Pain as a Matter of Overexcitability:

HCN2 Channels and TRIP8b Proteins are Key Regulators of Membrane Excitability in Sensory Neurons

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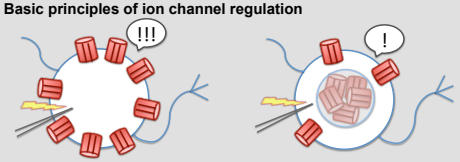
INTRODUCTION

Ion channels form tiny molecular pores on the outer surface of nerve cells, or neurons. These tiny pores control the passage of dissolved salts (ions) in and out of the cell. Because ions are electrically charged, **the electrical activity of neurons is governed by ion channels**. Several distinct types of ion channels have been discovered.

Here, we focus on **HCN2 channels**, which are found in neurons that communicate pain signals from the body to the brain. Studies have shown that HCN2 channels are involved in disease-related pain states, where neurons are thought to be hyperactive. In this study, we examined the effects of a **protein** called **TRIP8b(1b-2)** on the expression of HCN2 channels, as measured by the neuron's electrical activity. In addition, we demonstrated that the expression of HCN2 channels is required for converting "quiescent" neurons into repetitively firing, hyperactive neurons.

Basic principles of ion channel regulation



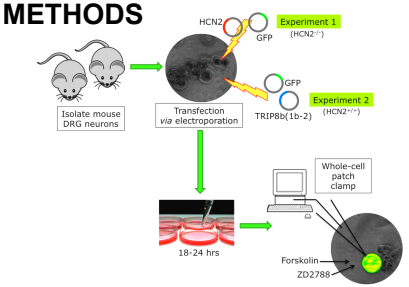
More "leaky"
More excitable

Less "leaky"
Less excitable

HCN2 Channels: What are they?

- **H**yperpolarisation-activated **C**yclic **N**ucleotide modulated channels (HCN**2** is a subtype)
- Modulated by cAMP
- When open, conducts inward Na+/K+ current (called I_h)
- 1st identified in heart muscle cells ("pacemaker current")
- Also found to have rhythmicity/pace-setting roles in thalamic, hippocampal, and cortical neurons

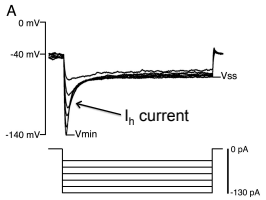
METHODS



EXPERIMENT 1: RECOVERY OF HCN2 EXPRESSION

Transfection of HCN2 cDNA into neurons lacking HCN2 channels:

- Enhances depolarizing rebound current (I_h) and raises RMP
- Restores cAMP-sensitive increases in membrane excitability, particularly in neurons that do not repetitively fire.

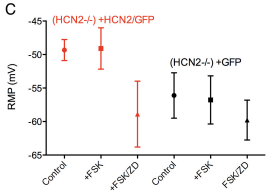


HCN2-/- = lacking HCN2 gene

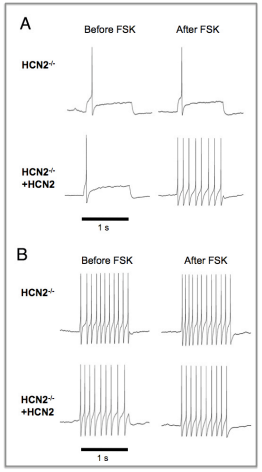
RMP = resting membrane potential (a measure of cell excitability)

FSK = forskolin (raises cAMP levels inside the cell, mimicking inflammation and injury)

ZD = ZD7288 (inhibits all HCN channels)



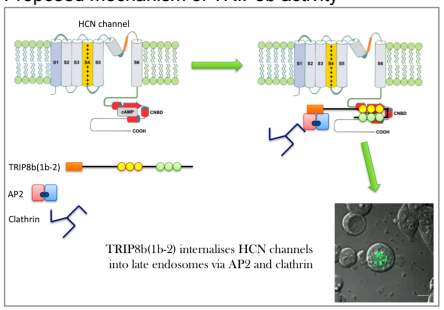
Sag ratio: quantitative measure of I_h

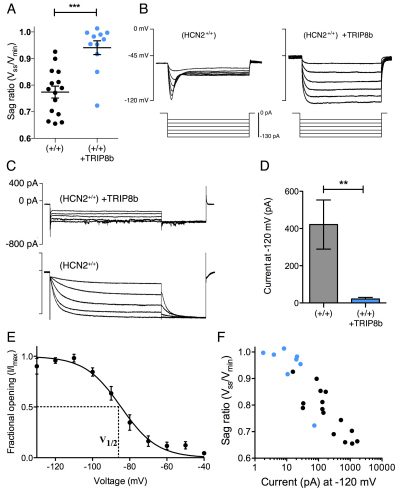


EXPERIMENT 2: INCREASING TRIP8b EXPRESSION

Transfection of **TRIP8b(1b-2)** cDNA into wild-type (+/+) neurons abolishes rebound current (I_h) as measured by sag ratio and voltage-clamped current.

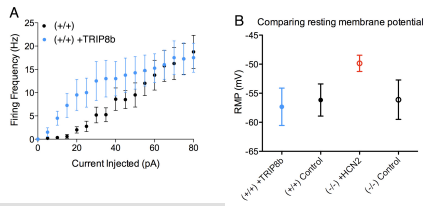
Proposed mechanism of TRIP8b activity





LIMITS OF TRIP8b(1b-2)

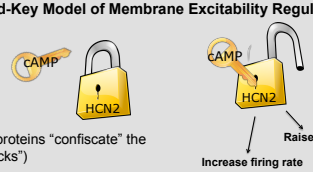
- Reducing HCN2 surface expression by TRIP8b(1b-2) does not inhibit repetitive firing
- Also does not alter resting membrane potential



SUMMARY OF FINDINGS

- Recovery of HCN2 channels in HCN2-lacking neurons restores ability to be sensitized, raises resting membrane potential, and increases rebound current.
- Increasing the amount of TRIP8b(1b-2) abolishes all HCN-channel currents in neurons, supporting its role in removing HCN channels from the membrane.

Lock-and-Key Model of Membrane Excitability Regulation



(TRIP8b proteins "confiscate" the HCN2 "locks")

FUTURE DIRECTIONS & APPLICATIONS

- Manipulate expression of HCN2 channels *in vivo*.
- See if HCN2-mediated sensitisation increases spontaneous firing, related to spontaneous pain.
- Development of novel analgesics that target HCN2 channels or TRIP8b proteins.

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