; thermal hyperalgesia was measured using the Hargreaves apparatus (littermate controls: $n = 10$; DTA: $n = 6$). A strong difference was observed [$P < 0.0001$, two-way repeated measured analysis of variance (ANOVA), $*P < 0.05$, $**P < 0.001$ for individual points using Student's t test]. (B) FCA; mechanical sensitivity was measured using von Frey filaments. (littermate controls: $n = 10$; DTA: $n = 6$). A strong difference was observed. ($P < 0.0001$, two-way repeated measured ANOVA, $**P < 0.001$, $***P < 0.0005$ for individual points using Student's t test). (C) Formalin test (littermate controls: $n = 6$; DTA: $n = 8$). 5% formalin was injected into the right hind paw and licking and biting of the injected paw was observed in 5-min intervals for 1 hour. No difference was observed for the first phase (0 to 10 min), whereas a strong resistance to inflammatory nociception was observed for the second phase (10 to 60 min) ($P < 0.0001$, two-way repeated measured ANOVA, $***P < 0.001$ for individual points using Student's t test). (D) Spontaneous pain after injections of FCA (littermate controls: $n = 5$; DTA: $n = 8$). The mice were observed for 5 min on each of the days ($***P < 0.0005$ for individual points using Student's t test). Neuropathic pain in littermate controls (white boxes) and DTA mice (black boxes). (E) Mechanical allodynia measured using von Frey filaments. No

difference was observed (littermate controls: $n = 13$; DTA: $n = 8$) ($P > 0.05$, two-way repeated measured ANOVA). No difference was observed over the time course ($P > 0.05$, two-way repeated measured ANOVA). (F) Thermal hyperalgesia using the Hargreaves apparatus (littermate controls: $n = 13$; DTA: $n = 8$). No difference was observed over the time course ($P > 0.05$, two-way repeated measured ANOVA). Error bars indicate SE.

Input from Na_v1.8-expressing neurons may also sensitize second-order dorsal horn neurons. The release of brain-derived neurotrophic factor from Nav1.8-expressing sensory neurons has been shown to play an important role in inflammatory hyperalgesia through effects on N-methyl-D-aspartate receptors (20). Both mechanisms may contribute to thermal hyperalgesia.

We used calcium imaging to examine whether TRPV1-positive neurons were present in the DTA knockout mouse. Heat sensing is known to still occur in TRPV1 null mice (7). Only a small number (4.5% of control) of capsaicin-sensitive neurons were still present in the DTA mouse (fig. S6), and capsaicin-evoked pain behavior (21) was suppressed (fig. S4). Quantitative reverse transcription polymerase chain reaction also demonstrated a substantial loss of TrpV1 transcript (figs. S7 and S8). TRPV1 is known to be important for inflammatory responses to heat, however, and the substantial loss of TRPV1-expressing cells correlates well with the loss of heat hyperalgesia (8). Responses to cooling are mediated mainly through activation of TRPM8 (22). We found that the levels of TRPM8 mRNA as well as the responses to menthol were barely altered in the DTA mouse (figs. S4, S7, and S8). The loss of noxious mechanosensation in the absence of Na_v1.8-expressing neurons is consistent with earlier data. There is a division between responses to noxious or non-noxious mechanical stimuli that correlates with the presence of Na_v1.8-expressing neurons. Na_v1.8 knockout mice are completely insensitive to mechanically evoked pain (13), as are Na_v1.7 knockout mice (10). However, neither channel is a primary mechano-transducer because mechanically gated currents in sensory

neurons are normal in Na_v1.7 and Na_v1.8 knockout mice (10).

Neuropathic pain is normal in the absence of Na_v1.8-expressing neurons. Although antisense oligonucleotides directed against Na_v1.8 have been claimed to reverse neuropathic pain, effects on other sodium channels cannot be ruled out (23). Neuromas show subtle alterations in excitability in the absence of Na_v1.8, and compounds with relative selectivity for Na_v1.8 have been shown to have some efficacy in neuropathic pain models (24), but it is clear that the principal conduit for many neuropathic pain conditions involves Na_v1.8-negative neurons.

We used microarrays to examine the altered repertoire of genes expressed in dorsal root ganglia (DRGs) from animals depleted of Na_v1.8-expressing neurons. These data are represented in fig. S9 and have been deposited in ArrayExpress. The loss of >96% of Na_v1.8 transcripts in DTA-mouse DRGs shows that the technique was effective. Both known nociceptor-specific transcripts (e.g., MrgA3, TRPA1, Na_v1.9, and P2X3) as well as many previously undescribed selectively expressed transcripts have been identified (fig. S9 and table S1).

In summary, acute heat and neuropathic pain do not require Na_v1.8-expressing neurons, but inflammatory, cold, and mechanical pain are absolutely dependent on these cells. The present study provides a repertoire of candidate molecules potentially involved in different aspects of somatosensation that can be examined experimentally through further tissue-specific ablation studies.

References and Notes

1. Y. Indo, *Hum. Mutat.* **18**, 462 (2001).
2. E. R. Perl, *Nat. Rev. Neurosci.* **8**, 71 (2007).
3. S. N. Lawson, *Exp. Physiol.* **87**, 239 (2002).

4. W. R. Lariviere *et al.*, *Pain* **97**, 75 (2002).
5. J. J. Lawson, S. L. McIlwrath, C. J. Woodbury, B. M. Davis, H. R. Koerber, *J. Pain* **9**, 298 (2008).
6. D. Julius, A. I. Basbaum, *Nature* **413**, 203 (2001).
7. C. J. Woodbury *et al.*, *J. Neurosci.* **24**, 6410 (2004).
8. M. J. Caterina, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R64 (2007).
9. Materials and methods are available as supporting material on Science Online.
10. M. A. Nassar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12706 (2004).
11. A. Ivanova *et al.*, *Genesis* **43**, 129 (2005).
12. M. E. Goldstein, S. B. House, H. Gainer, *J. Neurosci. Res.* **30**, 92 (1991).
13. A. N. Akopian *et al.*, *Nat. Neurosci.* **2**, 541 (1999).
14. J. S. Mogil *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12938 (2005).
15. K. Zimmermann *et al.*, *Nature* **447**, 855 (2007).
16. E. A. Matthews, J. N. Wood, A. H. Dickenson, *Mol. Pain* **2**, 5 (2006).
17. C. R. McNamara *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 13525 (2007).
18. L. Djouhri, S. Koutsikou, X. Fang, S. McMullan, S. N. Lawson, *J. Neurosci.* **26**, 1281 (2006).
19. S. G. Khasar, M. S. Gold, J. D. Levine, *Neurosci. Lett.* **256**, 17 (1998).
20. J. Zhao *et al.*, *Mol. Cell. Neurosci.* **31**, 539 (2006).
21. F. Amaya *et al.*, *J. Neurosci.* **26**, 12852 (2006).
22. D. M. Bautista *et al.*, *Nature* **448**, 204 (2007).
23. J. Lai *et al.*, *Pain* **95**, 143 (2002).
24. J. Ekberg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 17030 (2006).
25. We thank the Biotechnology and Biological Sciences Research Council, the Medical Research Council (UK), and the Wellcome Trust for supporting this work. We also thank the Institute of Child Health chimera production facility, which generated the DTA transgenic mouse line, and W. Huang for help and advice.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5889/702/DC1

Materials and Methods

Figs. S1 to S9

Table S1

References

25 February 2008; accepted 27 June 2008
10.1126/science.1156916