Identifying Cold Sensation Differentiation: Profiling Spinal Cord Neurons Based on Distribution and Excitatory/Inhibitory Neuronal Types using IHC

Adrienne Jung, Chenyu Yang, David McKemy Ph.D.

Dept of Biological Science, Bridge Institute, University of Southern California, Los Angeles, CA , USA

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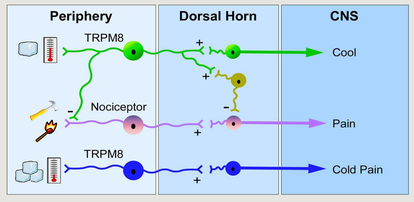
USC University of Southern California

Abstract

Amongst the family of TRP ion channels, TRPM8+ (Transient receptor potential melastatin subfamily 8), mainly functional in the peripheral nervous system, is involved in the human body's ability to perceive a wide range of cold sensations, including innocuous cool, noxious cold pain, and exertion of analgesic relief. Upon activation by a cold stimulus like menthol, TRPM8 + channels expressed in sensory nerve endings open for positively charged ions (Ca2+ and Na+) to enter, triggering action potentials that send impulses along sensory neurons, channeled by dorsal root ganglions to the spinal cord. Yet, how this single channel is capable of managing such a wide spectrum of cold sensations is still unknown. More specifically, where does the differentiation of cold signals happen along the circuit of cold signal transduction? The dorsal horn of the spinal cord is a site of converging sensory information detected by the peripheral nervous system to be then relayed to the brain for an output. The neurons in the spinal cord have different lamina distributions and neuronal types, including excitatory or inhibitory interneurons. These different features of spinal cord neurons have been associated with different functions. Therefore, we hypothesized that different spinal cord neurons relaying cold information could be responsible for differentiating cold signals, as shown by [Fig1]. It is significant to study spinal cord neurons that are downstream TRPM8 signaling. Hence, it is crucial to find a reliable method of determining different features of spinal cord neurons, including their distribution and neuronal types. By analyzing spinal cord cell identities using TRPM8 GFP mouse, we structure a protocol of detecting spinal cord neuron distribution and cell type, which enables future research on spinal cord circuits of cold sensation.



[Figure 1] Knowlton et al, J Neuroscience,2013

Objective

1. The main objective of this research is to determine TRPM8+'s involvement with the differentiation of cold-related sensations and neuron connectivity. What cells are involved with downstream TRPM8+ signaling?
2. The experimental objective is to find a dependable method of identifying distinct features of spinal cord neurons, such as their distribution and neuronal type.

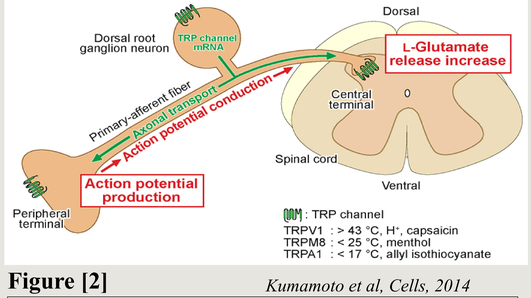
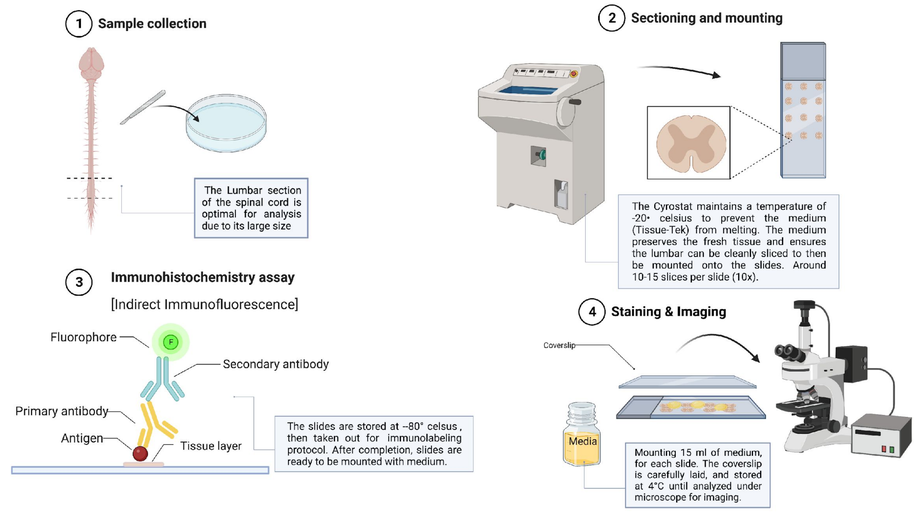


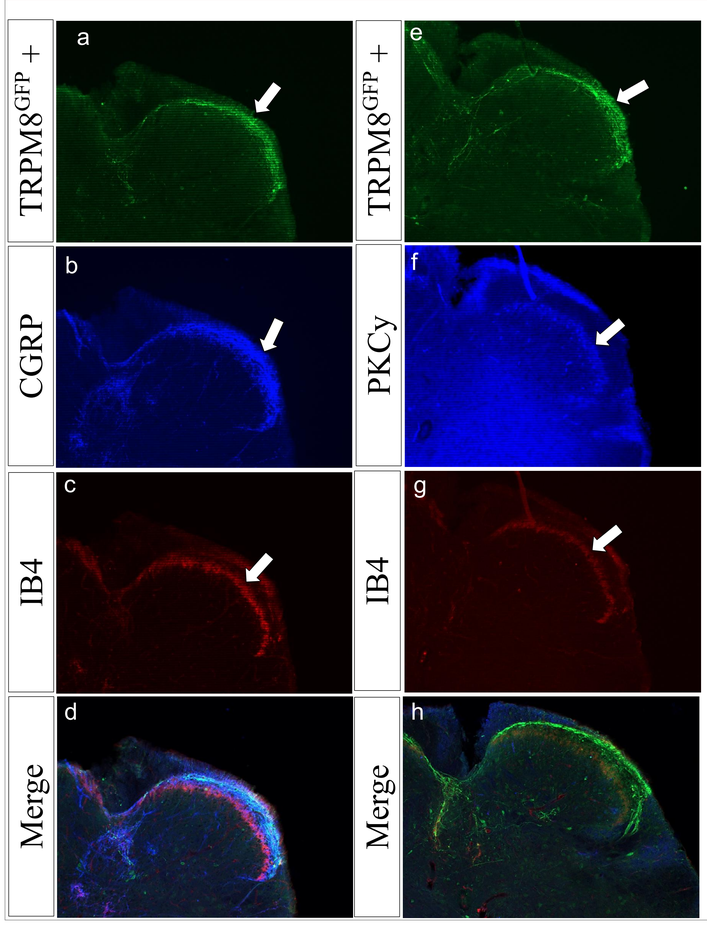
Figure [2] *Kumamoto et al, Cells, 2014*

[2] Demonstrating spinal circuit of temperature detection from peripheral to the spinal cord.

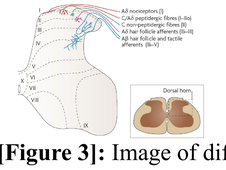
Methods: Immunohistochemistry



Immunofluorescence Imaging

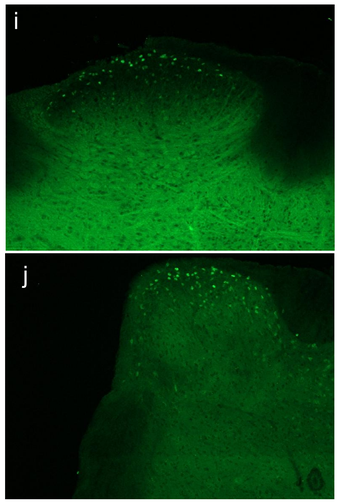


TRPM8GFP mouse:
Images (a-h) are taken of Superficial lamina in the mouse spinal cord dorsal horn.
Image a-b (Trpm8GFP and CGRP) overlap in lamina 1. However, IB4 axons do not overlap with lamina I (GFP and DAPI).
For images (e-h), Trpm8GFP layer is most superficial, while IB4 and PKCy layers are more inner laminae.
This information suggests that CGRP, IB4 and PKCy immunoshistochemistry labeling serves as am appropriate method of defining different layers of spinal cord dorsal horn and can be used to determine distribution of spinal cord neurons.



[Figure 3]: Image of different laminae layers in spinal cord. *Mytahir, Biology, 2015*

Inhibitory Neuronal Markers: Pax2, Wild type mouse



(i-j) Pax 2 targets inhibitory neurons shown under GFP fluorescence in dorsal horn.
Prescence of Pax2+ inhibitory neurons mostly lie in the Superficial Laminae (I-II)

This experiment suggests that Pax2 is a suitable target to define inhibitory neurons in superficial laminae of dorsal horn.

Summary and Significance

TRPM8+ cold signaling occurs at the spinal cord level.
-Lamina Layers have different projections, contributing to their distinct functions of relaying messages to the brain.
-The immunohistochemistry protocol targeting CGRP, IB4, and PKCy has been proven to be a suitable method for distinguishing distinct layers within the spinal cord dorsal horn. This enables us to identify the distribution of neurons in the spinal cord more effectively.

Significance

-Improves our understanding of how the body can have the ability to perceive cold temperatures and differentiate them into various responses.
-Knowledge of TRPM8+ differentiation can enhance drug development to target inhibiting nociceptors for cooling pain relief.

References

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