

Generally Physiological

On optimizing therapies, Orai, and ORNs

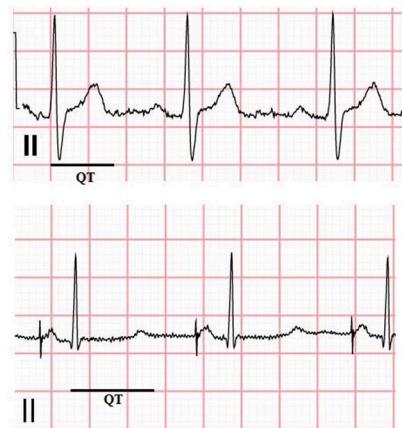


This month's installment of *Generally Physiological* considers individualized therapeutic strategies to treat the congenital heart condition long QT syndrome (LQTS), determination of the crystal structure of the Orai calcium channel, and nonsynaptic communication between olfactory receptor neurons (ORNs) in insect sensilla.

Defining individualized therapeutic strategies for channelopathies

Mutations that lead to ion channel dysfunction can result in human channelopathies; however, it's difficult to determine the precise relationship between clinical phenotype and specific pathophysiological mutations or the sensitivity of disease-associated mutations to therapeutic intervention. The development of techniques whereby differentiated human cells can be reprogrammed into induced pluripotent stem cells (iPSCs) and then redifferentiated into a specific cell type holds the promise of providing a customized approach to investigating how disease-causing mutations affect the cell physiology and therapeutic response of a given individual. LQTS, a congenital heart condition named for the characteristic prolongation of the QT interval seen on the electrocardiogram (associated with delayed ventricular repolarization), can lead to syncope, arrhythmias, and sudden cardiac death. QT prolongation can be caused by a decrease in repolarizing K^+ current or an increase in inward (Na^+ or Ca^{2+}) current, and LQTS can arise from mutation of any of various genes encoding cardiac ion channels or associated proteins.

The most common of these mutations involves *KCNQ1*, *KCNH2* (encoding the hERG K^+ channel), and *SCN5A* (encoding the $Na_v1.5$ Na^+ channel). Terrenoire et al. (2013) used iPSCs differentiated into cardiomyocytes (iPSC-CMs) to investigate the physiological basis for arrhythmias in a child with LQTS bearing a *de novo* mutation in *SCN5A* (F14773C) and a common polymorphism in *KCNH2*.



EKGs from individuals with (bottom) or without (top) LQTS. Both are lead II. (Credit: R.H. Pass, Albert Einstein College of Medicine, and The Children's Hospital at Montefiore.)

Voltage-clamp analyses of iPSC-CMs from the affected child and his parents indicated that the mutation in *SCN5A*, associated with defective Na^+ channel inactivation and a consequent increase in late Na^+ current (I_{NaL})—but not the *KCNH2* polymorphism—was responsible for generating arrhythmia. Pharmacological analysis indicated that, although high concentrations of the sodium channel

blocker mexiletine counteracted the pathophysiological effects of the F14773C mutation, they also inhibited current through hERG, limiting the drug's therapeutic range. Increasing stimulation frequency decreased I_{NaL} and enhanced its reduction by mexiletine, effects consistent with the most clinically effective paradigm for controlling this individual's arrhythmia (combining mexiletine treatment with atrial pacing). Thus, the *in vitro* data provide a mechanistic explanation for the clinical response, and support the use of this approach to define individualized therapies for LQTS and other channelopathies.

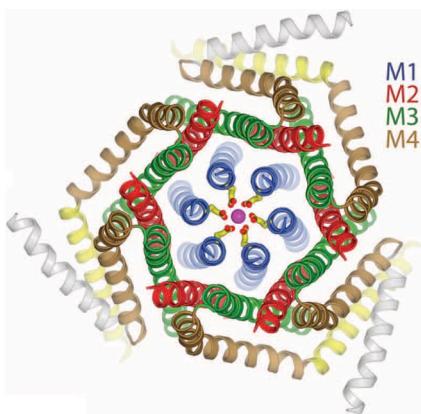
Insight into Orai

Calcium depletion from intracellular stores in the endoplasmic reticulum stimulates its influx through calcium release-activated calcium (CRAC) channels in the plasma membrane. Hou et al. (2012) determined the 3.35-Å resolution crystal structure of *Drosophila melanogaster* Orai (the CRAC channel pore), which has 75% sequence identity in the transmembrane region with human Orai1, as well as that of a

Treating channelopathies, the Orai structure, and nonsynaptic communication

nonconducting mutant corresponding to a human Orai1 mutant that causes immunodeficiency. Unexpectedly, the structure revealed that six Orai subunits surrounded a central pore, crossing the membrane to

form the channel. The authors identified four distinct regions of the \sim 55-Å-long pore: a ring of glutamates at the extracellular side that likely constitute the selectivity filter, a hydrophobic section in the transmembrane region, a basic section near the intracellular side that was capable of binding anions, and a cytosolic region. Flux assays indicated that the construct used for determination of the Orai structure (spanning residues 132–341 of *Drosophila* Orai, with four mutated residues) was in a closed conformation, as



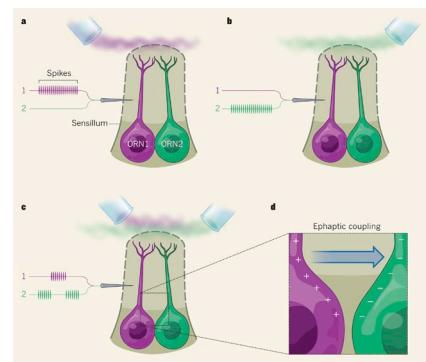
Orthogonal view of the Orai calcium channel, viewed from the extracellular side. (From Hou et al. 2012. *Science*. 338:1308–1313. Reprinted with permission from AAAS.)

would be predicted in the absence of the stromal interaction molecule (which senses endoplasmic reticulum calcium depletion to activate Orai). Analysis of the Orai architecture, which is distinct from that of other ion channels, together with available functional data, enabled

the authors to propose a model for Orai function and conformation of an open state.

Nonsynaptic communication between compartmentalized ORNs

ORNs in the fruit fly *Drosophila* are compartmentalized into sensilla, narrow, fluid-filled structures that encapsulate the ORN dendrites. Fruit fly antennal sensilla typically contain several ORNs that can be distinguished by spike amplitude and sensitivity to distinct odorants. For instance, type 3 antennal basiconic sensilla contain two ORNs, one of which (ab3A, the neuron with the larger action potential) is activated by methyl hexanoate, whereas the second (ab3B) is activated by 2-heptanone. Su et al. (2012) found that transient activation of either ab3A or ab3B inhibited tonic firing in its neighbor; for instance, a brief pulse of 2-heptanone interrupted the sustained train of action potentials elicited in ab3A neurons by prolonged exposure to methyl hexanoate. Lateral inhibition of neighboring ORNs was apparent when firing was driven by Channelrhodopsin2, in different types of fruit fly antennal sensilla (containing two or four ORNs), and in mosquito sensilla. Moreover, lateral inhibition could modulate behavior. Further analysis indicated that intra-sensillar communication of ORNs was independent of synaptic transmission. Lateral inhibition of neighboring ORNs occurred in isolated antennae (separated from central synaptic connections), when tetanus toxin was expressed in ORNs, and in



Lateral inhibition of an ORN by a neighboring neuron within the same sensillum. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. K. Shimizu and M. Stopfer. 492:44–45, copyright 2012.)

the presence of Cd^{2+} ; moreover, the patterns of spontaneous spikes in ab3A and ab3B were not coordinated. Thus, the authors propose that the compartmentalization of insect ORNs into sensilla enables their direct interaction through ephaptic coupling (see Shimizu and Stopfer, 2012, for a thoughtful discussion).

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