

Scn10a promoter studies:

Utilizing an *Scn10a*-EGFP reporter mouse to study changes in Na_v1.8 expression in peripheral sensory neurons and novel Na_v1.8 expression in the central nervous system



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and Alcoholism

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Why study promoters of ion channels?

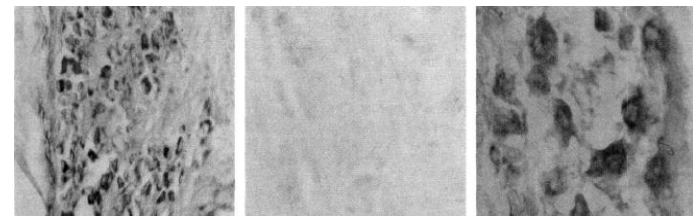
- Abnormal expression of ion channels contributes to human disease
 - E.g. $\text{Na}_v1.3$
 - E.g. $\text{Na}_v1.8$
- Tissue-selective targeting of ion channels

JOURNAL OF NEUROPHYSIOLOGY
Vol. 72, No. 1, July 1994. Printed in U.S.A.

RAPID PUBLICATION

Type III Sodium Channel mRNA Is Expressed in Embryonic But Not Adult Spinal Sensory Neurons, and Is Reexpressed Following Axotomy

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Department of Neurology, Yale University School of Medicine, New Haven, 06510; and Paralyzed Veterans of America/Eastern Paralyzed Veterans Association Neuroscience Research Center, Veterans Affairs Medical Center, West Haven, Connecticut 06516



E17
DRG

Adult
DRG

Adult
DRG (axotomy)

Why study promoters of ion channels?

- Abnormal expression of ion channels contributes to human disease
 - E.g. Na_v1.3
 - E.g. Na_v1.8
- Tissue-selective targeting of ion channels

Molecular and Cellular Neuroscience 10, 196–207 (1997)

Regulation of Expression of the Sensory Neuron-Specific Sodium Channel SNS in Inflammatory and Neuropathic Pain

Kenji Okuse, Sandra R. Chaplan,* Stephen B. McMahon,‡
Z. David Luo,* Nigel A. Calcutt,† Brian P. Scott,*
Armen N. Akopian, and John N. Wood¹

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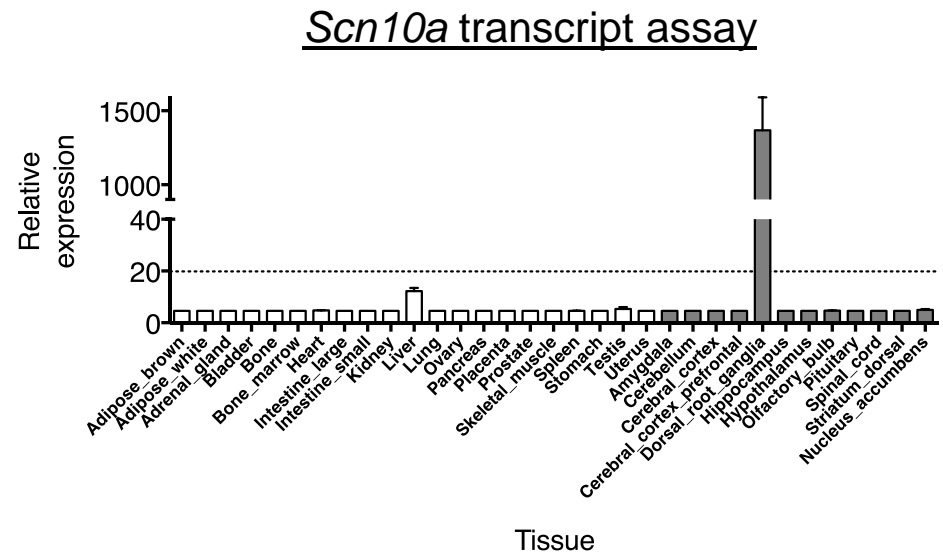
TABLE 1

Expression of SNS in Inflammatory and Neuropathic Pain Models

Treatment	% SNS mRNA normalized to controls
Freund's adjuvant 72 h	91% (SE 7) n = 9
NGF <i>in vivo</i> 4 h	95% (SE 10) n = 9
NGF <i>in vivo</i> 24 h	103% (SE 13) n = 9
Immunoreactive protein in NGF-treated DRG explants (7 days)	125% (SE 9) n = 3
NGF <i>in vitro</i> 7 days	117% (SE 8) n = 3
CGRP levels in the same cells	413% (SE 39) n = 3
Axotomy 7 days	36% (SE 4) n = 4
Axotomy 14 days	26% (SE 7) n = 4
Diabetic neuropathy 4 weeks	74% (SE 12) n = 4
Nerve ligation Holtzman strain 3 weeks	23% (SE 17) n = 4
Nerve ligation Harlan strain 3 weeks	58% (SE 24) n = 4
Neonatal capsaicin 7 weeks	47% (SE 4) n = 4
Neonatal capsaicin plus ligature at 5 weeks	27% (SE 7) n = 4
Ligature alone at 5 weeks	54% (SE 19) n = 3

Our approach: *Scn10a* promoter

- Develop tools to study regulation of *Scn10a* promoter
- Why *Scn10a*/ $\text{Na}_v1.8$?
 - Very selective expression pattern
 - Abnormal expression in human disease states
 - E.g. Multiple sclerosis



Our approach: *Scn10a* promoter

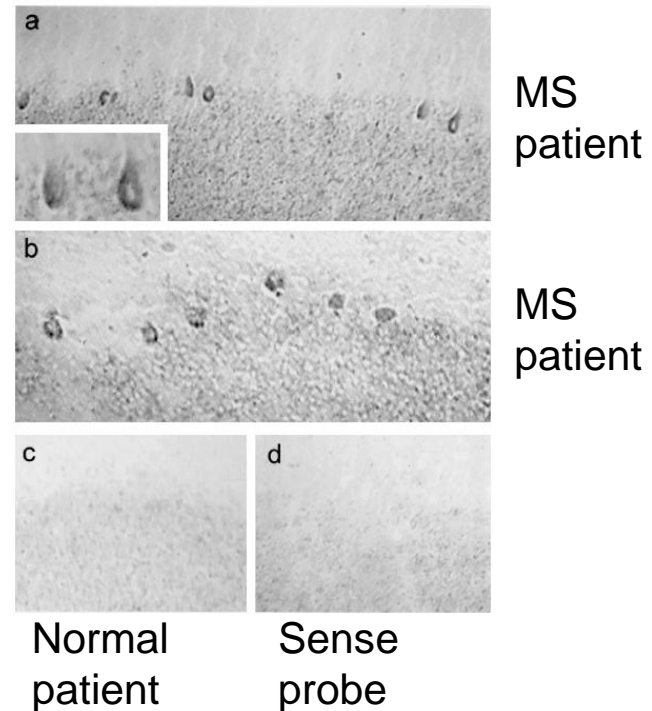
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- Why *Scn10a*/ $\text{Na}_v1.8$?
 - Very selective expression pattern
 - Abnormal expression in human disease states
 - E.g. Multiple sclerosis

11598–11602 | PNAS | October 10, 2000 | vol. 97 | no. 21

Sensory neuron-specific sodium channel SNS is abnormally expressed in the brains of mice with experimental allergic encephalomyelitis and humans with multiple sclerosis

Joel A. Black*, Sulayman Dib-Hajj*, David Baker[†], Jia Newcombe[†], M. Louise Cuzner[†], and Stephen G. Waxman**

*Department of Neurology and Paralyzed Veterans of America/Eastern Paralyzed Veterans Association (PVA/EPVA) Neuroscience Research Center, Yale School of Medicine, New Haven, CT 06510, and Rehabilitation Research Center, Veterans Affairs Hospital, West Haven, CT 06516; and [†]Department of Neurochemistry, Institute of Neurology, University College London, London WC1N 3BG, United Kingdom



Highlights of *Scn10a* promoter studies

I. *Scn10a* promoter constructs

- Identification of putative *Scn10a* promoter

II. *Scn10a*-EGFP transgenic mouse

- Validation of mouse model
- Growth factor regulation of expression
- Cell lineage-dependent regulation of expression
- Novel Na_v1.8 expression in CNS

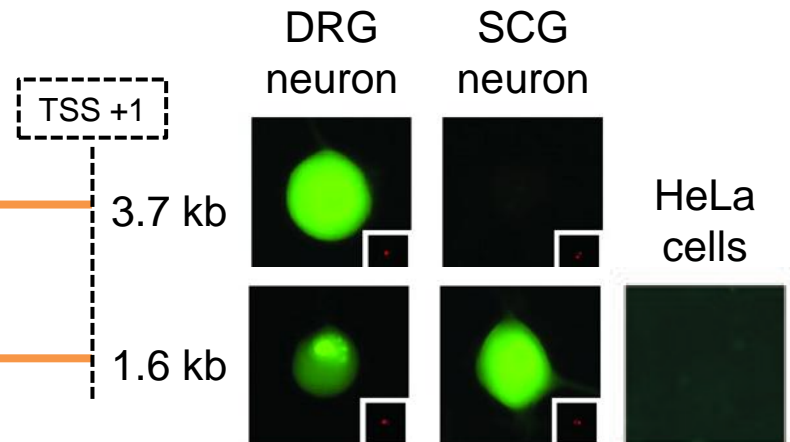
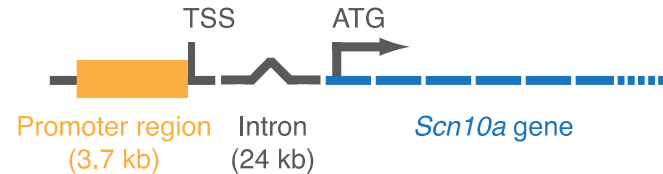
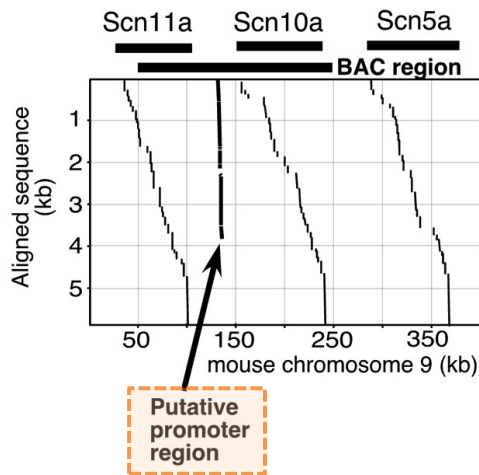
I. *Scn10a* promoter constructs

JOURNAL OF NEUROCHEMISTRY | 2008 | 106 | 1209-1224

Identification of the sensory neuron specific regulatory region for the mouse gene encoding the voltage-gated sodium channel $Na_v1.8$

Henry L. Puhl III and Stephen R. Ikeda

Laboratory of Molecular Physiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, USA



➤ Identification of putative *Scn10a* promoter

II. *Scn10a*-EGFP transgenic mouse

Cellular/Molecular

The Journal of Neuroscience, May 20, 2015 • 35(20):8021–8034 • 8021

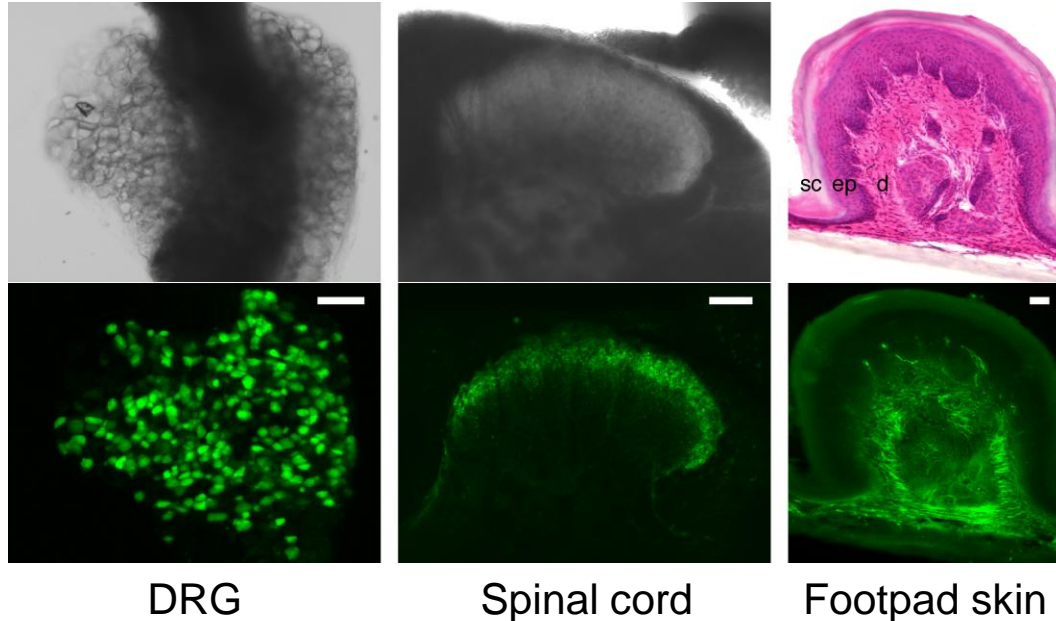


C57BL/6N-Tg(*Scn10a*-EGFP)ALmp/J
Stock #025400

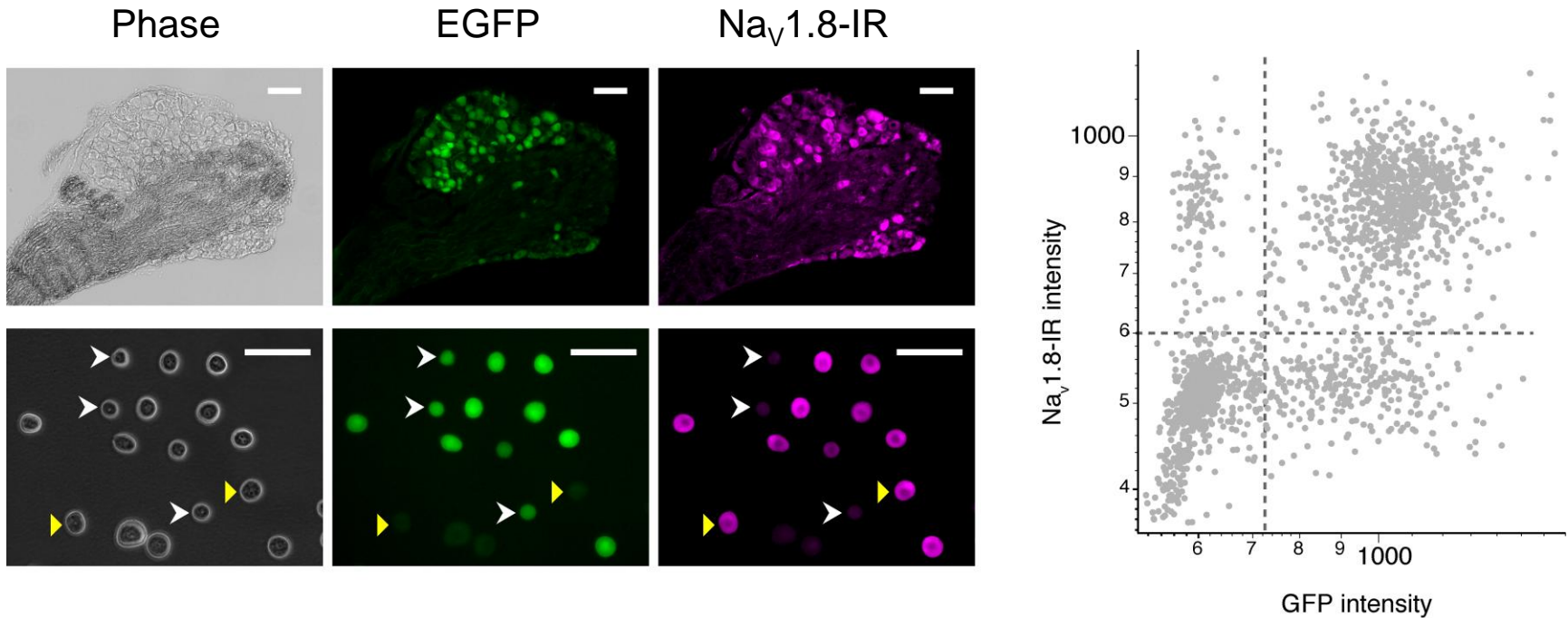
A 3.7 kb Fragment of the Mouse *Scn10a* Gene Promoter Directs Neural Crest But Not Placodal Lineage EGFP Expression in a Transgenic Animal

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Section on Transmitter Signaling, Laboratory of Molecular Physiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892-9411



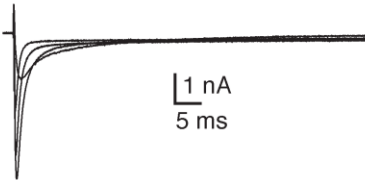
II. *Scn10a*-EGFP transgenic mouse



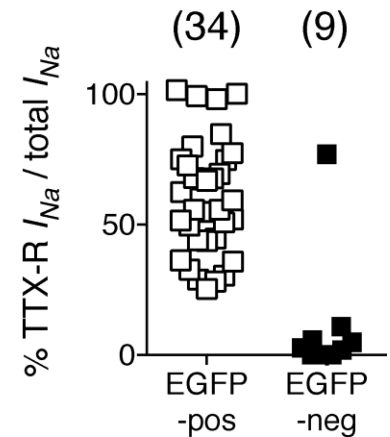
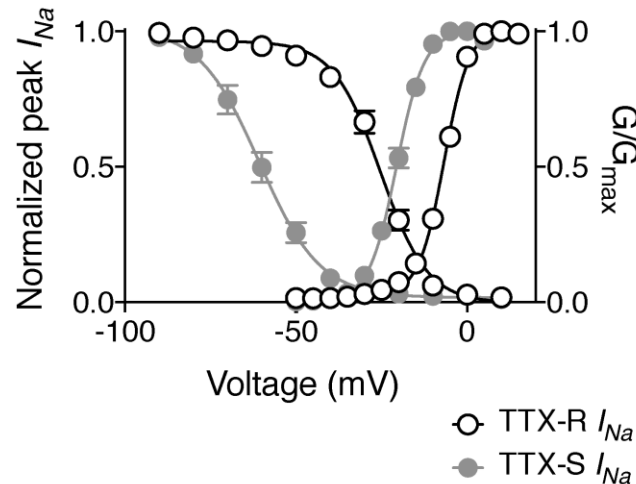
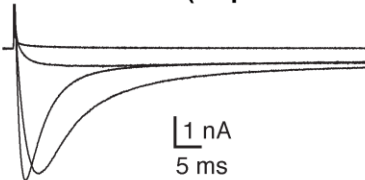
- 80% Na_v1.8-IR+ve cells are EGFP+ve
- 70% EGFP+ve cells are Na_v1.8-IR+ve

II. *Scn10a*-EGFP transgenic mouse

i. GFP-ve

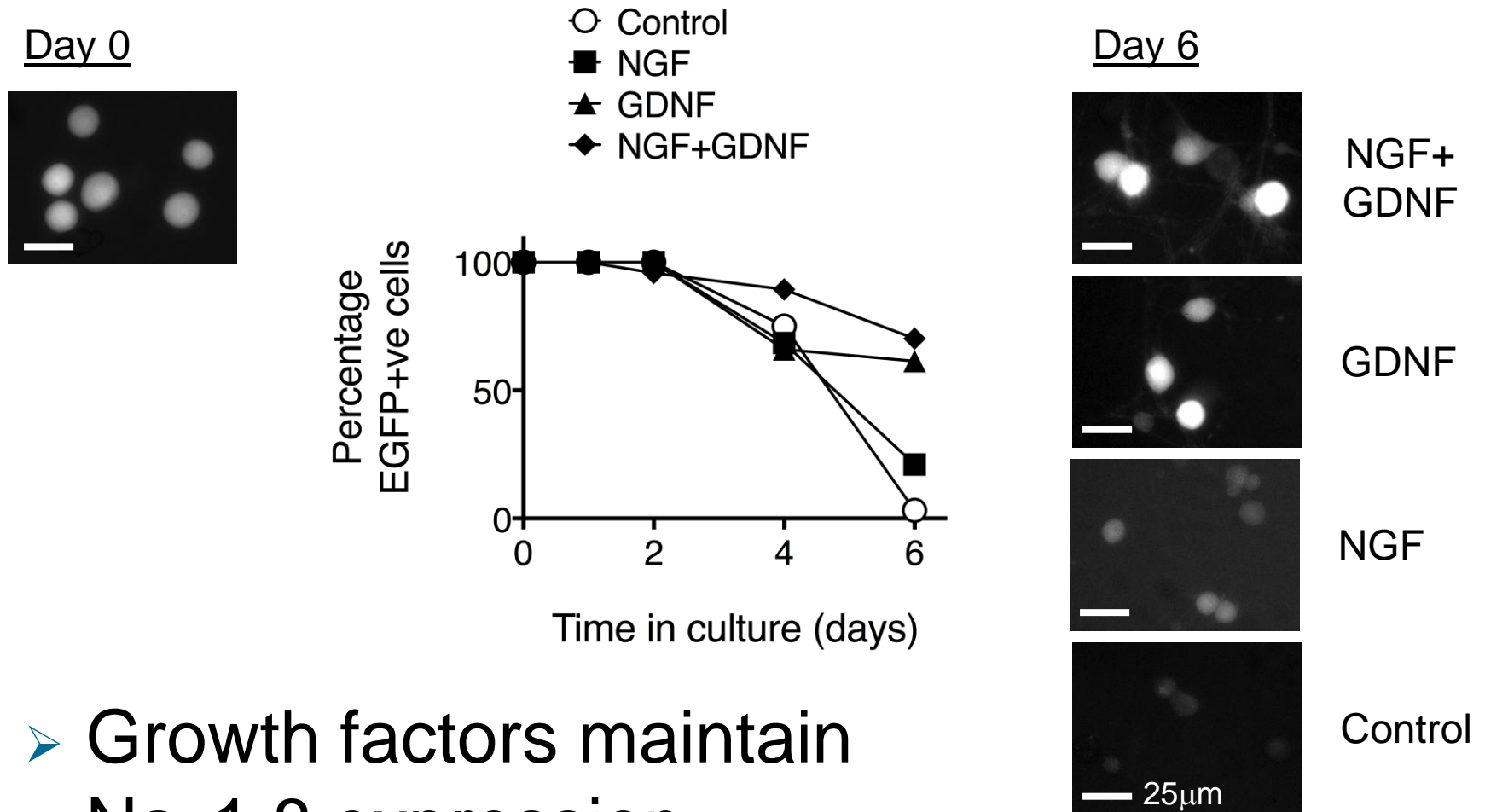


ii. GFP+ve (3 μ M TTX)



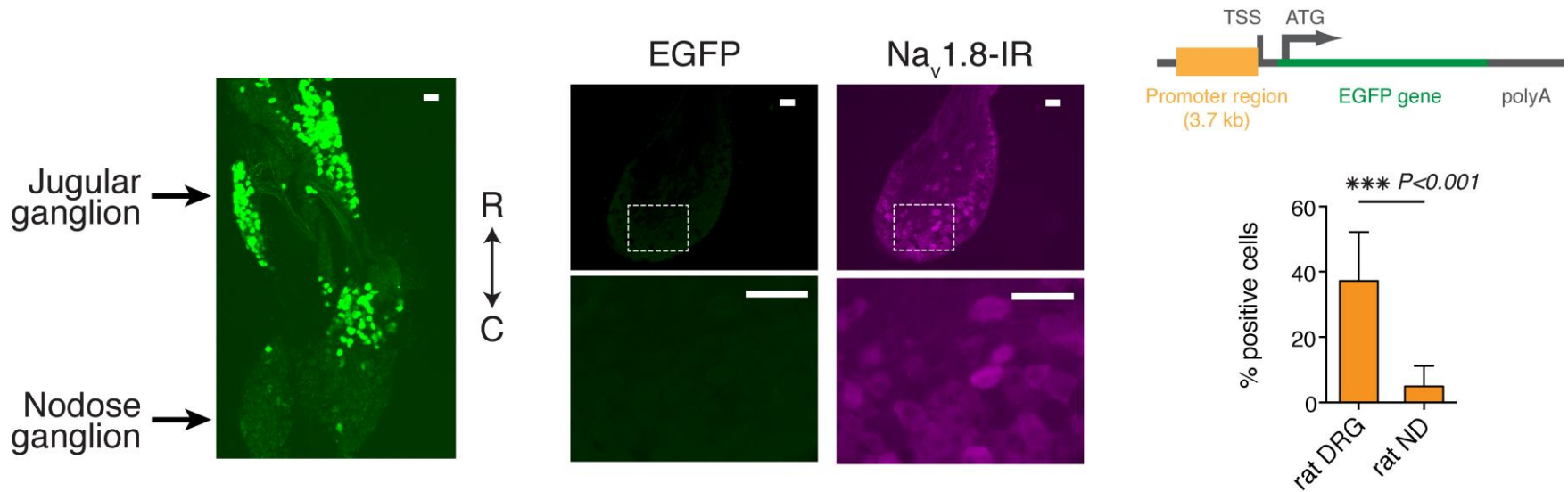
- A 3.7 kb fragment of the *Scn10a* promoter recapitulates most $Na_v1.8$ expression *in vivo*.

Regulation of Na_v1.8 expression



- Growth factors maintain Na_v1.8 expression

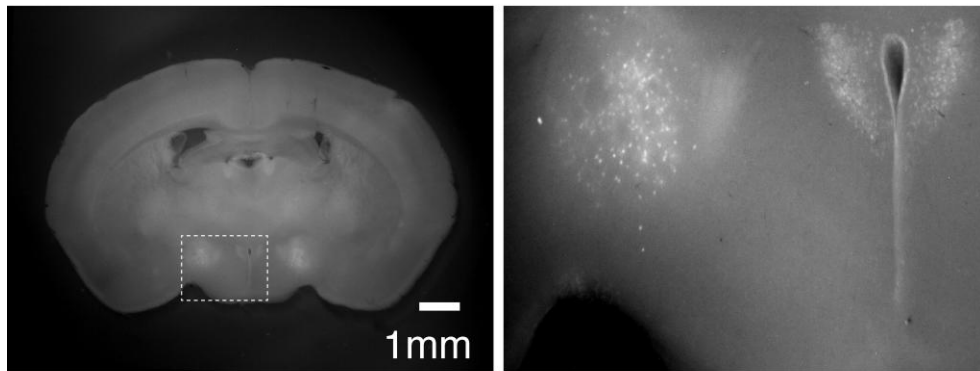
Regulation of Na_v1.8 expression



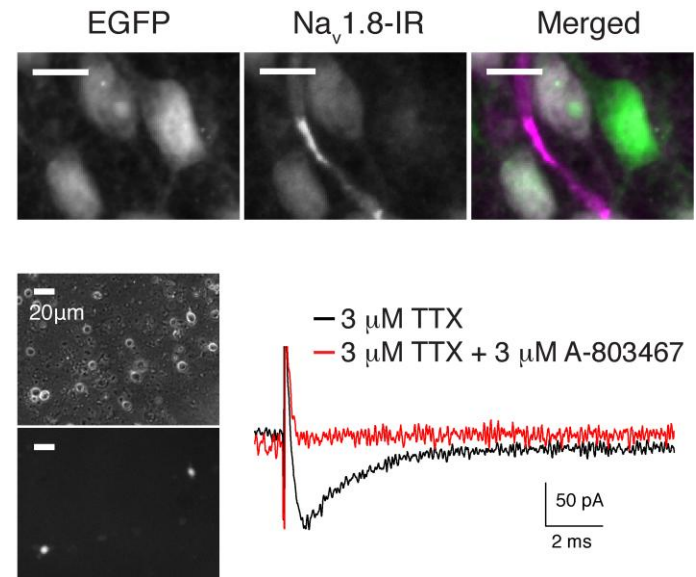
- Placodal-derived sensory neurons utilize additional promoter elements to regulate Na_v1.8 expression

Novel $\text{Na}_v1.8$ expression in the CNS

Hypothalamus:



Bregma -0.94mm

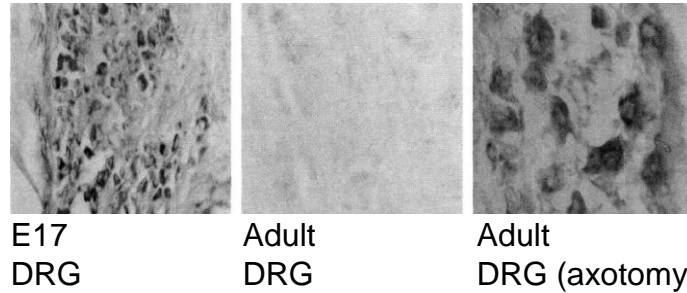


- Functional $\text{Na}_v1.8$ protein expressed in the CNS

Conclusions

- A 3.7 kb promoter fragment of the mouse *Scn10a* gene directs expression in sensory neurons of neural crest descent.
- Transgenic *Scn10a*-EGFP mouse a useful tool to study regulation of Na_v1.8 expression.
 - Real-time changes in Na_v1.8 expression
 - Novel expression of Na_v1.8

Targeting ion channels to treat pain?

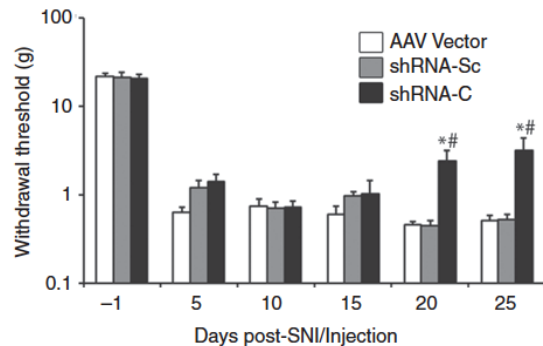


Molecular Therapy vol. 21 no. 1 jan. 2013

Virus-mediated shRNA Knockdown of Na_v1.3 in Rat Dorsal Root Ganglion Attenuates Nerve Injury-induced Neuropathic Pain

Omar A Samad^{1,2}, Andrew M Tan^{1,2}, Xiaoyang Cheng^{1,2}, Edmund Foster¹⁻³, Sulayman D Dib-Hajj^{1,2} and Stephen G Waxman^{1,2}

¹Department of Neurology, Yale University School of Medicine, New Haven, Connecticut, USA; ²Center for Neuroscience and Regeneration Research, Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut, USA; ³Present address: Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland



Molecular Pain 2006, 2:33

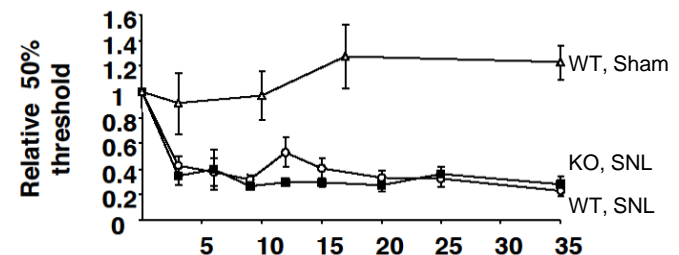
Research

Open Access

Nerve injury induces robust allodynia and ectopic discharges in Na_v1.3 null mutant mice

Mohammed A Nassar¹, Mark D Baker¹, Alessandra Levato¹, Rachel Ingram², Giovanna Mallucci³, Stephen B McMahon² and John N Wood^{1*}

Address: ¹Molecular Nociception Group, Department of Biology, University College London WC1E 6BT, UK; ²Centre for Neuroscience Research, Kings College London, London SE1 7EH, UK and ³MRC Prion Unit and Department of Neurodegeneration, Institute of Neurology, Queen Square, London WC1N 3BG, UK



Acknowledgements

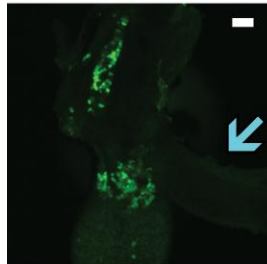
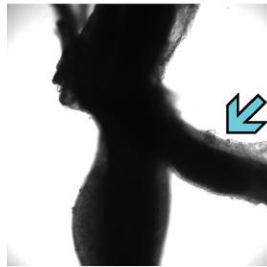
- LMP members
 - Stephen R. Ikeda
 - Henry L. Puhl
 - Daniel J. Liput
 - Claudia Colina-Prisco
 - Steven S. Vogel
 - Tuan Nguyen
 - Elizabeth Kirby
- Laboratory for Integrative Neuroscience (LIN), NIAAA
 - Margaret I. Davis
- NIMH Transgenic core facility
 - Jim Pickel
- Funding sources
 - Intramural research program (IRP), NIAAA, NIH
 - Department of Defense



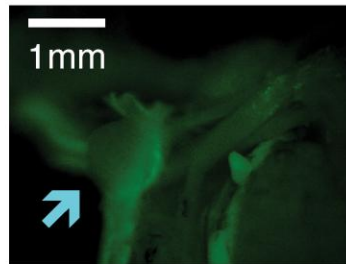
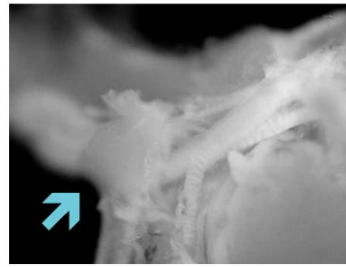
C57BL/6N-Tg(Scn10a-EGFP)ALmp/J
Stock #025400

Other placodal-derived ganglia

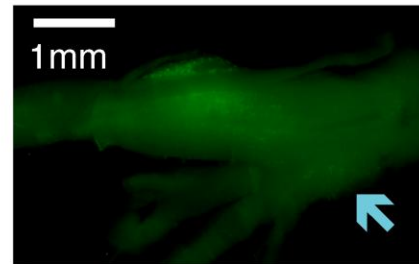
Petrosal ganglion



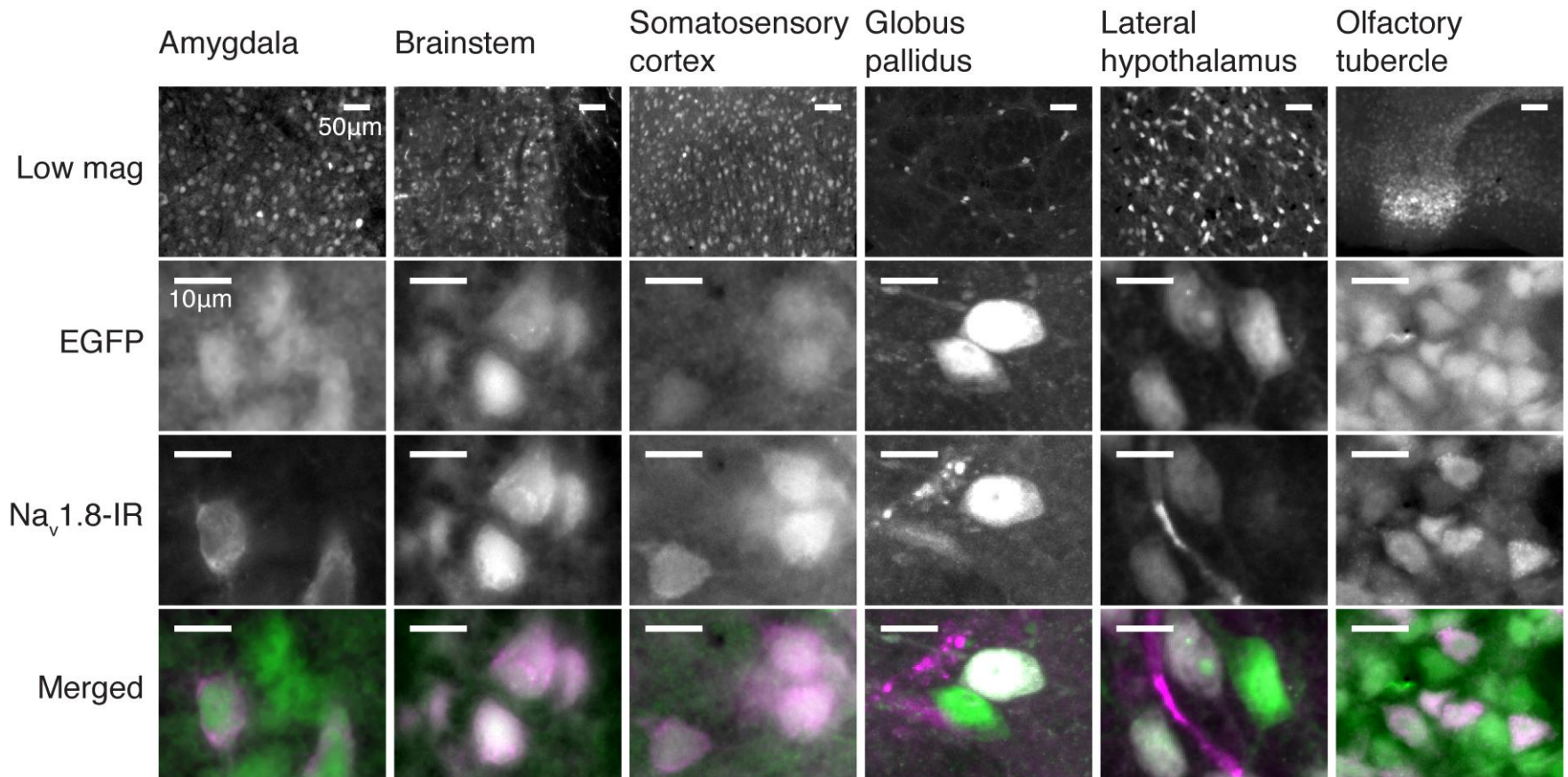
Geniculate ganglion



Trigeminal ganglion

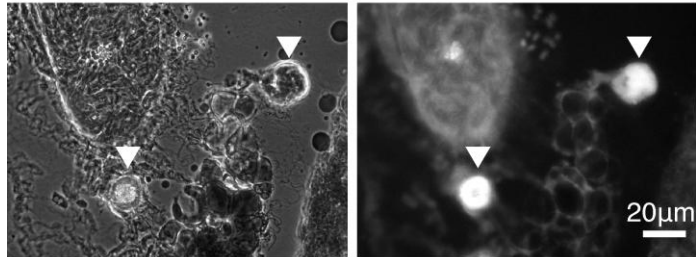


Na_v1.8 in CNS tissues

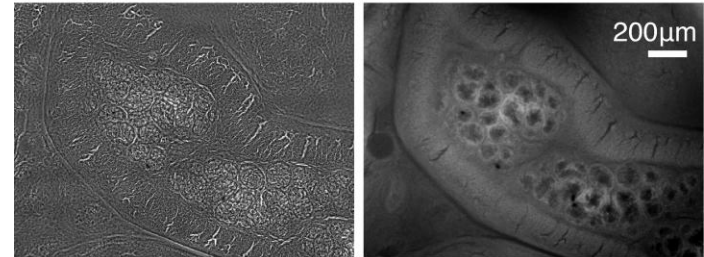


Ectopic expression in *Scn10a*-EGFP mouse

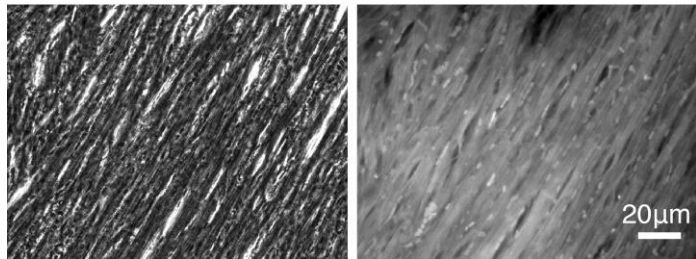
Cardiac ganglion



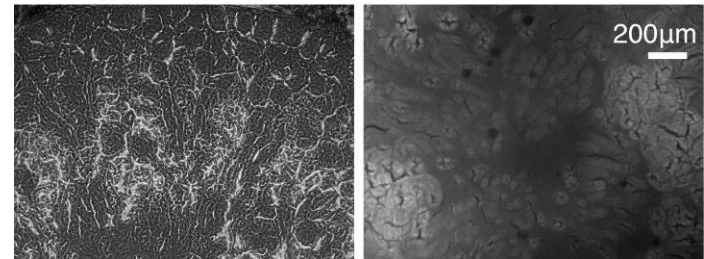
Pancreas



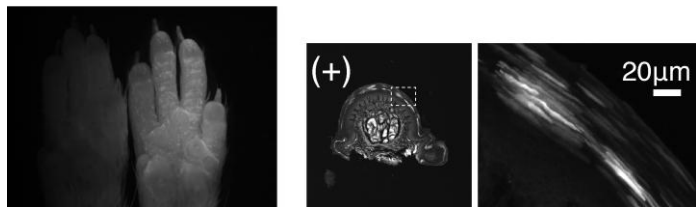
Cardiac muscle



Kidney



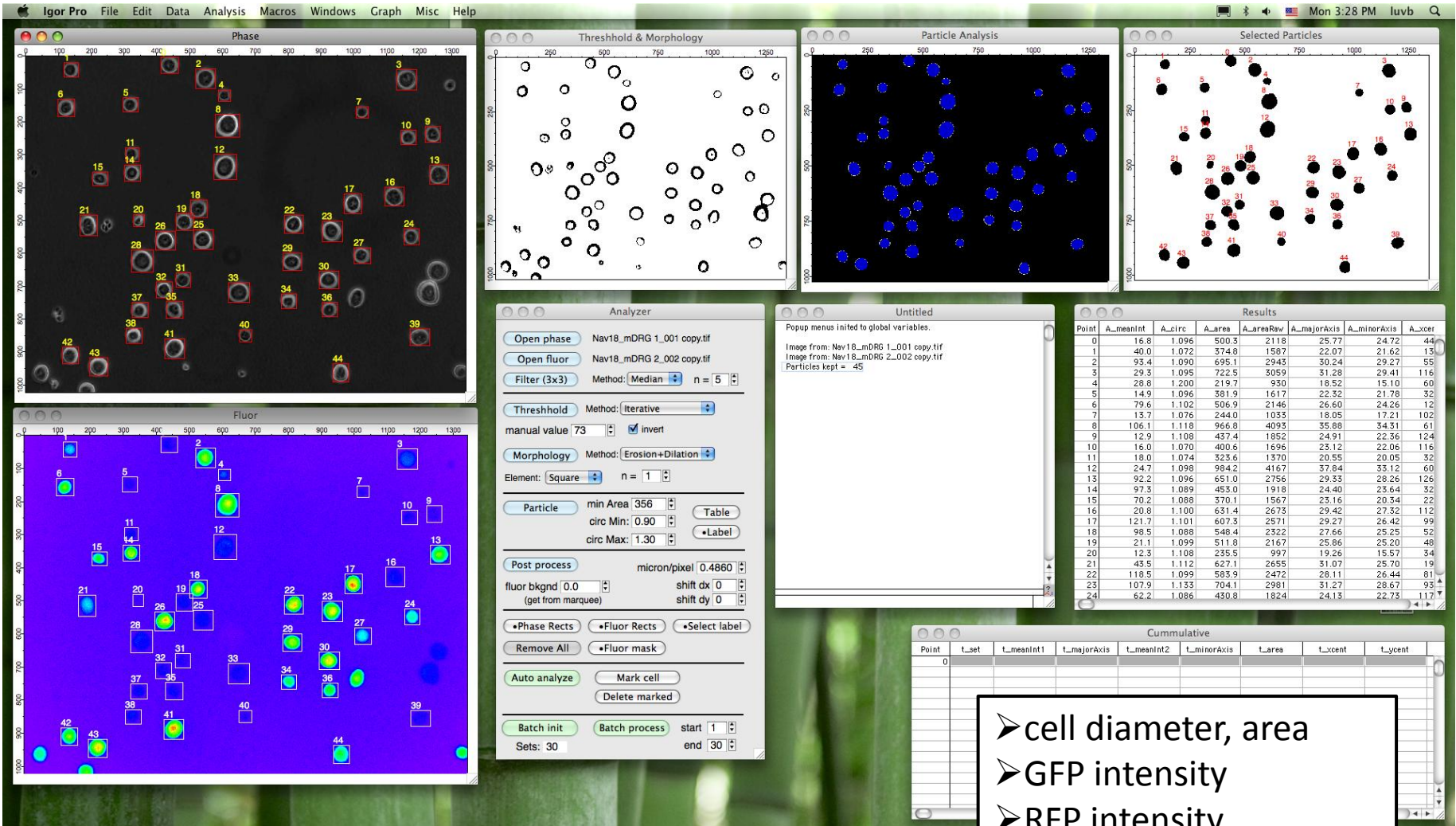
Epidermal skin layer



Grueneberg ganglion



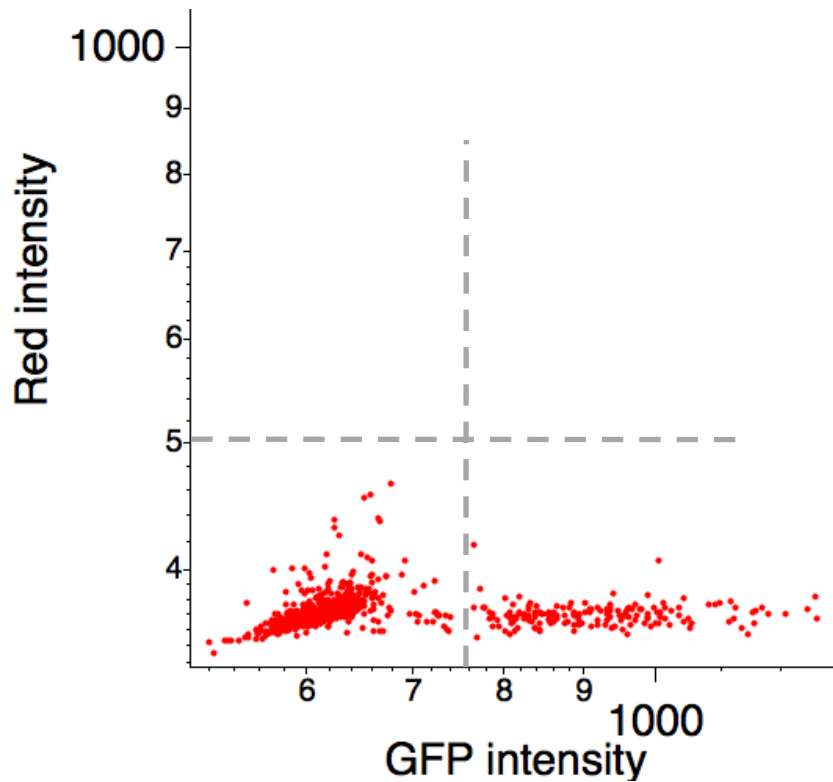
Analysis program for ICC experiments



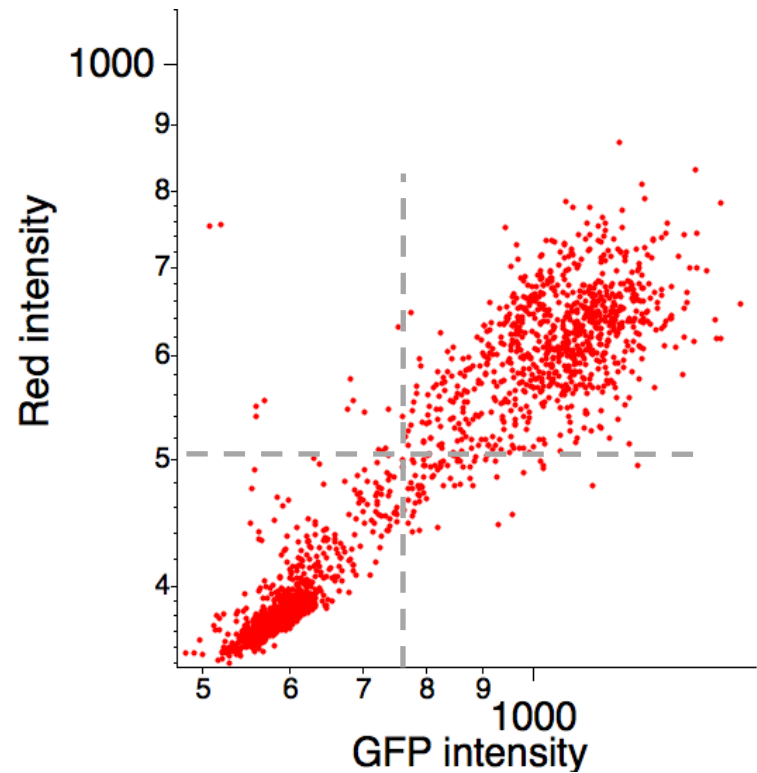
- cell diameter, area
- GFP intensity
- RFP intensity
- coordinates (location)

Controls for ICC experiments

No primary Ab



GFP 1° Ab



Characterization of EGFP+ve DRG neurons

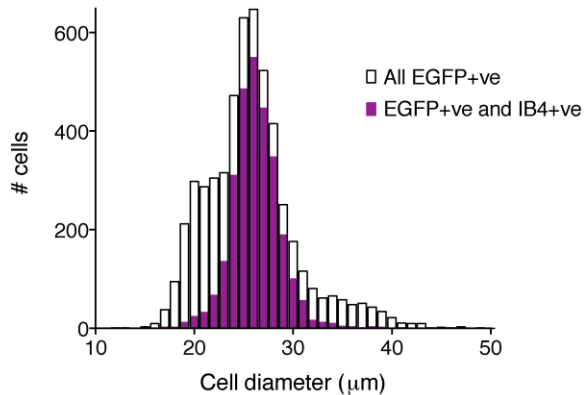
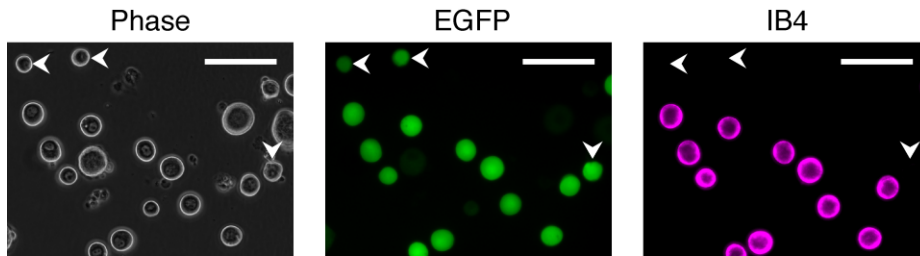
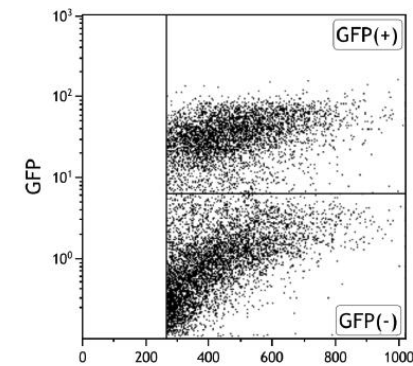


Table 1. Characterization of the EGFP-positive peripheral sensory neuron population^a

Label	% positive for label	% double-labeled cells (as % EGFP-positive cells)	% double-labeled cells (as % label-positive cells)
For DRG neurons			
EGFP, transgene expression	45.7 ± 2.2 (40)		
EGFP-IR	35.5 ± 3.6 (4)	97.9 ± 1.2	90.7 ± 2.8
Na _v 1.8	44.6 ± 2.2 (12)	69.7 ± 1.6	80.2 ± 4.6
IB4	35.4 ± 1.5 (18)	56.7 ± 1.7	75.2 ± 4.2
CGRP	14.6 ± 1.1 (5)	18.7 ± 0.9	61.4 ± 4.0
Substance P	8.0 ± 0.8 (4)	7.2 ± 0.9	45.9 ± 4.7
CtB	36.5 ± 1.3 (12)	9.6 ± 1.0	11.4 ± 1.7
NF200	25.9 ± 1.1 (3)	8.6 ± 1.4	10.8 ± 3.3
TH	9.0 ± 0.7 (5)	5.6 ± 0.3	32.2 ± 3.9
Na _v 1.7	91.3 ± 3.0 (9)	95.8 ± 1.7	41.9 ± 5.0
For ND neurons			
EGFP, transgene expression	7.0 ± 1.2 (13)		
Na _v 1.8	46.1 ± 9.0 (5)	82.9 ± 10.2	14.6 ± 3.8



Gate	%Total	%Gated
All	4.65	100.00
GFP(-)	2.60	55.89
GFP(+)	2.05	44.11