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Investigating neuronal cell types through comparative cellular physiology

Russell W Teichert
Department of Biology; University of Utah; Salt Lake City, UT USA

Comprehensive understanding of nervous systems would require the unambiguous identification of all neuronal cell types, which are distinguished by gene-expression differences, particularly in plasma-membrane receptors and ion channels. Toward this ultimate goal, a recent paper initiated an approach to identify and study divergent neuronal cell types through comparative cellular physiology.

In our recent paper (by R. Teichert, T. Memon, J. Aman, and B. Olivera),[1] we compared somatosensory neurons that responded to innocuous cool temperatures (17 °C) from mouse and rat dorsal-root and trigeminal ganglia at multiple developmental stages.[1] We considered these neurons "cold thermosensors," in contrast to "cold nociceptors" that responded to colder temperatures, presumably to mediate the sensation of painfully cold temperatures to the brain.

Our comparison of cold thermosensors revealed multiple similarities and differences between these neurons[1] (**Fig. 1**). All cold thermosensors expressed transient receptor potential (TRP) subfamily M member 8 channels (TRPM8 channels). However, only neonatal cold thermosensors from mouse and rat expressed ATP receptors; the expression was lost during postnatal development. This loss of ATP-receptor expression occurred earlier in low-threshold than high-threshold cold thermosensors. High-threshold cold thermosensors also expressed relatively high levels of $K_V1.1/1.2$ potassium channels, which are involved in setting the higher cold threshold of these neurons. Additionally, cold thermosensors only expressed the α7 subtype of nicotinic acetylcholine receptor (nAChR), whereas other somatosensory neurons expressed nAChRs that include α6, α3 and β4 subunits. Finally, most cold thermosensors from rat expressed TRPA1 channels, but the vast majority of mouse cold thermosensors did not. Although we do not understand the physiological relevance of each gene-expression difference, discovering the differences is an essential first step.

For our comparative study, we utilized a novel pharmacological approach called "constellation pharmacology"[1,2]. Constellation pharmacology is the use of subtype-selective pharmacological agents in combination with calcium imaging to identify the cell-specific combinations, or "constellations," of receptors and ion channels that are expressed in different neuronal cell types. The advantage of calcium imaging is that we can monitor individual responses to pharmacological challenges from ~200 cells simultaneously, enabling us to infer which receptor and ion-channel subtypes are expressed in each neuronal (or glial) cell type. However, constellation pharmacology could also potentially be accomplished through voltage imaging, high-throughput electrophysiology, etc.

Notably, plasma-membrane receptors and ion channels are among the most important determinants of cellular identity for neuronal cell types.[3] The cell-specific integration of receptor and ion-channel subtypes creates functional divergence between neuronal cell types, conferring specific physiological properties upon each type of neuron. Consequently, comparative cellular physiology may become essential for understanding the integrated molecular signaling components of different neuronal cell types and for translational applications.

In our paper,[1] we addressed the fundamental scientific question: what is a cell type? We defined "cell type" by invariant core components. Presumably every terminally differentiated cell type has invariant core components that distinguish each cell type from other cell types. Others have similarly defined "cell type" as "a shared, stable, molecular ground state,"[3]. Notably, each cell type also has variable ancillary signaling components. Understanding the identity of a cell type requires elucidating both constant and variable components and features.

In our paper,[1] we also extended the definition of "cell type" through the concept of homologous cell types. Although the concept of homology pre-dates Darwin and has been used extensively in comparative biology to make inferences about relationships between genes, tissues, organs and organisms, the concept has only recently been applied to the comparison of cell types in animals.[4] It is now possible to obtain molecular fingerprints from single cells, which has enabled such comparative cell biology.[1,4]

In contemporary biology, the concept of homology is commonly applied to genes. The relationships between gene-homologs are inferred from sequence similarity. For example, the classification of a gene as "*Scn9a*" (which encodes the voltage-gated sodium channel, $Na_V1.7$) in both mouse and rat is based on the inference that *Scn9a* is the same entity, inherited

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Correspondence to: Russell W Teichert; Email: Russ.Teichert@utah.edu

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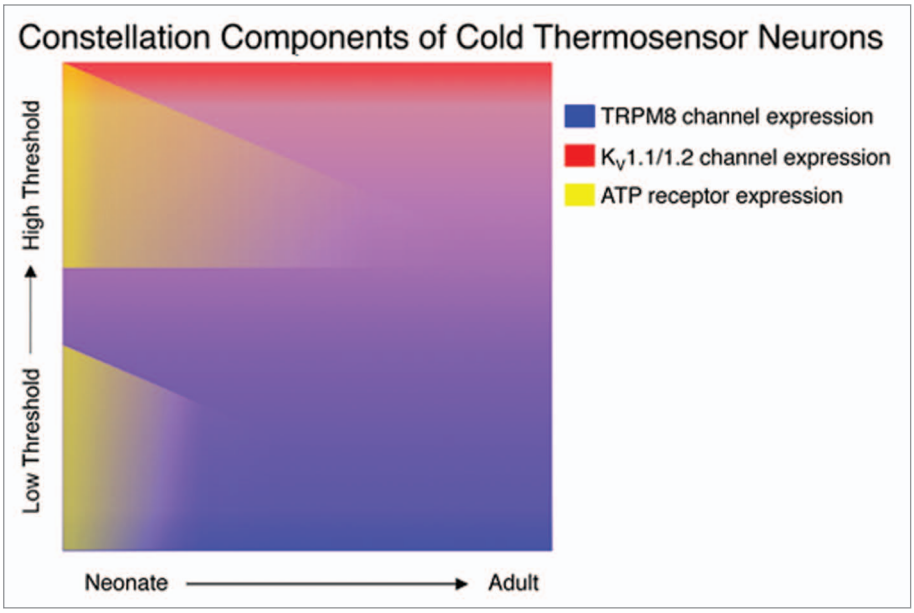

Figure 1. A few selected constellation components of cold thermosensor neurons are depicted. All cold thermosensors appear to express TRPM8 channels (blue) but they vary in expression levels of K_V1.1/1.2 potassium channels (Red). The higher expression of K_V1.1/1.2 potassium channels appears to correlate with higher cold threshold. Neonatal cold thermosensors express ATP receptors (yellow) but the expression is lost over developmental time and occurs earlier in low-threshold than high-threshold cold thermosensors (loss of expression depicted by both color and gradient).

from a common ancestor of mouse and rat (orthologs). Similarly, the classification of the genes encoding Na_V1.7 and 1.8 in mouse is based on the inference that these genes are closely related and arose through a gene-duplication event, followed by functional divergence (paralogs). Thus, a gene is defined, in part, by its inferred relationship to other genes within and among species. In principle, cell types may also be defined in this way. Presumably, homologous cell types in different species were inherited from a common ancestor (orthologous cell types) and homologous cell types within a single species (i.e., "sister cell types"[4]) evolved through a cell-type duplication from a common progenitor cell (paralogous cells types). Comparative cellular physiology enables us to infer evolutionary relationships between cell types and their respective physiological roles.[1]

Physiologists have long known that some somatosensory neurons specialize in reporting the sensation of cold temperature to the brain.[5] Nevertheless, there are unanswered questions and controversies regarding the molecular and cellular mechanisms mediating the neuronal signaling associated with the detection of cold temperature.[1,6] One controversy is related to the roles of TRPM8 and TRPA1 channels as detectors of painfully cold temperature.[6] Among the possible reasons for this unresolved controversy is that various studies have focused on different subsets of somatosensory neurons from different animal species at different developmental time points.[1,6] Hypothetically, TRPM8 and TRPA1 channels may serve somewhat different roles in different somatosensory neurons, in different species, at different developmental stages, where they may be functionally coupled to different sets of receptors and ion channels. Comparative studies may help to resolve this controversy.

Another possible explanation for the controversy is that only homomeric TRP channels are considered as transducers of cold-pain signaling, despite increasing evidence suggesting that a variety of heteromeric TRP channels are expressed in vivo.[7] Speculatively, there may by heterotetramers comprised of TRPM8, TRPA1 and/or other TRP channel subunits that mediate cold-pain signaling in certain types of cold nociceptors (a hypothesis suggested to me by Tosifa Memon). The elucidation of this potential molecular complexity may be facilitated by subtype-selective pharmacological agents that can distinguish between homomeric and heteromeric ion channels. Accordingly, there is a great need to discover and develop more highly selective pharmacological agents as research tools and therapeutic drugs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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