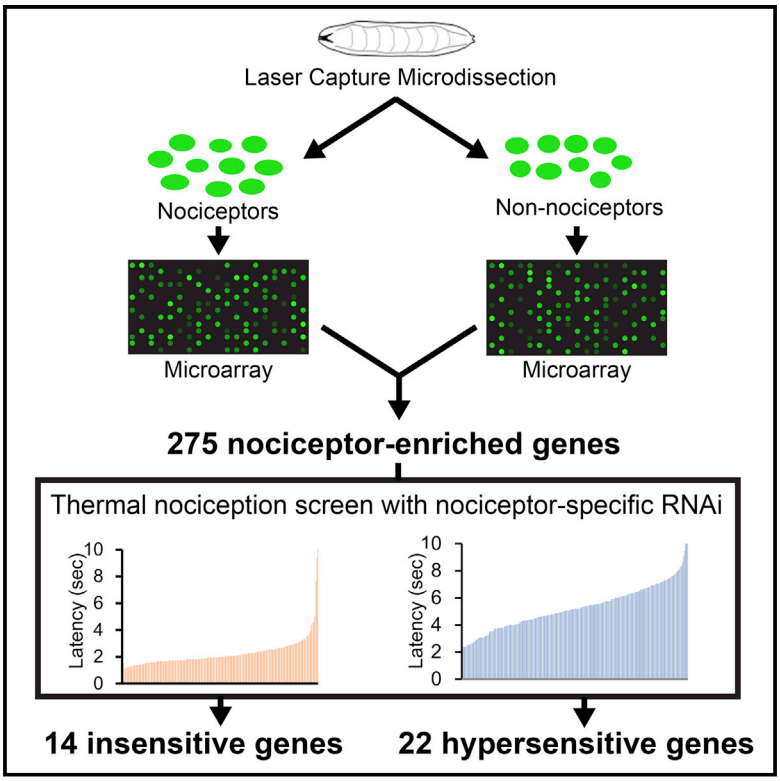
Report

Cell Reports

Nociceptor-Enriched Genes Required for Normal Thermal Nociception

Graphical Abstract



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In Brief

Using tissue-specific microarray analyses and nociceptor-specific RNAi screens in *Drosophila*, Honjo et al. identify genes that both show enriched expression in nociceptors and are functionally important for thermal nociception. Many of genes uncovered by the screen are evolutionarily conserved.

Highlights

- Laser capture microarray analyses identify 275 nociceptor-enriched genes
- Nociceptor-specific RNAi screens implicate 36 genes in thermal nociception
- The screens uncover genes affecting nociception signaling
- Homologs of nociception genes are enriched in mammalian nociceptors

Accession Numbers

E-MTAB-3863

Honjo et al., 2016, Cell Reports *16*, 295–303
July 12, 2016
http://dx.doi.org/10.1016/j.celrep.2016.06.003

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Nociceptor-Enriched Genes Required for Normal Thermal Nociception

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http://dx.doi.org/10.1016/j.celrep.2016.06.003

SUMMARY

Here, we describe a targeted reverse genetic screen for thermal nociception genes in *Drosophila* larvae. Using laser capture microdissection and microarray analyses of nociceptive and non-nociceptive neurons, we identified 275 nociceptor-enriched genes. We then tested the function of the enriched genes with nociceptor-specific RNAi and thermal nociception assays. Tissue-specific RNAi targeted against 14 genes caused insensitive thermal nociception while targeting of 22 genes caused hypersensitive thermal nociception. Previously uncategorized genes were named for heat resistance (i.e., *boilerman*, *fire dancer*, *oven mitt*, *trivet*, *thawb*, and *bunker gear*) or heat sensitivity (*firelighter*, *black match*, *eucalyptus*, *primacord*, *jet fuel*, *detonator*, *gasoline*, *smoke alarm*, and *jetboil*). Insensitive nociception phenotypes were often associated with severely reduced branching of nociceptor neurites and hyperbranched dendrites were seen in two of the hypersensitive cases. Many genes that we identified are conserved in mammals.

INTRODUCTION

The pomace fly *Drosophila melanogaster* has been developed as a robust system to study nociception (Babcock et al., 2009, 2011; Im et al., 2015; Neely et al., 2010; Tracey et al., 2003). *Drosophila*, with its unparalleled genetic tools, is an excellent model to uncover nociception genes. *Drosophila* larvae rotate along the long body axis in a corkscrew like fashion in response to noxious stimuli such as heat (>39°C) or harsh mechanical stimuli (Tracey et al., 2003). This highly stereotyped response to harmful stimuli, named nocifensive escape locomotion (NEL) or rolling, serves as a robust behavioral readout of nociception since it is specifically triggered by noxious stimuli, and it is clearly distinguishable from normal locomotion and other somatosensory responses.

Several lines of evidence indicate that class IV multidendritic (md) neurons are polymodal nociceptive sensory neurons responsible for larval thermal and mechanical nociception. The *pickpocket* and *balboa/ppk-26* genes show highly specific expression in these neurons and are required for mechanical nociception (Gorczyca et al., 2014; Guo et al., 2014; Mauthner et al., 2014; Zhong et al., 2010). Similarly, reporter genes for specific *dTRPA1* transcripts are specifically expressed in the class IV cells, and *dTRPA1* is required for both mechanical and thermal nociception (Zhong et al., 2012). Genetic silencing of class IV neurons severely impairs thermal and mechanical nociception behavior, and optogenetic activation of these neurons is sufficient to evoke NEL (Hwang et al., 2007; Zhong et al., 2012).

RESULTS AND DISCUSSION

Laser Capture Microdissection and Microarray Analyses Identify 275 Nociceptor-Enriched Genes

Genes involved in nociception are often preferentially expressed in nociceptors (Akopian et al., 1996; Caterina et al., 1997; Chen et al., 1995; Dib-Hajj et al., 1998; Mauthner et al., 2014; Nagata et al., 2005; Zhong et al., 2010, 2012). Thus, to identify *Drosophila* nociceptor-enriched genes we performed laser capture microdissection to isolate RNAs from nociceptive and non-nociceptive neurons (Mauthner et al., 2014). We then performed microarray analyses on the isolated samples (Mauthner et al., 2014). We compared the gene expression profiles of nociceptive class IV multidendritic (md) neurons to class I md neuron profiles (Mauthner et al., 2014) as class IV md neurons are polymodal nociceptors (their output is both necessary and sufficient for triggering larval nociception behaviors), and class I md neurons are functionally dispensable for nociception (Hwang et al., 2007). Indeed, as internal validation of these methods, this microarray study successfully detected the enrichment of genes previously thought to be preferentially expressed in class IV relative to class I neurons, such as *cut*, *knot*, *Gr28b*, *ppk*, and *balboa/ppk26* (Mauthner et al., 2014) (Table S1).

To further identify nociceptor-enriched genes, we made a side-by-side comparison of the normalized hybridization intensity between class IV and class I neurons for all Affymetrix probe sets, and identified 278 probe sets corresponding to 275 genes that showed a greater than 2-fold higher expression in class IV neurons in comparison to class I neurons (class IV/class I >2; $p < 0.05$ with Welch t test) (Figure S1; Table S1).