Mini-Symposium

A Double TRPtych: Six Views of Transient Receptor Potential Channels in Disease and Health

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At the 2008 Annual Meeting of the Society for Neuroscience, a Mini-Symposium entitled "Contributions to TRP Channels to Neurological Disease" included talks from six heads of newly established laboratories, each with a unique research focus, model system, and set of experimental tools. Some of the questions addressed in these talks include the following. What is the role of transient receptor potential (TRP) channels in pain perception? How do normally functioning TRP channels contribute to cell death pathways? What are the characteristics of TRPpathies, disease states that result from overactive or underactive TRP channels? How are TRP channels regulated by signal transduction cascades? This review summarizes recent results from those laboratories and provides six perspectives on the subject of TRP channels and disease.

Key words: **cation channel; melanocyte; mucolipidosis type IV; nociception; pain; TRP; amyotrophic lateral sclerosis/parkinsonism dementia complex; taste; cell death; lysosomal storage disease; TRPA1; TRPM5; TRPM7; TRPM8; TRPML1; TRPML3**

Transient receptor potential (TRP) channels comprise a large family of cation channels. The founding member of this family, *Drosophila* TRP, is essential for phototransduction and is opened in response to rhodopsin-coupled phospholipase C (PLC) signaling (for review, see Montell, 2005). To date, >100 TRP channel genes have been identified, in organisms ranging from yeast to mice, and they are divided into seven distinct subclasses based on their primary sequences. Equally diverse are the physiological functions of TRP channels, in processes including taste, thermosensation, hearing, and calcium and magnesium homeostasis (for review, see Flockerzi, 2007). The breadth of TRP channel functions, and the intensity of investigations into these functions, is reflected in the large number of recent reviews covering aspects of TRP biology, such as TRP channel pharmacology (Okuhara et al., 2007), TRP channel structural biology (Gaudet, 2008), connections of TRP channels and cell death (McNulty and Fonfria, 2005), trafficking of TRP channels (Ambudkar, 2007), and TRP channels in disease (Nilius, 2007). The objective of the current review is to summarize results presented, in six presentations, at a Mini-Symposium at the 2008 Annual Meeting of the Society for Neuroscience entitled "Contributions of TRP Channels to Neurological Disease" (Table 1). This field is moving rapidly and seems likely to be the focus of energetic investigation for years to come.

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TRP channels contribute to pain sensation

Pain is a clinically important element of countless diseases. Several members of the TRP channel family have been proposed to play key roles in pain and inflammation (Jordt et al., 2003; Wang and Woolf, 2005; Dhaka et al., 2006). In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as nociception, is mediated by specialized somatosensory neurons called nociceptors. Natural plant-derived irritants have served as powerful pharmacological tools for elucidating the molecular mechanisms underlying nociception, as illustrated by the use of capsaicin to identify the heat-activated ion channel TRPV1 (Caterina et al., 1997). The characterization of TRPV1-deficient animals has demonstrated an essential role for TRPV1 in both heat transduction and inflammation-evoked thermal hypersensitivity (Caterina et al., 2000). The menthol receptor TRPM8 and the wasabi receptor TRPA1 have also been proposed to play key roles in nociceptor function (McKemy et al., 2002; Story et al., 2003; Jordt et al., 2004; Nagata et al., 2005). To probe the physiological roles of these channels, Diana Bautista (University of California, Berkeley, Berkeley, CA) and her colleagues in David Julius' laboratory at the University of California, San Francisco (San Francisco, CA) have generated TRPM8- and TRPA1-deficient mice and have tested them in a variety of *in vitro* and *in vivo* assays.

TRPA1 is activated by a number of environmental chemicals that induce inflammatory pain. These include allyl isothiocyanate and allicin, the pungent compounds found in mustard and

Table 1. TRP channels studied in our laboratories

TRP family member	Connection to disease or pathology	Reviewed by
TRPM7	Wild-type form promotes excitotoxic cell death (Aarts et al., 2003)	Aarts and Tymianski, 2005
	Hypomorphic variant associated with Guamanian amyotrophic lateral sclerosis/parkinsonism-dementia complex (Hermosura et al., 2005)	Hermosura and Garruto, 2007
	Hypomorphic alleles cause melanocyte cell death and paralysis in zebrafish mutants (Kelsh et al., 1996; Arduini and Henion, 2004; Cornell et al., 2004; Elizondo et al., 2005).	Iuga and Lerner, 2007
TRPML1	Hypomorphic form causes MLIV (Bargal et al., 2000; Bassi et al., 2000; Sun et al., 2000)	Slaugenhaupt, 2002; Bach, 2005
TRPA1	Wild-type form mediates inflammatory pain in mice (Bautista et al., 2006)	Story, 2006
TRPM8	Wild-type form mediates cold-induced pain in mice (Bautista et al., 2007)	Story, 2006
TRPML3	Mutant forms cause death of hair cells and melanocytes in mouse (Grimm et al., 2007; Kim et al., 2007; Xu et al., 2007; Nagata et al., 2008)	Cuajungco and Samie, 2008
TRPM5	TRPM5 signal transduction pathways are relevant to drugs that modulate taste	Liman, 2007

garlic extracts, acrolein, an α,β-unstaurated aldehyde that acts as an irritant in tear gas, vehicle exhaust and burning vegetation, and volatile general anesthetics, such as isofluorane. In addition, TRPA1 is a target of endogenous inflammatory agents, including products of lipid peroxidation and the proalgesic agent bradykinin (Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2006; Trevisani et al., 2007; Andersson et al., 2008). TRPA1-deficient neurons show little to no response to these compounds, and TRPA1-deficient animals display attenuated pain behaviors or hypersensitivity after exposure to these irritants (Bautista et al., 2006; Kwan et al., 2006; Matta et al., 2008). These findings demonstrate that TRPA1 is the main molecular site through which a variety of environmental irritants and endogenous inflammatory mediators activate the pain pathway.

Both TRPA1 and TRPM8 had been proposed previously to mediate cold nociception (McKemy et al., 2002; Peier et al., 2002; Story et al., 2003). To elucidate the role of these channels in cold sensation, cellular and behavioral cold responses were measured in mice lacking TRPA1 or TRPM8. Cultured sensory neurons and intact sensory nerve fibers from TRPM8-deficient mice were found to exhibit profoundly diminished responses to cold. Consistent with this finding, these animals themselves showed clear behavioral deficits with respect to their ability to discriminate between cold and warm surfaces. TRPA1-deficient mice, however, displayed no such deficits (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). These findings demonstrate an essential and predominant role for TRPM8 in thermosensation, over a wide range of cold temperatures.

TRPM7 contributes to ischemic cell death of neurons

Death of neurons occurs in neurodegenerative diseases and in stroke, and there is evidence that normally functioning TRP family members promote cell death in certain circumstances. Ischemic cell death is thought to involve a Ca^{2+} signaling, the generation of intracellular free radicals, and mitochondrial dysfunction. The clinical failure and nonspecific effects of calcium channel blockers and free radical scavengers highlight the importance of determining the molecular events that cause ischemic death; only in this way will valid therapeutic targets be identified. Several channels of the TRPM family have been implicated in ischemic cell death and represent novel targets for therapeutic research. Indeed, TRPM7 knockdown both *in vitro* and *in vivo* can prevent neuronal death in experimental models of stroke (Aarts et al., 2003) (M. Aarts, unpublished observations). Dr. Michelle Aarts (University of Toronto, Scarborough, Toronto, Canada) has been studying the mechanism by which TRPM7 facilitates cell death in these models. Cation channels are believed to regulate acute neuronal death, via both the regulation of intracellular Ca^{2+} and intracellular signaling. However, what interactions exist between TRPM channel proteins and what part each protein plays in ischemic disease remain unknown. In this context, it is intriguing that the processes in anoxic injury as resolved by TRPM7 knockdown are attributed to the TRPM2 channel *in vitro*. Recombinant TRPM2 is activated by the direct application of both hydrogen peroxide and ADP ribose, the latter being a cleavage product released from mitochondria during oxidative stress (Kraft et al., 2004; Miller, 2004; Kolisek et al., 2005). Recently, TRPM2 was shown to be upregulated in brain ischemia, and its activation has been linked to the activity of polyADP ribose polymerase, a nuclear enzyme that is activated in ischemic injury (Fonfria et al., 2006). These findings suggest that TRPM7 and TRPM2 functionally interact during anoxia-induced cell death *in vitro*.

TRPM channels within the cerebrovascular network may also mediate constrictive forces that exacerbate tissue death after ischemic injury. This possibility is supported by new evidence that TRPM members act as mechano-stimulated channels. Vascular (smooth muscle) damage has been shown to lead to dramatic enhancement of TRPM7 channel expression at the cell surface (Numata et al., 2007b), and TRPM7 can be activated by cellular swelling (Numata et al., 2007a). This vascular role may be linked to the interaction of TRPM7 with cytoskeletal elements and to its proposed role in regulating cell morphology (Dorovkov and Ryazanov, 2004; Clark et al., 2006). TRPM4 activation by $[Ca^{2+}]_i$ has also been shown to induce contraction in cerebral vessels (Reading and Brayden, 2007). Together, these finding suggest that TRPM channels may exacerbate ischemic conditions by decreasing critical blood flow to the brain. The identification of new protein interactions will lead to discovery of the pathways that are downstream of TRPM proteins and govern cell survival.

Mutations in the TRPML1 gene cause a lysosomal storage disease

The functions of TRP channels in normal physiology are widespread, and so an association between a variety of disease and mutant TRP channel genes might be expected. One example is a lysosomal storage disease, which include genetic conditions that impair the function or localization of proteins that are responsible for the digestion or absorption of endocytosed materials. The resulting cellular "indigestion" causes a buildup of intracellular storage inclusions that contain unprocessed lipids, proteins, or macromolecular complexes. Most lysosomal storage diseases are associated with degenerative processes and cause severe developmental delays, cognitive disabilities, blindness, and early death. An example of such a disease is mucolipidosis type IV (MLIV) (Slaugenhaupt, 2002; Bach, 2005), which is caused by mutations

in a gene termed *MCOLN1* (Bargal et al., 2000; Bassi et al., 2000; Sun et al., 2000). This gene encodes the ion channel TRPML1, which is localized in lysosomes. The debate over TRPML1 function focuses primarily on whether this channel (1) directly modulates membrane traffic within the lower portion of the endocytic pathway (Piper and Luzio, 2004) or (2) regulates lysosomal ion homeostasis (Miedel et al., 2008) (this controversy reviewed by Zeevi et al., 2007). Identifying the role of TRPML1 in the endocytic pathway will be absolutely crucial, because it will define the direction of the future search for pharmacological interventions for MLIV.

Membrane traffic delays have been demonstrated in human skin fibroblasts affected by MLIV; this led to conclusion that TRPML1 directly regulates membrane traffic (LaPlante et al., 2004; Treusch et al., 2004; Bach, 2005; Pryor et al., 2006). This is the first indication that ion channels may be involved in intracellular membrane fusion/fission events. However, a serous limitation of this experimental system is that the chronic accumulation of undigested lipids in these cells may affect the ability of traffic markers to enter organelles, misleadingly manifesting as traffic delays. In order to circumvent this problem, the group of Dr. Kirill Kiselyov (University of Pittsburgh, Pittsburgh, PA) developed a small interfering RNA-driven, TRPML1 acute knockdown system and tested the immediate effects of TRPML1 knockdown on membrane traffic (Miedel et al., 2008). No membrane traffic delays were detected in acutely TRPML1-deficient cells, which argues that the basis of this disease is metabolic rather than a defect in trafficking. This finding suggests that enzyme replacement therapies for the treatment of MLIV should focus on the formulation of modified enzymes to work in the MLIV-specific ionic environment and emphasizes the need for a deeper inquiry into TRPML1 permeability and regulation.

Like most lysosomal storage diseases, MLIV is a neurodegenerative disorder. Although degenerative processes have been shown in all lysosomal storage diseases, a correlation between the number of storage inclusions and the severity of the degenerative processes is not always apparent. This suggests that a specific mechanism set in motion by lysosomal deficiencies drives degenerative processes in these diseases. Dr. Kiselyov's group found that the suppression of lysosome function in lysosomal storage diseases inhibits the utilization of aged mitochondria (Jennings et al., 2006). The resulting buildup of effete mitochondria promotes the proapoptotic effects of Ca^{2+} and results in a higher percentage of cell death when the cells are stimulated by hormones and neurotransmitters (Kim et al., 2007; Kiselyov and Muallem, 2008). Similar results have been reported recently in other experimental systems (Pacheco et al., 2007; Settembre et al., 2008; Vergarajauregui et al., 2008). These results explain the specificity of neurodegeneration in lysosomal storage diseases and suggest that caspase inhibitors may be used as complimentary treatments for lysosomal storage diseases.

Deafness in *varitint-waddler* mutant mice results from constitutive activation of TRPML3

Although no disease has yet been associated with TRPML3, the phenotype of mouse *Trpml3* mutants suggest that this gene should be considered a candidate locus in congenital diseases that include sensorineural hearing loss. Thus, *varitint-waddler* (*Va*) mutant mice, which are deaf and have vestibular impairment, bear a semidominant mutation in the TRP channel-encoding gene *Trpml3* (Cloudman and Bunker, 1945; Deol, 1954; Cable and Steel, 1998; Di Palma et al., 2002). *Varitint-waddler* mice display several inner ear defects, including (1) reduction or elimination of the endocochlear potential, (2) anatomical alteration of the stria vascularis, the cochlear structure that generates this potential, with its marginal cells rounding up and losing their cytoplasmic processes, and (3) degeneration and loss of sensory hair cells, which display apical deformations at embryonic stages and are later extruded from the sensorineural epithelium (Deol, 1954; Cable and Steel, 1998). The *Va* mutation results in an alanine-to-proline substitution at residue 419 (A419P) of TRPML3, and this is thought to break the α helix of the fifth transmembrane domain (S5), near the pore (Di Palma et al., 2002; Grimm et al., 2007). How this form of TRPML3 contributes to cell death is unknown. Jaime García-Añoveros (Northwestern University, Evanston, IL) and his coworkers have been studying this issue.

Is the effect of TRPML3 on cell viability cell autonomous? The García-Añoveros group demonstrated that many epithelial cells that line the cochlear scala media and the vestibular endolymphatic compartments of the inner ear express *TRPML3* mRNA (Nagata et al., 2008). These include the marginal cells of the stria vascularis and the equivalent dark cells of the vestibule, as well as the cochlear and vestibular mechanosensory hair cells, which degenerate in *varitint-waddler* mice. Furthermore, this group showed that, when heterologously expressed in LLC–PK1–CL4 epithelial cells, which serve as a culture model for hair cells, a TRPML3::GFP (green fluorescent protein) fusion protein accumulated in lysosomal vacuoles as well as in espin-enlarged microvilli that resemble stereocilia (Nagata et al., 2008). When these cells express the mutant TRPML3 (A419P), they die and are extruded from the epithelium in a manner reminiscent of the degeneration of hair cells in *Va* mice (Nagata et al., 2008). Together, these findings suggest that hair cell death in *varitint-waddler* mice occurs because of cell-autonomous expression of mutant TRPML3.

What effect does the A419P mutation have on TRPML3 function? Like many other TRP channels, TRPML3 forms cation channels that normally open only in response to high positive potentials and display outer rectification. However, these channels can also open at negative potentials generating double rectification (Kim et al., 2007; Nagata et al., 2008). TRPML3 channels have a preference for calcium over sodium and potassium and are blocked by gadolinium and verapamil but not by ruthenium red, gentamycin, or amiloride (Xu et al., 2007; Nagata et al., 2008). They have permeabilities ranging from 50 pS (at negative potentials) to 70 pS (at positive potentials) (Nagata et al., 2008). The A419P mutation does not affect either the conductance or permeability of the TRPML3 channel. Instead, this gain-of-function mutation greatly enhances the open probability of the channel at hyperpolarized potentials (Nagata et al., 2008). The result of this hyperactivity is a large inwardly rectifying cationic current and severe cellular depolarization (Grimm et al., 2007; Kim et al., 2007; Xu et al., 2007; Nagata et al., 2007, 2008). Of note, recent work from the Clapham laboratory indicates that the pale coat color of *varitint-waddler* mutants likely results from the death of melanocytes (Xu et al., 2007). In summary, these findings suggest that constitutive activity of TRPML3 (A419P) channels at physiological potentials likely underlies the melanocyte cell loss, hair cell degeneration, and deafness that characterize *varitint-waddler* mice.

TRPM7 prevents melanin-synthesis-dependent death of embryonic melanocytes

TRPM7 seems a prime candidate to be a disease locus because it is required for viability of several cell types. Specifically, *TRPM7*

knockdown in B-cells, retinoblastoma, and smooth-muscle cell lines causes growth arrest and/or cell death (Nadler et al., 2001; Hanano et al., 2004; He et al., 2005). Supporting an essential role for TRPM7 in normal development, *trpm7* mutants have been isolated several times in phenotype-based mutagenesis screens in zebrafish. Two of these alleles have been molecularly characterized; both carry mutations that cause a frame shift in sequence encoding the intracellular C terminus (Elizondo et al., 2005). The phenotype of embryos homozygous for either of these alleles resembles that in embryos injected with *trpm7* antisense oligonucleotides, implying that the alleles are hypomorphs (loss-of-function) (Elizondo et al., 2005). Zebrafish embryos homozygous for mutant alleles of *trpm7* display a range of phenotypes at various developmental stages. At embryonic stages, *trpm7* mutants are characterized by the death of embryonic melanocytes and a transient period of paralysis (Kelsh et al., 1996; Arduini and Henion, 2004; Cornell et al., 2004); at larval stages and adult stages, they display dwarfism, abnormal skeletogenesis, and kidney stones (Elizondo et al., 2005). Of note, knockdown of TRPM7 expression caused concomitant reduction of TRPM2 expression in cultured cortical neurons (Aarts et al., 2003); it is an interesting and testable possibility that reduction of *trpm2* expression occurs in zebrafish *trpm7* mutants and contributes to phenotypes therein.

Robert Cornell's group (University of Iowa, Iowa City, IA) has been investigating the mode of melanocyte cell death and the cellular underpinnings of paralysis in zebrafish *trpm7* mutant embryos. Application of a broad-specificity caspase inhibitor, which prevents melanocyte cell death in zebrafish embryos mutant for the gene encoding the receptor tyrosine kinase Kit, does not have this effect in *trpm7* mutants, implying that melanocyte death in these mutants does not occur by apoptosis (McNeill et al., 2007). In contrast, supplementing embryo medium with magnesium, but not calcium, rescued melanocyte cell death in *trpm7*, but not *kit*, mutants. Interestingly, the inhibition of melanin synthesis via application of a tyrosinase inhibitor also served to prevent melanocyte cell death in *trpm7* mutants (McNeill et al., 2007). Combined with the fact that the intermediates of melanin synthesis are toxic reactive oxygen species, these findings imply that loss of Trpm7 leads to magnesium deficiency in melanophores, resulting in a buildup of toxic intermediates of melanin synthesis that induce necrotic cell death. Notably, the loss of TRPM7 and excess TRPML3 activity both result in the death of melanocytes (McNeill et al., 2007; Xu et al., 2007), possibly revealing a complex interaction between these channels.

Paralysis in zebrafish *trpm7* mutant embryos is intriguing because of the association of *TRPM7* with a neurodegenerative disease in humans, but its cellular basis remains unknown. Thus, a hypomorphic variant of TRPM7, encoding a channel with reduced propensity to close in response to intracellular magnesium, is associated with increased risk for the neurodegenerative disease *lytico bodig*, a disease with neurofibrillary tangles and features of amyotrophic lateral sclerosis and parkinsonism (Hermosura et al., 2005) [TRPM2 may also be associated with this disease (for review, see Hermosura and Garruto, 2007)]. Dopaminergic neurons share similar metabolic chemistry with melanocytes, and the byproducts of dopamine metabolism are also known to be toxic. Therefore, it is possible that, like melanocytes, dopaminergic neurons require TRPM7 to prevent toxic buildup of dopamine metabolites. An alternative explanation for paralysis in TRPM7 mutants is abnormal cholinergic signaling at the neuromuscular junction or perhaps in the brain because cholinergic neurons in the sympathetic neurons require TRPM7 for normal synaptic transmission (Krapivinsky et al., 2006; Brauchi et al., 2008). Additional exploration of the cellular basis of paralysis in zebrafish *trpm7* mutants may yield insight into the etiology of *lytico bodig* and potentially other neurodegenerative diseases.

Some TRP channels are regulated by G-protein-coupled receptors

The accumulating evidence that TRP channels contribute to disease processes motivates an improved understanding of how TRP channels are regulated. The prototypical TRP channel, dTRP, mediates phototransduction in *Drosophila*, and similarly many vertebrate TRP channels are involved in sensory transduction. At least six TRP channels are directly gated by sensory stimuli, whereas others, such as dTRP, are activated downstream of G-protein-coupled receptors (GPCRs). This latter class includes TRPC2, an ion channel expressed in the pheromone-sensing vomeronasal organ of mammals (Liman et al., 1999; Stowers et al., 2002), TRPM5, an ion channel that is primarily restricted to chemosensory cells (Perez et al., 2002; Zhang et al., 2003), and TRPA1 and TRPV1, two ion channels that are involved in nociception (Jordt et al., 2003; Dhaka et al., 2006). In nonsensory tissues, including muscle and brain, some TRP channels may, likewise, transduce the binding of neurotransmitters to GPCRs into electrical responses (Clapham, 2003).

The general model for understanding the GPCR-based mechanism of TRP channel activation is based on extensive work in the fly photoreceptor (Montell, 1999; Hardie, 2007). A key component of this system is PLC (NorpA), which is activated after absorption of light by rhodopsin, and is essential for phototransduction (Bloomquist et al., 1988). PLC activation leads to the hydrolysis of phosphatidyl inositol (4,5) bisphospate [PI(4,5)P2] into diacylglycerol (DAG) and inositol trisphosphate (IP_3), as well as to the release of intracellular Ca^{2+} (Berridge, 1993); any of these products might be the one that activates the *Drosophila* TRP channels. Several lines of evidence suggest that it is the lipid metabolites of DAG that activate the fly TRP channels and mediate phototransduction (Chyb et al., 1999; Leung et al., 2008). Unfortunately, the failure of these compounds to activate native channels has impeded progress in confirming this possibility (Hardie, 2007).

Taste is an excellent system in which to study the regulation of TRP channels that lie downstream of GPCR signaling because many of the molecular components of taste transduction have been identified, and the sensory stimuli are well characterized. Bitter is detected by a small family of GPCRs, whereas sweet and umami are each detected by a heterodimeric GPCR (Chandrashekar et al., 2006). Receptors for bitter, sweet, and umami tastes are coupled through trimeric G-proteins to the enzyme PLCβ2, whose activity is essential for taste transduction (Zhang et al., 2003). Also essential is the ion channel TRPM5 (Perez et al., 2002; Zhang et al., 2003; Damak et al., 2006), as revealed by the near insensitivity of TRPM5 knock-out mice to both bitter and sweet substances (Zhang et al., 2003; Damak et al., 2006).

How, then, does PLC activation lead to a change in the gating of TRPM5 channels? In the laboratory of Emily Liman (University of Southern California, Los Angeles, CA), this question has been addressed by studying the responses of TRPM5 channels, in both cells expressing heterologous TRPM5 and taste cells expressing native TRPM5, to putative second messengers. Initial studies by this group and others have shown that, when expressed heterologously, TRPM5 forms a nonselective cation channel that is activated by the elevation of intracellular Ca^{2+} (Hofmann et al., 2003; Liu and Liman, 2003; Prawitt et al., 2003) or by the

depletion of Ca^{2+} stores (Perez et al., 2002). More recently, the Liman group showed that intracellular Ca^{2+} released from IP_3 stores gates TRPM5 in native taste receptor cells (Zhang et al., 2007) and that, after sustained activation, TRPM5 channels, in both native and heterologous cells, desensitize by a process that may be mediated be the depletion of PI(4,5)P2 (Liu and Liman, 2003). Together, these data suggest a model for taste transduction whereby elevation of IP_3 and the ensuing release of intracellular Ca^{2+} gates TRPM5, leading to membrane depolarization. Although this mechanism cannot explain phototransduction in *Drosophila*, in which Ca^{2+} has no direct activating effect (Ranganathan et al., 1994; Hardie, 1995) and the IP_3 receptor is dispensable (Acharya et al., 1997; Raghu et al., 2000), it may be applicable to other systems in which Ca^{2+}-activated TRP channels participate.

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