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Sensory Transduction

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1. Direct Patch-Clamp Recordings from Melanosomes Reveal a Chloride Channel Required for Pigmentation.
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Pigment cells contain unique lysosome-related organelles called melanosomes that are responsible for the production and storage of melanin, the major pigment in animals. The oculocutaneous albinism 2 (OCA2) gene encodes a putative melanosomal ion transporter critical for pigmentation. Indeed, mutations in OCA2 result in absent or decreased pigment in the eyes and skin, causing pronounced visual deficits and increased skin cancer risk. However, the molecular function of OCA2 remains unknown. Here we used patch-clamp measurements of endolysosomes containing heterologously expressed OCA2 to record, for the first time, the physiological properties of OCA2. We found that OCA2 expression is required for a chloride-selective current with ion channel properties. OCA2-mediated currents were significantly reduced by a prevalent mutation found in patients with oculocutaneous albinism. Direct patch-clamp recordings from melanosomes reveal that endogenous OCA2 contributes to a similar chloride conductance required for pigmentation. Moreover, organelles expressing OCA2 have higher luminal pH, suggesting that the chloride conductance may affect melanin synthesis by regulating melanosomal pH. Our results identify OCA2 as an essential component of a melanosomal anion channel, and suggest that regulation of melanosome pH is critical for melanogensis.

2. Ultraviolet Light Detection in Human Skin via a Novel Phototransduction Pathway.
NICHOLAS W. BELLONO, JULIA A. NAJERA, and ELENA V. OANCEA, *Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI 02912*

Human skin is constantly exposed to light, but is only known to elicit a physiological response to ultraviolet (UV) radiation, suggesting that it is able to detect UV wavelengths. Solar UV radiation is a powerful environmental carcinogen comprising ∼95% UVA and ∼5% UVB at the Earth's surface. Human skin has the unique ability to respond to UV by synthesizing the pigment melanin, which protects the skin by absorbing UV photons and reactive oxygen species. UVB is directly absorbed by DNA and causes damage, which activates a pathway resulting in increased melanin within days after exposure; this delayed UVB-activated response is not likely to function as the detection mechanism for solar UV. The effects of UVA on human skin are more elusive, and no receptor-mediated pathway activated by UVA has been identified.

We investigated the mechanism by which human epidermal skin detects and responds to UV light by simultaneously stimulating epidermal melanocytes with physiological doses of UV and performing fluorescence calcium imaging or electrophysiological recordings. We found that stimulation of melanocytes with UV evoked rapid intracellular calcium responses that were mediated by release from internal calcium stores and influx from that extracellular medium. This signaling pathway is retinal-dependent, mediated by activation of a Gαq protein and PLCβ, and leads to activation of the transient receptor potential A1 (TRPA1) ion channels, which mediate the calcium influx. The resulting elevation in cytosolic calcium is critical for increased cellular melanin within minutes of exposure, an order of magnitude faster than previously reported. Our results reveal a novel UV signaling mechanism in human melanocytes and have broad implications for understanding light-induced signal transduction and its physiological consequences on human skin.

3. Depletion of Phosphoinositides by TRPV1 Activation Inhibits Piezo Channels.
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Capsaicin, the activator of the noxious heat sensor TPRV1, is clinically used to relieve chronic pain. Desensitization of TRPV1 involves downstream PLCδ activation, but the mechanism by which capsaicin treatment can also alleviate mechanical allodynia is unknown. Piezo2 encodes rapidly adapting mechanically activated currents in sensory neurons. Expression of these novel mechanosensitive ion channels in a subset of TPRV1 positive neurons suggests their role in pain-related sensory mechanotransduction.

Our study shows that capsaicin application inhibited rapidly adapting mechanically activated currents in TRPV1 expressing sensory neurons. We also show that TRPV1 activation inhibited heterologously expressed Piezo1 and Piezo2 ion channels in whole-cell patch clamp experiments. Inclusion of either phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] or PI(4)P in the patch pipette alleviated this inhibitory effect. Besides, activation of PLCβ by stimulating muscarinic receptors only marginally inhibited mechanically activated Piezo1 currents. Phosphoinositide sensors revealed that activation of PLCδ by a robust calcium influx through TRPV1 severely depleted $PI(4,5)P_2$ and PI(4)P. On the other hand, muscarinic stimulation of PLCβ significantly decreased $PI(4,5)P_2$ levels, but only induced a small decrease of PI(4)P. This differential activation of PLC isoforms may explain the difference between the

inhibitory effects of the two PLC pathways. Targeted depletion of $PI(4,5)P_2$ and its precursor PI(4)P using a chemically inducible lipid phosphatase replicated the inhibition of Piezo1 currents. Additionally, $PI(4,5)P_2$ and PI(4)P applied to excised inside-out patches inhibited the rundown of Piezo1 activity, further emphasizing the significance of these phosphoinositides in Piezo channel regulation.

Here we demonstrate that the activity of Piezo channels requires the presence of either $PI(4,5)P_2$ or PI(4)P, and severe depletion of both phosphoinositides by TRPV1 activation limits channel activity. In conclusion, our data may explain how capsaicin alleviates mechanically induced pain.

4. Structural and Functional Insights into Mechanosensitivity of the Human K^+ Channel TRAAK. STEPHEN G. BROHAWN[1] and RODERICK MACKINNON,[1,2] *[1]The Rockefeller University and [2]Howard Hughes Medical Institute, New York, NY 10065*

Mechanosensitive ion channels underlie such fundamental processes as our ability to feel and hear, but the molecular basis of their mechanosensitivity is poorly understood. TRAAK and TREK are members of the two-pore domain K^+ (K2P) channel family. They are biophysically mechanosensitive, and their ablation results in mechanical allodynia in mice. Here we present our current molecular and biophysical understanding of the mechanosensitivity of these channels. We show that among all K2P channels, only TRAAK and TREK are mechanosensitive. Mechanically activating TRAAK can electrically counter the depolarizing mechanosensitive channel Piezo1 expressed in the same cell. We demonstrate with pure reconstituted TRAAK and TREK1 that mechanosensitivity is mediated directly by the lipid membrane in these channels. This extends the force–from–lipid paradigm discovered in unrelated bacterial channels to eukaryotes, contrary to the widely held assumption that eukaryotic mechanosensitive channels rather work through tethers or upstream force sensors. We have solved x-ray crystal structures of TRAAK to investigate K2P structure and gating principles in general and mechanical force sensation of TRAAK in particular. These structures show remarkable divergence from the canonical K^+ channel architecture. Two unique features of TRAAK are intimately associated with the membrane and may be important for gating and mechanosensitivity. First, TRAAK presents a wide lateral opening from the channel cavity to the lipid membrane that can be sealed by protein conformational changes in a transmembrane helix. Second, two diagonally opposed helices form extended membrane-interacting structures at the cytoplasmic leaflet in TRAAK and TREK. We propose a model for gating of TRAAK K^+ channels based on these data.

5. *Drosophila* Rhodopsin 6 Required for Temperature Preference in Larvae: Multiple Rhodopsins Required for Temperature Discrimination in *Drosophila melanogaster*. HSIANG-CHIN CHEN, TAKAAKI SOKABE, and CRAIG MONTELL, *Neuroscience Research Institute, MCDB Department, University of California, Santa Barbara, Santa Barbara, CA 93106*

Until recently, rhodopsins were thought to function exclusively in light sensation. However, our laboratory reported that one of the six known *Drosophila* rhodopsins (Rh1) was required in larvae for fine temperature discrimination in the comfortable range (18 vs. 23°C). Here, we report that distinct rhodopsins were required during different stages of larval development. We found that Rh1 was essential predominantly during the early third instar, but not during the late third instar. Therefore, we screened for a requirement for other rhodopsins, and found that Rh6 functioned in discriminating between 18 and 23°C during the late third instar. Unexpectedly, Rh6 played a dominant role in temperature preference throughout the second to third instar. Rh6 is known to be expressed in larval photo-sensitive tissue—the Bolwig organ. However, the function of Rh6 in thermosensation was Bolwig organ independent, and was not affected by acute light. Instead, we found that Rh6 was required in *trpA1*-positive neurons in the brain, rather than peripheral neurons. Thus, we conclude that multiple rhodopsins have unconventional roles in thermosensation.

H.-C. Chen and T. Sokabe contributed equally to this work.

6. TRP Trafficking in *Drosophila* Photoreceptor Cells Dependent on XPORT Proteins. ZIJING CHEN and CRAIG MONTELL, *Neuroscience Research Institute, MCDB Department, University of California, Santa Barbara, Santa Barbara, CA 93106*

TRP channels play important roles in transducing sensory signals initiated by light, odorants, tastants, mechanical stimuli, and changes in temperature. To function in sensory transduction, most TRP channels need to be trafficked to the plasma membrane within sensory receptor cells. We are exploiting *Drosophila* photoreceptor cells to dissect the mechanisms underlying TRP channel trafficking. Within *Drosophila* photoreceptor cells, TRP needs to target to a specialized membrane organelle—the rhabdomere. Here we report the discovery of a single-pass transmembrane protein (CG42508; XPORTB) that is involved in TRP trafficking in *Drosophila* photoreceptor cells. XPORTB was encoded by a previously unrecognized bicistronic mRNA that also encoded another unrelated single-transmembrane protein, XPORT (CG4468), which Nansi Colley and colleagues reported was essential for trafficking of TRP (Rosenbaum et al. 2011. *Neuron.* 72:602–615). We found that mutation of *xportB* caused a transient light response indistinguishable from *trp* null

mutants. TRP protein levels in *xportB* mutants were reduced to 3% the levels expressed in wild type. The residual TRP was mislocalized outside of the rhabdomeres, where TRP normally functioned during phototransduction. The XPORTB protein was localized in intracellular compartments, suggesting that it was required for trafficking TRP to the rhabdomeres. We are exploring the underlying mechanistic functions of XPORT and XPORTB. We propose that clarifying the roles of these proteins will illuminate similar mechanisms controlling the intracellular trafficking of other TRPs, including those in mammalian cells.

7. Long-Term Potentiation of Glycinergic Synapses Triggered by Interleukin-1β. A.M. CHIRILA,[1] T.E. BROWN,[2] R.J. STEVENSON,[1] R.A. BISHOP,[1] and J.A. KAUER,[1] *[1]Department of Molecular Pharmacology and Physiology, Brown University, Providence, RI 02912; [2]School of Pharmacy, University of Wyoming, Laramie, WY*

Although glycine is a major inhibitory neurotransmitter in key areas of the CNS, little is known about the regulation of glycinergic synaptic strength. Here we report that in the spinal cord dorsal horn, glycinergic synapses on GAD65-EGFP lamina II inhibitory interneurons exhibit LTP, triggered rapidly after exposure to the inflammatory cytokine interleukin-1β (IL-1β, 10 ng/ml; IPSC amplitudes: 165.0 ± 11% of pre–IL-1β values, $n = 24$, $P < 0.0001$).

Our data suggest that glycine receptor LTP (GlyR LTP) results from an increase in postsynaptic GlyR number or function. To probe this, we first disrupted membrane fusion reactions in GAD65-EGFP neurons. Blocking postsynaptic SNARE proteins with *N*-ethylmaleimide (NEM; 5 mM) in the recording pipette prevented GlyR LTP (IPSC amplitudes: 88.9 ± 9.3% of pre–IL-1β values, $n = 9$, n.s.). We next recorded glycinergic IPSCs for a baseline period, followed by application of the high affinity GlyR antagonist strychnine (2 μM) to block all surface GlyRs. Slices were then washed for 20 min and either IL-1β or BSA was bath-applied. Recovery from strychnine block was significantly greater in slices treated with IL-1β compared with BSA controls (percent recovery at 40–46 min after washing strychnine: control 8.7 ± 3.7%; IL-1β: 28.8 ± 7.1%, $n = 5$; $P < 0.05$). Together, our data indicate that IL-1β may promote exocytosis of intracellularly sequestered GlyRs.

Notably, peripheral inflammation in vivo might trigger GlyR LTP. GABAergic neurons from saline-treated mice had robust GlyR LTP, whereas those from formalin-treated hyperalgesic mice did not (IPSC amplitudes: saline-injected: 176.6 ± 16.7%, $n = 9$; formalin-injected: 104.1 ± 10.5%, $n = 7$; $P < 0.005$). Furthermore, glycinergic mIPSCs in neurons from formalin-treated mice were significantly larger compared with saline-treated controls (mIPSC amplitudes, formalin-treated, $n = 8$: 148 ± 12% of saline-treated animals, $n = 5$; $P < 0.05$), suggesting that GlyR LTP is triggered during inflammatory peripheral injury. Blocking glycine receptor LTP may represent a useful therapeutic strategy in the treatment of inflammatory pain.

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8. Heterodimeric Sweet Taste Receptor Signaling through Cis- and Trans-Activation Mechanisms. MENG CUI and DIOMEDES E. LOGOTHETIS, *Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA 23298*

Sweet taste receptors (STRs) belong to the family of G protein–coupled receptors (GPCRs). Structurally, the STR is an obligate heterodimer of taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3). Growing evidence shows that the STR has multiple ligand binding sites for different sweeteners. For example, the receptor can be activated through (1) the Venus Flytrap Module (VFTM) of T1R2 by aspartame, neotame, and sucralose; (2) the Transmembrane Domain (TMD) of T1R2 by perillartine and SWT819; (3) the TMD of T1R3 by cyclamate and NHDC; and (4) the combined extracellular domains of T1R2/T1R3 by the sweet proteins, thaumatin and monellin. Interestingly, all sweet responses can be blocked by the sweet taste inhibitor lactisole that binds to the TMD of T1R3. Thus, the interaction between the two monomers and intersubunit cross-talk contributes importantly to the function of the receptor. However, molecular mechanisms of STR intersubunit cross-talk and sweet taste signaling remain unclear. Here, we present a characterization of the intracellular signaling of STRs using an ion channel activity assay. We used the *Xenopus laevis* oocyte expression system to coexpress ion channels, sweet taste receptors, T1R2 and T1R3, and G proteins, including $G\alpha i_1$, $G\alpha i_2$, $G\alpha i_3$, $G\alpha_s$, $G\alpha_q$, $G\alpha_o$, or $G\alpha_{gustducin}$. We used Girk1 (Kir3.1) and Girk2 (Kir3.2) heteromeric ion channels as reporters of the Gi, Go, and Gs signaling pathways, and IRK3 (Kir2.3) ion channels as reporters of the Gq signaling pathway. Together with the ion channels and G proteins, we expressed either T1R2 or T1R3 alone or together to study the signaling of each T1R subunit homomer or T1R2/T1R3 heteromer. Our results indicate that the $G\alpha_{gus}$ and $G\alpha i_2$ proteins couple to the T1R2 subunit and transduce signals through the Gi signaling pathway.

9. Pharmaceutical Manipulation of ANO1 Ca^{2+}-gated Cl^- Channel Activates and Modulates Nociceptive Neurons and Behaviors. FARAH DEBA and BRET BESSAC, *Rangel College of Pharmacy, Texas A&M Health Science Center, Kingsville, TX*

Tissue injury and potential hazards, such as heat and reactive chemicals, are detected by specific receptors of nociceptor neurons that signal via action potentials to

central neurons for protective responses, such as pain perception. Sensory neuronal Cl^- channel ANO1 is directly gated by noxious heat and intracellular Ca^{2+}, $[Ca^{2+}]_i$, released from internal stores by factors signaling cell damage activation of G protein–coupled receptors. Because of the relatively high intracellular Cl^-, $[Cl^-]_i$, ANO1 gated Cl^- efflux depolarizes sensory neuron membranes and subsequently propagates action potentials, resulting in pain perception. Here, we show that direct activation of ANO1 by 10 µM Ano1-act (TMEM16A Activator, Eact) induces action potential propagation of mouse dorsal root ganglia sensory neurons, and intraplantar injection of 5 mM Ano1-act induces mouse nociceptive behaviors, in vivo. Detection of cold, hot, and reactive chemicals by sensory neuronal cation channels also increases $[Ca^{2+}]_i$. Potentially, $[Ca^{2+}]_i$ from these cation channels activates ANO1. This would allow ANO1 to compound with the cation influx to depolarize the membrane and trigger action potentials. Inflammation and other conditions increase $[Cl^-]_i$, which would increase ANO1 facilitation of membrane depolarization by cation channels, and *visa versa*, ANO1 opening would inhibit membrane depolarization in conditions that decrease $[Cl^-]_i$. We show that blocking ANO1 activity with 20 µM ANO1-inh (T16Ainh-A01) will diminish action potential propagation of mouse sensory neurons induced by 15 µM capsaicin, an agonist of the TRPV1 cation channel that is crucially involved in noxious heat perception. Furthermore, the nocifensive behavioral responses to intraplantar injections of 50 µM capsaicin are attenuated by coapplication of 1.3 mM Ano1-inh. Our results indicate that direct activation of Ano1 can propagate sensory neuronal action potentials, resulting in nocifensive behaviors, and ANO1 facilitates TRPV1-mediated activation of sensory neurons and nocifensive behaviors. Thus, blocking Ano1 attenuates TRPV1-mediated activation of sensory neurons and nocifensive behaviors.

10. Diacylglycerol Activates the Light-dependent Channels TRP and TRPL in *Drosophila* Photoreceptors.

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Drosophila TRP and TRPL channels are confined to the light-sensitive microvilli of the photoreceptor's rhabdomere. Photoactivated rhodopsin triggers a signaling cascade involving phospholipase C, which cleaves phosphatidylinositol bisphosphate (PIP_2) into IP_3, diacylglycerol (DAG), and H^+. DAG kinase (DGK) turns DAG into phosphatidic acid. Additionally, a DAG lipase may cleave DAG generating polyunsaturated fatty acids (PUFAs). PIP_2, DAG, H^+, and PUFAs have been considered as possible TRP/TRPL activators; however, the identity of the activator had not been determined. We tested each one of these candidates in inside-out microvilli patches containing the TRP channel, making use of mutants and pharmacology. In patches excised under illumination, TRP was constitutively active and could be closed with ATP. In contrast, the channel was closed in patches excised in darkness and could be opened with DAG. The ATP effect was abolished by inhibiting DGK (R59022) and was absent in *rdgA* (DGK mutant). This evidence shows that excised patches retained DGK and that the level of activator was locked as it was upon excising the patch. DAG activated TRP under a DAG-lipase inhibitor (RHC-80267), suggesting that PUFAs are not involved. PIP_2 had no affect and acidification (pH 6.4) irreversibly activated TRP, inconsistent with a regulatory role of the channel. We also studied the lipid profile of a rhabdomere-enriched membrane preparation derived from light- and dark-adapted eyes, by liquid chromatography–mass spectrometry, and detected a light-dependent increment in six (of seven) unsaturated DAG species, no changes in saturated DAGs (three), and no changes in PUFAs. These results strongly support DAG as the TRP/TRPL activator and are inconsistent with a role of PUFAs, PIP_2, and H^+.

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11. Different Classes of Sensory Neurons Visualized and Controlled in Spinal Dorsal Horn by Optogenetic Methods.

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Analysis of synaptic events in the spinal dorsal horn arising from distinct, identified populations of sensory neurons is critical though technically complex. Sensory neuron populations in dorsal root ganglia are heterogeneous and have been grouped according to cell size, neuropeptide content, genetic markers, ionic current, and axonal conduction velocity. Conventional electrical stimulation methods are unable to selectively activate presynaptic fibers from specific subpopulations of neurons because sensory fibers comingle in the dorsal root. Here, we used optogenetic methods to elicit synaptic events in spinal cord slices and paw withdrawal responses in awake, behaving mice by activation of TRPV1-expressing or $Ca_V3.2$-expressing afferent fibers. We generated two mouse strains expressing a channelrhodopsin-YFP (ChR2-EYFP) fusion protein in either TRPV1- or $Ca_V3.2$-expressing neurons by mating existing TPRV1-cre mice (JAX no. 17769) and a new *Cacna1h*-cre strain generated by our laboratory to cre-dependent ChR2-EYFP–expressing mice (JAX no. 012569).

$Ca_V3.2$-expressing neurons are characterized by large, low-threshold T-type currents, medium-sized cell bodies, mechanosensitivity, and a lack of capsaicin response. We found that the TRPV1-expressing fibers that detect noxious thermal stimuli terminate primarily in laminae I/II and colocalize with CGRP, as previously reported, whereas $Ca_V3.2$-expressing fibers terminate throughout superficial laminae of the dorsal horn and do not colocalize with CGRP. We recorded light-evoked, postsynaptic AMPAR and NMDAR currents in retro-labeled projection neurons and lamina II interneurons from TRPV1/ChR2-EYFP acute spinal cord slices. Additionally, an LED lamp applied to the ventral surface of the hindpaw elicited a nocifensive response in both TRPV1/ChR2-EYFP and $Ca_V3.2$/ChR2-EYFP mouse strains, but not in control mice. Using these strains, we can now activate specific subpopulations of sensory neurons and study the resulting synaptic currents in acute spinal cord slices and behavioral responses in awake, behaving mice.

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12. Melanopsin Tristability for Sustained and Broadband Photoreception. ALAN J. EMANUEL and MICHAEL TRI H. DO, *F.M. Kirby Neurobiology Center, Boston Children's Hospital; Program in Neuroscience, Harvard Medical School; Center for Brain Science, Harvard University; Division of Sleep Medicine, Brigham and Women's Hospital, Boston, MA*

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are mammalian photoreceptors that use a receptor pigment called melanopsin to capture light. Melanopsin-based phototransduction is required for normal operation of many visual responses, particularly "non-image" functions such as regulation of the circadian clock, sleep, and hormone levels. We have studied the intrinsic light responses of ipRGCs by performing patch-clamp electrophysiology in the mouse retina and using numerical modeling. Our investigations suggest that melanopsin is unlike any other known pigment in that it has three stable conformational states: one that signals electrically and two that are silent. Thermal stability of the signaling state allows ipRGCs to remain activated for minutes after illumination ceases. The magnitudes of these persistent responses are graded with wavelength. Shorter wavelengths produce larger persistent responses because they establish photoequilibria in which the signaling state dominates; conversely, longer wavelengths produce smaller persistent responses because they establish smaller fractions of the signaling state. Thus, the level of ipRGC activity can be acutely controlled by delivering monochromatic lights of different wavelengths. Persistent responses are also driven by white light that has a spectrum and intensity similar to sunlight; repeated presentations of white light cause increasing levels of the persistent response. Therefore, melanopsin's stable signaling state allows ipRGCs to sum photons over extended periods of time under natural conditions. Furthermore, activation from two silent states allows ipRGCs to integrate over wavelength because these states are spectrally distinct. Taken together, our experiments indicate that melanopsin is a tristable visual pigment, and that tristability enhances the integrative capacity of ipRGCs in both temporal and chromatic domains.

13. Processing and Modulation of the Primary Olfactory Signal. F. GENOVESE, H.G. BAUERSACHS, F. MÖHRLEN, and S. FRINGS, *Department of Molecular Physiology, Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany*

Psychophysical studies on humans have provided evidence for interactions between the trigeminal and the olfactory systems, although the cellular-processing pathways underlying these interactions are not understood yet. Our aim is to study the generation of the olfactory signal and the impact of the trigeminal system on its processing in molecular detail. Virtually all odorants can act as irritants, and most irritants have an odor. To generate olfactory signals without coactivation of the trigeminal system, we use a genetically modified mouse (Smear et al. 2011. *Nature.* 479:397). This mouse line expresses the light-sensitive channel rhodopsin (ChR2) in all mature olfactory receptor neurons (ORNs). Histological characterization of ChR2 Venus mice shows that ChR2 is expressed in the entire ORN with the exception of the olfactory cilia. Expression could be detected up to the synaptic terminals in the glomeruli of the olfactory bulb. Functional studies (electro-olfactogram) revealed that graded signals could be reproducibly triggered by light (470 nm) in the olfactory epithelium and that ORNs respond to the trigeminal neuropeptide CGRP (Daiber et al. 2013. *Eur. J. Neurosci.* 37:572). Trigeminal fibers were detected in the glomerular and olfactory nerve layers of the olfactory bulb. We therefore qualitatively and quantitatively analyzed the association between glomeruli and trigeminal fibers along the rostral–caudal axis. Immunohistochemical and electrophysiological techniques will be used to investigate the possible targets of trigeminal neuropeptides in the olfactory bulb and their modulatory effects on the olfactory circuitry. So far, trigeminal innervation of the olfactory system and its modulation by trigeminal neuropeptides indicate an interaction between those two systems, and the ChR2 mouse is the appropriate tool to study this phenomenon.

14. Taking "A Shotgun Approach": A New High-Throughput Mechanical Nociception Paradigm in *Drosophila.* MELISSA GOTTRON CHRISTIANSON and W. DANIEL TRACEY, *Department of Anesthesiology,*

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Drosophila larvae offer an excellent system in which to study molecular pathways responsible for nociception, but the available methods for studying mechanical nociception in larvae are prohibitively slow and inconsistent. Therefore, we developed a high-throughput mechanical nociception paradigm in which wandering third instar *Drosophila* larvae were ballistically stimulated with12-micron tungsten particles that were fired from an air gun.

We found that ballistically stimulated larvae exhibit the characteristic nocifensive rolling response that is observed with other noxious stimuli. This behavior depended on both tungsten density and particle emission pressure, and larval viability was unaffected by the ballistic stimulus. Importantly, the response required Class IV multidendritic neuron nociceptors. Silencing of these neurons with tetanus toxin or *paralytic* RNAi, Class IV–specific RNAi knockdown of *piezo*, *pickpocket*, or *painless*, and genetic null mutation of *painless* all blocked ballistically induced nociception responses.

We used this paradigm to conduct an RNAi screen to identify specific genetic pathways important for mechanical nociception. We first identified nociceptor-enriched genes with laser capture microdissection of nociceptive Class IV multidendritic neurons and non-nociceptitve Class I multidendritic neurons. Four biological replicates of each neuron type were used to probe Affymetrix microarrays. 231 nociceptor-enriched were then tested for function in nociceptors by tissue-specific RNAi knockdown. Knockdown in either Classes I–IV md neuron (md-GAL4) or Class IV md neurons (ppk-GAL4) was performed for all 231 genes. After testing 45,978 larvae, we identified 17 and 8 candidate genes from the md-GAL4 and ppk-GAL4 screens, respectively.

Interestingly, our screen identified previously known mechanical nociception genes such as *pickpocket* and *piezo* in a blinded fashion. Our characterization of other interesting genes in the pathway will be discussed.

15. Connecting Temperature Preference to Neural Circuit Activity Using Calcium Imaging in Thermotaxis Mutants. JOSH D. HAWK,[1] VIVEK VENKATACHALAM,[2] ARAVI D. SAMUEL,[2] JOEL GREENWOOD,[2] MIRIAM B. GOODMAN,[3] and DANIEL A. COLÓN-RAMOS,[1] *[1]Yale University, New Haven, CT; [2]Harvard University, Cambridge, MA; [3]Stanford University, Stanford, CA*

Experience-dependent changes in behavior rely upon reshaping of neural network activity through synaptic plasticity. For this reason, synaptic plasticity is evolutionarily conserved and contributes to virtually all non-instinctive behavioral patterns. Yet, a substantial gap in knowledge remains regarding the molecular mechanisms that allow synaptic plasticity to reshape patterns of neural activity to achieve behavioral changes. Experimental advantages in the nematode *C. elegans* provide an opportunity to close this gap. *C. elegans* performs a number of learned behaviors including thermotaxis, a behavior resulting in experience-dependent changes in temperature preference. The neurons involved in thermotaxis have been identified by cell-specific ablation and mutation studies, and the genetic underpinnings of this behavior are being determined by forward and reverse genetic approaches (Garrity et al. 2010. *Genes Dev.* 24:2365–2382). The connection between these genetic lesions and neural circuit logic is less clear. To address this gap, we are examining worms with mutations in the *protein kinase C-1* (*pkc-1*) gene. As previously reported (Okochi et al. 2005. *EMBO J.* 24:2127–2137), we have found that *pkc-1* loss-of-function mutations produce constitutive warmth-seeking behavior, whereas gain-of-function *pkc-1* mutations produce constitutive cold-seeking behavior. Each of these effects on temperature preference is caused by PKC-1 function in the thermosensory neuron AFD. To connect these findings to neural circuit activity, we examined thermally induced calcium responses in AFD. In contrast to the earlier study of PKC-1 function in thermotaxis, we observed AFD calcium signals in *pkc-1* mutant worms that are identical to wild-type responses. We also found that PKC-1 localizes in the AFD axon in a pattern resembling presynaptic sites, consistent with the role of PKC-1 in regulating vesicle release in other neurons (Sieburth et al. 2007. *Nat. Neurosci.* 10:49–57). Together, these findings suggest that PKC-1 may regulate AFD synaptic output to control thermal preference.

16. Mechanisms Underlying the Transduction and Encoding of Tactile Stimuli in Merkel Discs of Mammals. RYO IKEDA, MYEOUNGHOON CHA, JENNIFER LING, ZHANFENG JIA, DENNIS COYLE, and JIANGUO G. GU, *Department of Anesthesiology, The University of Cincinnati College of Medicine, Cincinnati, OH 45267*

The sense of touch is indispensable for environmental exploration, social interaction, tactile discrimination, and other tasks in life. Mammals have developed complicated tactile end-organs including Merkel discs, Pacinian corpuscles, Meissner's corpuscles, Lanceolate endings, and Ruffini endings. Merkel discs, also known as Merkel cell–neurite complexes, are formations of Merkel cells and Aβ-afferent nerve endings in synapse-like structures. They are highly abundant in fingertips of humans, whisker hair follicles of nonhuman mammals, and other touch-sensitive spots throughout mammalian body. Tactile stimuli to Merkel discs in the skin elicit slowly adapting type I responses (SAI) in Aβ-afferent fibers. This tactile-induced SAI response allows fingertips of humans and whiskers of nonhuman mammals to perform tactile discrimination of an object's shape, curvature, texture, and other physical properties. Although Merkel discs and Merkel cells were discovered 139 years ago, cellular and molecular mechanisms

underlying tactile transduction in Merkel discs remain unclear after over a century of studies. In this talk, I will present our recent work performed on rat whisker hair follicles to address several key issues such as whether tactile stimuli are transduced by Merkel cells in Merkel discs, what molecules are involved in the tactile transduction in Merkel discs, and how tactile stimuli are encoded by Merkel discs to drive SAI impulses in Aβ-afferent endings. Our key findings are (1) Merkel cells are primary sites of tactile transduction, (2) the Piezo2 ion channel is the key Merkel cell mechanical transducer, (3) Piezo2 transduces tactile stimuli into Ca^{2+} action potentials in Merkel cells, (4) the Ca^{2+} action potentials in Merkel cells drive Aβ-afferent nerve endings to fire slowly adapting impulses, and (5) Piezo2 and Ca^{2+} action potentials in Merkel cells are required for whisker behavioral tactile responses.

17. Single-Point Mutations in Ankyrin Repeat Six Make Mouse TRPA1 Sensitive to Warm Temperatures.
SAIRAM JABBA,[1] RAMAN GOYAL,[1] JASON O. SOSA-PAGÁN,[1] HANS MOLDENHAUER,[2] JASON WU,[1] BREANNA KALMETA,[1] MICHAEL BANDELL,[3,4] RAMON LATORRE,[2] ARDEM PATAPOUTIAN,[3,4] and JÖRG GRANDL,[1] *[1]Department of Neurobiology, Duke University Medical Center, Durham, NC 27710; [2]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaiso 2349400, Chile; [3]Department of Cell Biology, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037; [4]Genomics Institute of the Novartis Research Foundation, La Jolla, CA 92037*

Several transient receptor potential (TRP) ion channels are activated with high sensitivity by either cold or hot temperatures. However, structures and mechanism that determine temperature-directionality (cold vs. heat) are not established. Here we screened 12,000 random mutant clones of the cold-activated mouse TRPA1 ion channel with a heat stimulus. We identified three single-point mutations that are individually sufficient to make mouse TRPA1 warm-activated, while leaving sensitivity to chemicals unaffected. Mutant channels have high temperature-sensitivity of voltage-activation, specifically of channel opening, but not channel closing, which is reminiscent of other heat-activated TRP channels. All mutations are located in ankyrin repeat six, which identifies this domain as a sensitive modulator of thermal activation. We propose that a change in the coupling of temperature-sensing to channel-gating generates this sensitivity to warm temperatures. Our results demonstrate that minimal changes in protein sequence are sufficient to generate a wide diversity of thermal sensitivities in TRPA1.

S. Jabba and R. Goyal contributed equally to this work.

18. Heterogeneity of P2X2, P2X3, and 5-HT3 Receptors on Gustatory Ganglion Neurons.
ERIC D. LARSON, TOM E. FINGER, and SUE C. KINNAMON, *Rocky Mountain Taste and Smell Center, University of Colorado School of Medicine, Aurora, CO 80045*

Taste receptor cells detect taste stimuli and release transmitters to communicate with afferent fibers innervating the taste bud. One such transmitter is ATP, released from Type II cells in response to bitter, sweet, and umami stimuli. Genetic deletion of P2X2 and P2X3 purinergic receptors (P2X2/P2X3 DKO mice) results in absence of afferent nerve response to all taste qualities, implicating ATP in transmission of sour and salty sensations that arise from Type III cells. Although ATP release has not been measured from Type III cells, these cells release 5-HT upon stimulation, but whether 5-HT contributes to nerve fiber activation is unknown. Here, we investigated whether ATP and 5-HT activate different gustatory ganglion cells using isolated, geniculate ganglion neurons loaded with Fura-2-AM. To identify cells with 5-HT receptors, we used a mouse expressing GFP under the control of the 5-HT3A receptor promoter (GENSAT: 5-HT3A-GFP mice). Although all ganglionic neurons increased intracellular calcium in response to ATP, only those neurons expressing GFP responded to 5-HT, and those responses were blocked by ondansetron, a 5-HT3 antagonist. To identify the receptors mediating the ATP responses, neurons from P2X2/P2X3 DKO mice and wild-type (WT) mice were tested with ATP. No responses were obtained from the DKO mice, indicating that P2X2 and P2X3 are the only purinergic receptors involved in ATP activation of gustatory afferents. In WT mice, ATP responses were blocked by AF-353, an antagonist of P2X3-containing receptors. Interestingly, some neurons were more sensitive to the antagonist than others, suggesting that they may contain more P2X3 subunits. This was confirmed by immunocytochemistry, showing that although all neurons express P2X3, about half contain little or no P2X2. These data confirm the role of P2X2 and P2X3 in gustatory activation, but also suggest that 5-HT3 may play a role in activation of fibers that contact Type III taste cells.

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19. Food Odors Trigger *Drosophila* Males to Secrete a Pheromone That Guides Female Egg Laying Decisions.
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Animals use olfactory signals to directly interpret the external world. Pheromones are specialized animal-derived odorants that convey critical social information between members of the same species. However, connections between environmental olfactory stimuli and pheromone signaling remain largely unexplored. Here, we report the novel finding that flies actively secrete an attractive pheromone onto their surroundings upon odor stimulation. Pheromone secretion is rapid (within 5 min) and depends on food odors (such as apple cider vinegar, yeast, banana) but is not triggered by stimulation with individual food odorant components (such as humidified air, ethyl acetate, or acetic acid). Additional tests indicate that males are the source of the pheromone, and that secretion and detection of the pheromone requires the olfactory, but not gustatory, system. To identify the pheromone, we use gas chromatography–mass spectrometry to compare the secretions from flies stimulated with food odors to those stimulated with control odorants. We find a single molecule is enriched after the food odor stimulation: 9-tricosene (9-T). Because only males secrete 9-T, we examine if this pheromone might be influencing female behaviors. We find that 9-T actively enhances courtship behavior and females preferentially oviposit on agarose gel containing 9-T. This is surprising as it was previously thought that females alone decided egg-laying sites by probing for appropriate locations. Our findings instead suggest that males actively participate in egg-laying site decisions by depositing this novel food odor–induced pheromone. We use electrophysiological and behavior approaches to show that 9-T activates basiconic Or7a+ olfactory neurons (ab4A), and we show that loss of these neurons abolishes pheromone attraction and oviposition site selection. These studies link food odor perception to male pheromone secretion and subsequent female oviposition decision-making, providing a framework for understanding dynamic interactions between external odors and pheromone responses in animals.

20. TRPA1 Controls Inflammation and Pruritogen Responses in Allergic Contact Dermatitis. BOYI LIU and SVEN-ERIC JORDT, *Department of Anesthesiology, Duke University, Durham, NC*

Allergic contact dermatitis is a common skin disease associated with inflammation and persistent pruritus. Transient receptor potential (TRP) ion channels in skin-innervating sensory neurons mediate acute inflammatory and pruritic responses after exogenous stimulation and may contribute to allergic responses. Genetic ablation or pharmacological inhibition of TRPA1, but not TRPV1, inhibited skin edema, keratinocyte hyperplasia, nerve growth, leukocyte infiltration, and antihistamine-resistant scratching behavior in mice exposed to the haptens, oxazolone, and urushiol, the contact allergen of poison ivy. Hapten-challenged skin of TRPA1-deficient mice contained diminished levels of inflammatory cytokines, nerve growth factor, and endogenous pruritogens, such as substance P (SP) and serotonin. TRPA1-deficient sensory neurons were defective in SP signaling, and SP-induced scratching behavior was abolished in Trpa1(−/−) mice. SP receptor antagonists, such as aprepitant, inhibited both hapten-induced cutaneous inflammation and scratching behavior. These findings support a central role for TRPA1 and SP in the integration of immune and neuronal mechanisms leading to chronic inflammatory responses and pruritus associated with contact dermatitis.

21. Functional Characterization of Rhodopsins in Gustatory Signaling of *Drosophila melanogaster*. CHAO LIU, *Department of Molecular, Cellular & Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA 93106*

Rhodopsin is the visual pigment in retinal photoreceptor cells, and is the classical seven-transmembrane G protein–coupled receptor. In flies, light activation of rhodopsin is linked to a Gq/phospholipase C pathway. Until recently, it was thought that rhodopsins function exclusively in light sensation. However, our laboratory demonstrated that one of the six known rhodopsins in *Drosophila*, Rh1, is also required for thermal discrimination (Shen et al. 2010. *Science*. 331:1333–1336). Loss of Rh1 impairs the ability of larvae to select the optimal temperature within the comfortable range. This finding raises the possibility that rhodopsins are also used in other sensory modalities. Here, we describe a requirement for Rh1 in the taste system of *Drosophila melanogaster*. We found that Rh1 was expressed in gustatory receptor neurons within the major taste organ, the labelum. Of primary significance, Rh1 was required for both behavioral and electrophysiological responses in response to low concentrations of the bitter plant-derived compound, aristolochic acid. However, Rh1 was not required for responding to high concentrations of aristolochic acid. Detecting low levels of aristolochic acid also requires PLCβ and TRPA1 (Kim and Lee. 2010. *PNAS*. 107:8440–8445). We conclude that the rhodopsin-PLCβ-TRPA1 signaling allows the flies to sense low concentrations of aristolochic acid through signal amplification. However, avoiding high concentrations of this chemical is Rh1-independent.

22. Elucidating Mechanisms of Touch-Receptor Plasticity during Target-Organ Remodeling. KARA L. MARSHALL,[1] YOSHICHIKA BABA,[1] BLAIR A. JENKINS,[1,3] YUXIANG WANG,[4] DAINE R. LESNIAK,[4] GREGORY J. GERLING,[4] and ELLEN A. LUMPKIN,[1,2,3] *[1]Department of Dermatology, [2]Department of Physiology & Cellular Biophysics, and [3]Department of Neurobiology & Behavior Program, Columbia University, New York, NY; [4]Department of Systems and Information Engineering, University of Virginia, Charlottesville, VA*

Touch receptors are embedded in skin, our body's barrier to the outside world. Skin is a dynamic organ

that continually remodels during epidermal renewal and cyclical hair growth. The receptors that transduce touch stimuli are often intimately associated with skin appendages, such as hair, which raises a key question: how do touch receptors maintain reliable firing during normal skin remodeling? We address this question by using mouse Merkel cell–neurite complexes. Merkel cells are innervated by slowly adapting type I (SAI) afferents, which encode information about object features and static pressure. Mouse hair growth occurs in synchronous waves. Our previous studies of mouse skin mechanics showed that skin thickens and becomes more compliant during hair growth phases. Because tissue mechanics sets the transfer function from skin displacement to touch-receptor deformation, we hypothesized that touch-evoked firing will change over the hair cycle unless compensatory mechanisms, such as neuronal remodeling, occur. To test this hypothesis, we investigated SAI-afferent structure across hair-cycle phases. During hair growth, Merkel cells increased in number by 50% and were incorporated into SAI arbors, suggesting that they are functional transduction units. Neuronal branching complexity increased correspondingly, with 40% more terminal branches and higher branching orders in growth phases. To test the functional consequences of this neuronal plasticity, we performed ex vivo recordings of SAI afferents during hair growth and resting phases. Firing rates did not differ between hair-cycle phases in the dynamic (mean ± SEM: 122 ± 28 spikes/s, $n = 4$ and 132 ± 23 spikes/s, $n = 7$, respectively) or static phase of firing (49 ± 15 spikes/s and 70 ± 10 spikes/s, respectively). Adaptation rates and interspike-interval distributions were also indistinguishable between groups. Mechanical sensitivity, as measured by the slope of displacement–response curves, was greater during the resting phase but not statistically different. Thus, these data suggest that the neuronal remodeling compensates, in part, for changes in skin mechanics during hair growth.

23. Balboa(Ppk-26) Interacts with Pickpocket In Vivo and Is Required for Mechanical Nociception in *Drosophila* Larvae. STEPHANIE E. MAUTHNER,[1] RICHARD Y. HWANG,[2] QI XIAO,[2] and W. DANIEL TRACEY,[1,2,3,4] *[1]University Program in Genetics and Genomics, [2]Department of Neurobiology, [3]Department of Cell Biology, and [4]Department of Anesthesiology, Duke University, Durham, NC 27710*

The *Drosophila* gene *pickpocket (ppk)* encodes an ion channel subunit of the Degenerin/Epithelial Sodium Channel (DEG/ENaC) family. Although PPK is specifically expressed in nociceptor neurons and is required for mechanical nociception, the currents that it mediates have not been detected in heterologous expression studies. Thus, we hypothesized that interacting proteins may be needed for functional expression of PPK and PPK-mediated currents. To find such partners, we performed a genome-wide genetic screen of ion channel subunits, and identified those required for mechanical nociception. This led us to a novel gene that we named *balboa* (a.k.a. *CG8546, ppk-26*). The *balboa* locus encodes a DEG/ENaC ion channel subunit that is closely related to PPK. Laser-capture isolation of RNA from larval neurons and microarray analyses revealed that *balboa* is highly expressed in nociceptive neurons. Additionally, a *balboa-GAL4* reporter supported this finding by showing exclusive expression in larval nociceptors. Remarkably, subcellular localization of Balboa::GFP proteins was dependent on expression of PPK. The Balboa::GFP protein was uniformly distributed in the dendrites of Class IV neurons (which also express PPK), but it was localized to discrete punctae when ectopically expressed in Class I, II, and III multidendritic neurons. Ectopic coexpression of *ppk* in Class I, II, and III neurons altered the localization of Balboa::GFP, converting it to the uniform pattern of Class IV neurons. Furthermore, RNAi knockdown of *ppk* in Class IV neurons converted the uniform Balboa::GFP pattern to a punctate distribution. Finally, using a GFP-reconstitution approach in transgenic larvae, we directly detected an in vivo physical interaction between PPK and Balboa. In summary, we identified a novel protein important for mechanical nociception, and we demonstrate that this protein physically interacts with PPK in vivo.

24. Cloning and Characterization of the Ca^{2+}-activated Cl^- Channel ClCa4l from Rat Olfactory Sensory Neurons. CASILDA MURA,[1] RICARDO DELGADO,[1] DIEGO RESTREPO,[2] and JUAN BACIGALUPO,[1] *[1]Universidad de Chile, Chile; [2]University of Colorado, Denver, CO 80202*

Odor binding to G protein–coupled receptors in olfactory cilia triggers a cAMP cascade, where cAMP gates the nonselective cation channel CNG. CNG opening increases intraciliary Ca^{2+}, which gates Ca^{2+}-activated Cl^- channels (CaCCs) that amplify the depolarizing current. This channel is thought to be Anoctamine 2 (Ano2). With PCR and immunochemistry, we found the ClCa4l channel in olfactory cilia, belonging to the ClCa family of CaCCs (Gonzalez-Silva et al. 2013. *PLoS One.* 8:e69295). We further characterized the channel by cloning, sequencing, and heterologous expression. Total olfactory epithelium mRNA extracted by Trizol was treated with RNase-free DNase. mRNA was transcribed with the reverse transcription Superscript III and oligodT to generate the first cDNA strand. For cDNA amplification by PCR, we used several sets of specific ClCa4l primers. Full-length cDNA was obtained after several PCR rounds. The products inserted in pUC18 were amplified and sequenced. Homology search was performed on the BLAST server. The coding sequence showed 99% homology with ClCa4l. HEK293 cells were

transfected with EGFP-encoded vector pIRES-EGFP-ClCa4l plasmid. The transfected cells were used 3–9 days after transfection for patch-clamp characterization of expressed ClCa4l. The transfected ClCa4l cDNA conferred to the cells a Ca^{2+}-activated Cl^--selective current, sensitive to the Cl^- channel blocker niflumic acid. Excised patches from ClCa4l-expressing HEK293 cells and from rat olfactory cilia revealed a Ca^{2+}-activated Cl^- channel with similar characteristics (10–20 pS, $K_{0.5}$ = ~100 nM). The results strongly support the presence of CaCl4l in olfactory cilia, suggesting its participation in odor transduction.

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25. Optogenetic Activation of Small, Steady-State Currents in Merkel Cells Is Sufficient to Elicit Action Potentials in Tactile Afferent Neurons. MASASHI NAKATANI,[1,4] SRDJAN MAKSIMOVIC,[1] and ELLEN A. LUMPKIN,[1,2,3] *[1]Department of Dermatology, [2]Department of Physiology & Cellular Biophysics, and [3]Program in Neurobiology & Behavior, Columbia University, New York, NY; [4]Graduate School of System Design and Management, Keio University, Yokohama, Japan*

Merkel cell–neurite complexes are touch receptors that mediate slowly adapting type I (SAI) responses. We recently reported that epidermal Merkel cells are mechanosensory cells necessary and sufficient to excite sustained firing in SAI afferents (Maksimovic et al. 2014. *Nature.* 509:617–621; Woo et al. 2014. *Nature.* 509:622–626). Merkel cells exhibit *Piezo2*-dependent, rapidly adapting mechanotransduction currents ($\tau_{inactivation}$ = 8 ± 2 ms; n = 6) with small steady-state components (20 ± 6 pA, V_{hold} = −70 mV; n = 6). In contrast, SAI spike trains adapt slowly over hundreds of milliseconds to minutes. Thus, an important question is whether rapidly adapting inward currents in Merkel cells can excite sustained SAI firing. To address this question, we analyzed physiological responses from $Cck^{Cre/+};ChR2^{loxP/+}$ mice, which selectively express Channelrhodopsin-2 (ChR2) in Merkel cells. Using skin-nerve preparation recordings, we found that 250-ms light pulses activated phase-locked, sustained discharges in SAI afferents. This demonstrates that depolarizing Merkel cells for <1 s is sufficient to excite SAI afferents. To analyze ChR2-mediated currents, we purified ChR2-expressing Merkel cells by flow cytometry and cultured them for 1–2 days. Whole-cell voltage-clamp recordings from Merkel cells showed blue light–induced currents (I_{peak} = 38 ± 2 pA, $I_{steady\text{-}state}$ = 18 ± 1 pA; V_{hold} = −70 mV; n = 56 trials from 5 cells). Steady-state magnitudes of the Merkel cell's light- and touch-evoked currents were not significantly different. Moreover ChR2 current amplitudes were indistinguishable 50 and 1,000 ms after the onset of light stimulation (n = 4 cells, 24 trials, P = 0.96, paired t test). Thus, ChR2 activation of Merkel cells induces picoampere currents that persist for up to 1 s. Taken together, these data demonstrate that inward currents with small, steady-state components in Merkel cells are capable of stimulating prolonged SAI spike trains. Because each afferent innervates multiple Merkel cells, we propose that small inputs from several Merkel cells are integrated to produce robust SAI firing.

26. Opsin Expression in Human Epidermal Skin. RANA N. OZDESLIK, KIRK HALTAUFDERHYDE, and ELENA V. OANCEA, *Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI 02912*

Opsins are light-sensitive members of the superfamily of G protein–coupled receptors (GPCRs). Visual pigments include rhodopsin (OPN2) and the cone opsins (OPN1) and are expressed in rods and cones of the retina to mediate dim-light and color vision, respectively. In addition to the classical visual opsins, a number of nonvisual opsins have been recently described including encephalopsin (OPN3), melanopsin (OPN4), and neuropsin (OPN5) (Kumbalasiri and Provencio. 2005. *Exp. Eye Res.* 4:368–375). Our laboratory is interested in light detection in the skin, and we recently showed that OPN2 contributes to detection of ultraviolet radiation in human epidermal melanocytes (Wicks et al. 2011. *Curr. Biol.* 22:1906–1911). However, the expression profile of other light-sensitive proteins in human skin remains unknown.

Here, we used reverse transcription polymerase chain reaction and quantitative PCR analysis to show that four opsin receptors, i.e., OPN1-SW, OPN2, OPN3, and OPN5, are expressed in the two major epidermal cell types of human skin, melanocytes and keratinocytes. We found that in both cell types, two OPN3 transcripts are expressed in similar amount. We also detected three OPN5 splice isoforms, two of which result in truncated proteins. Interestingly, quantitative PCR revealed that OPN3 mRNA was significantly more abundant in epidermal cells than other opsins. The expression of opsins in skin suggests that they may function as epidermal photoreceptors; further studies will uncover their physiological roles.

27. Calcium Is a Critical Photoexcitation Messenger in Melanopsin-expressing Microvillar Photoreceptors of Amphioxus. GABRIEL PEINADO,[1] TOMÁS OSORNO,[1] MARÍA DEL PILAR GOMEZ,[1,3] and ENRICO NASI,[2,3] *[1]Departamento de Biología and [2]Instituto de Genética, Universidad Nacional de Colombia, Bogotá, Colombia; [3]Marine Biological Laboratory, Woods Hole, MA 02543*

Melanopsin, the photopigment of the "circadian" receptors that regulate the biological clock and the pupillary reflex in mammals, is homologous to invertebrate

rhodopsins. Evidence supporting the involvement of phosphoinositides in light signaling has been garnered, but the downstream effectors that control the light-dependent conductance remain poorly understood. Photosensitive microvillar neurons in the neural tube of amphioxus—the most basal chordate—also express melanopsin, which colocalizes with a G_q. Moreover, the light response is accompanied by Ca mobilization from internal stores, and is highly susceptible to PLC inhibitors. In previous work, the application of diverse DAG analogues proved inert, whereas IP_3R antagonists depressed the photoresponse. We therefore examined the role of calcium in activating the photoconductance. High time-resolution simultaneous measurements of membrane current and $\Delta[Ca^{2+}]$ revealed that the light-induced calcium rise slightly precedes the onset of the photocurrent, making it a viable candidate in the activation chain; in support of a causal link, Ca chelators greatly reduced light responsiveness. On the other hand, photolysis of caged Ca elicits an inward current of similar size and time course as the physiological light response, but with a much shorter latency. Comparison of the kinetics of the induced Ca elevation and the resulting current suggests a highly cooperative process. Ion substitutions and pharmacological manipulations show that the Ca-triggered current does not reflect the activation of an electrogenic Na/Ca exchanger, and is independent of PLC activity. The sodium dependency and reversal potential of this current, and its susceptibility to blockers of TRP-class ion channels, are similar to the native light-evoked current. Calcium thus emerges as a possible key downstream messenger to initiate the opening of light-dependent channels in melanopsin-expressing photoreceptors of early chordates.

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28. In Vivo Kinetics of the Vertebrate Rod Photoresponse. GABRIEL PEINADO, EDWARD N. PUGH JR, and MARIE E. BURNS, *University of California, Davis, Davis, CA*

The kinetics of the light response elicited by rod photoreceptors has been extensively studied ex vivo by means of the suction electrode recording technique. This technique requires dissecting the retina from the eye, which could potentially alter retinal function from that in its native physiological environment. To study the kinetic properties of rod photoreceptor responses in vivo, the electroretinograms of C57BL/6J mice were measured under improved recording conditions, which included the use of a mild gaseous (isoflurane) anesthesia and electrical isolation of the potential at the corneal surface. The increase in the signal-to-noise ratio of the recordings allowed the application of the paired-flash protocol (Lyubarsky et al. 1999. *J. Neurosci.* 19:442–455) to measure the time course of the rod photoresponse to dim flashes that suppressed only 15% of the dark current. As compared with ex vivo experiments, the in vivo rod responses exhibited a faster time-to-peak of 80–85 ms for the dim-flash response, and an accelerated time course of recovery. To understand the cause of the faster kinetics in vivo, a Pepperberg analysis of the responses to saturating flashes was performed in wild-type animals and in mice with rods that overexpressed Rgs9, the complex that speeds G protein deactivation. Surprisingly, this experiment revealed that the time constant of deactivation for the molecular step that rate-limits recovery after saturation of wild-type rods was 135 ± 13 ms (mean ± sem, $n = 4$) in vivo, 45% faster than ex vivo. Mice with rods that overexpressed Rgs9 had even faster recovery time constants: 46 ± 1.5 ms (mean ± sem, $n = 3$), indicating that G protein deactivation is the molecular step that normally rate-limits rod recovery in vivo, and partially explaining the faster kinetics of the photo response.

29. Exposure to Nicotine and Ethanol Modulates nAChR Subunit Expression Levels in STC-1 Cells. JIE QIAN, SHOBHA MUMMALANENI, and VIJAY LYALL, *Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA 23112*

Taste responses to nicotine and ethanol involve both TRPM5-dependent and TRPM5-independent transduction pathways.TRPM5-independent responses depend upon the presence of nicotinic acetylcholine receptors (nAChRs) in taste cells. We used STC-1 cells, an enteroendocrine cell line from mouse small intestine, as a model to investigate the effects of ethanol and nicotine exposure on nAChRs in taste cells. STC-1 cells respond to all five basic taste stimuli. We detected the mRNA expression of T2R38, α-ENaC, TRPV1, TRPM5, α3, α4, α5, α6, α7, α9, β2 and β4 nAChR subunits by RT-PCR. The α3, α4, β2, and β4 antibody binding was observed in STC-1 cells. The β2 antibody binding was colocalized with T2R38 and TRPM5 antibody in STC-1 cells. Treating STC-1 cells with 250 and 500 nM nicotine for 24 h induced a dose-dependent increase in the α6 nAChR mRNA expression. Relative to α6, the increase in α4, α5, and β4 nAChRs was smaller. At 1,000 nM nicotine, the mRNA expression levels for the above nAChRs were significantly lower. After 4-day exposure, there was a dose-dependent increase in α6 nAChR mRNA, but it was significantly lower than that observed after 24-h treatment. Exposing STC-1 cells to 50 mM ethanol enhanced α6 nAChR mRNA that was higher than that produced by 100 mM ethanol. Western blot data demonstrated that nicotine and ethanol exposure increased α4 and α5 nAChR protein levels, but not the β2 nAChR protein level. The nicotine- and ethanol-induced increase in nAChR protein was blocked by treating the cells with mecamylamine, a nAChR antagonist. We conclude that exposure to nicotine or ethanol up-regulates selective

nAChRs in STC-1 cells in a dose- and time-dependent manner. Thus, STC-1 cells are a model for investigating nAChR gene expression profiling before and after nicotine and ethanol exposure in taste bud cells.

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30. Molecular Identification of SWELL Protein Family As the Volume-regulated Anion Channel (VRAC).
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How does cell sense its volume change and keep it constant? The plasma membrane of most animal cells is highly permeable to water. Decrease in extracellular osmolality or increase in intracellular osmolality during various physiological and pathological conditions induces a rapid water influx across the cell membrane, resulting in swelling of the cell. This volume increase activates a ubiquitously expressed volume-regulated anion channel (VRAC), which mediates the efflux of intracellular Cl^- and other organic osmolytes, such as amino acids, and constitutes one of the major pathways responsible for cell volume homeostasis. The activity of VRAC was identified more than 25 years ago, but its molecular identity in vertebrates remains unknown. We recently developed a yellow fluorescent protein–based cellular assay for VRAC-mediated ion flux and performed a genome-wide RNAi screen to find components of VRAC. We identified SWELL1 (LRRC8A), a member of a four-transmembrane (TM) protein family with unknown function, as essential for VRAC activity. SWELL1 is localized to the plasma membrane, and its knockdown dramatically reduces endogenous VRAC currents and regulatory cell volume decrease in various cell types. Furthermore, point mutations in threonine at position 44 (T44) of SWELL1 TM1 domain cause a significant change in VRAC anion selectivity, suggesting that SWELL1 is close to or forms the VRAC pore. Affinity purification of SWELL1 and proteomic analysis reveal a membrane protein complex consisting of SWELL1 and its four other closely related homologues (LRRC8B-E). The combination of these SWELL family members determines the degree of outward rectification of VRAC current. The identification of the SWELL protein family as an integral part of VRAC provides the basis to perform the structure–function analysis of VRAC, to explore how cell volume increase opens the channel, and to investigate the role of VRAC and cell volume regulation in normal physiology and disease.

31. Piezo2 Is Required for Touch Sensation in Mice.
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Somatic sensory neurons relay critical information about our physical environment by transforming mechanical and thermal energy into electrical signals via specialized ion channels. Thermosensitive ion channels have been described; however, the identity of mammalian mechanically activated (MA) ion channels responsible for touch in sensory neurons is unknown. Piezo2 is a MA ion channel present in a heterogeneous population of sensory neurons. Here, we analyze mice in which Piezo2 is ablated in sensory neurons and Merkel cells in the adult. Most rapidly adapting MA currents in sensory neurons were absent in Piezo2-deficient mice. Furthermore, over half of Piezo2-deficient cutaneous mechanoreceptors were activated by electrical stimuli but not by mechanical stimuli. The remaining innocuous mechanosensitive afferent fibers did not respond or responded poorly to moving stimuli. Behavioral analyses indicate that touch sensation largely depends on Piezo2. Therefore, Piezo2 is a key mechanotransducer for innocuous touch sensation in mice.

32. Degenerin Channel of *Caenorhabditis elegans* Is Activated by Shear Stress.
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The epithelial Na^+ channel (ENaC)/degenerin family encodes a group of structurally related ion channels that are highly selective for Na^+ and sensitive to amiloride and its derivatives. Members of this family are involved in many fundamental biological processes, including Na^+ absorption and volume regulation (ENaCs), nociception (acid-sensing ion channels), and mechanosensation (ENaCs and *Caenorhabditis elegans* degenerins). Within kidney tubules, laminar shear stress (LSS) activates ENaC by increasing channel open probability. MEC-4 and MEC-10 of *C. elegans* are the pore-forming subunits of the mechanosensitive ion channel complex required for the worm's gentle touch response. Our previous studies have identified that multiple sites and regions of ENaC subunits are required the channel's response to LSS. To gain insights regarding the structural features of MEC-4 and MEC-10 that are important

for mechanosensing, we have begun to explore the response of these channels to LSS. We discovered that, similar to ENaCs, *C. elegans* degenerins responded to LSS with increases in channel activity. In *Xenopus* oocytes expressing MEC-4d/10/2/6, whole cell currents increased approximately twofold by LSS (0.12 dynes/cm^2). This change in channel activity is reversible and relatively stable to repetitive LSS stimulations. MEC-10 is required for a robust LSS response, as MEC-4 homomeric channels exhibited only a modest response to LSS. In addition, the LSS response is substantially diminished in channels bearing touch-insensitive mutations of MEC-10, suggesting that MEC-10 plays an essential role in the channel's regulation by mechanical forces. As there is abundant structural conservation among members of the ENaC/degenerin family, *C. elegans* degenerin channels provide a model to investigate how ENaC and related ion channels are regulated by mechanical forces in the whole organism.

33. Magnetosensitive Neurons Mediate Geomagnetic Orientation in *Caenorhabditis elegans*. ANDRES VIDAL-GADEA,[1] KRISTI WARD,[1] CELIA BERON,[1] JOSHUA RUSSELL,[1] NAVID GHORASHIAN,[2] NICHOLAS TRUONG,[1] ADHISHRI PARIKH,[1] ADELA BEN-YAKAR,[2] and JONATHAN PIERCE-SHIMOMURA,[1] *[1]Department of Neuroscience; Center for Brain, Behavior & Evolution; Center for Learning and Memory; Waggoner Center for Alcohol and Addiction Research; and Institute of Cell & Molecular Biology; and [2]Mechanical Engineering Department, The University of Texas at Austin, Austin, TX 78712*

Many animals from butterflies to birds use the magnetic field of the earth as aide in navigation. Although the list of organisms known to sense and orient to magnetic fields continues to grow, no magnetosensory neuron or transduction mechanism has been described in any animal. We provide behavioral and physiological evidence that the nematode *Caenorhabditis elegans* has an identified sensory neuron pair that detects and enables orientation to earth-strength magnetic fields. Worms use the earth's magnetic field during vertical burrowing migrations that are directionally influenced by their satiation state. Populations isolated from different parts of the world migrate at angles to the magnetic vector that would optimize vertical translation in their native soil, with worms from the southern and northern hemispheres displaying opposite migratory preferences. The ability of these different populations to migrate up a magnetic gradient correlated with the vertical, but not the horizontal, component of their native magnetic field. Magnetic orientation and vertical migrations require the TAX-4/TAX-2 cyclic nucleotide–gated ion channel in an identified sensory neuron pair. These neurons respond to magnetic fields even when synaptic input is impaired by mutations. Thus, like magnetotactic bacteria, worms use the earth's magnetic field to simplify their vertical migratory behavior by reducing the orientation problem from three dimensions to one.

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34. *Pkd2l1* Expression in Type III Taste Cells Varies across Oral Taste Fields. COURTNEY E. WILSON,[1,2,3] WALLACE S. CHICK,[1,4] and SUE C. KINNAMON,[1,2,3] *[1]Neuroscience Program, [2]Department of Otolaryngology, [3]Rocky Mountain Taste and Smell Center, and [4]Department of Cell and Developmental Biology, University of Colorado School of Medicine, Aurora, CO 80045*

Taste buds contain three major types of cells with different chemosensitivities and functions. Type III taste cells respond to sour and salty stimuli, and unlike Type II cells, form distinct synapses with afferent nerves. Although aspects of sour transduction and signaling are known, many details remain obscure. Other investigators have described polycystic kidney disease 2-like 1 (PKD2L1), an ion channel found in sour-responsive cells, as a marker for Type III taste cells. We have used immunocytochemistry to examine expression of PKD2L1 against that of other Type III cell markers, e.g., SNAP-25, 5-HT, Car4, and HCN4, in taste buds across the different oral taste fields: fungiform, foliate, circumvallate, and palate. Results confirmed that although SNAP-25 and PKD2L1 immunoreactivity is largely coincident in both the circumvallate and foliate papillae, PKD2L1-immunoreactive cells are only a small subset of SNAP-25–immunoreactive cells in fungiform and palatal taste buds. Comparisons of PKD2L1 immunoreactivity with that of 5-HT, Car4, and HCN4 yielded similar results. Our findings, then, suggest a more nuanced expression profile of various Type III cell markers, and thus a more heterogeneous cell population than previously thought. To further elucidate the role of PKD2L1 positive cells in taste signaling, we have created a knock-in mouse that contains a bicistronic IRES Cre recombinase construct directly following the *Pkd2l1* stop codon. This mouse will allow us to genetically manipulate sour responsive cells specifically. PKD2L1-Cre mice crossed with floxed tomato reporter mice indicate that the reporter signal (1) appears in all taste fields; (2) appears in PKD2L1 immunoreactive cells with varying efficiency across taste fields; and (3) does not colocalize with TrpM5-GFP, a marker of Type II taste cells. The data suggest that the PKD2L1-Cre mice will provide a useful tool to manipulate gene expression in sour responsive cells.

35. Piezo2 Is Required for Merkel Cell Mechanotransduction. SEUNG-HYUN WOO,[1] SANJEEV S. RANADE,[1] ANDY D. WEYER,[2] ADRIENNE E. DUBIN,[1]

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How we sense touch remains fundamentally unknown. The Merkel cell–neurite complex is a gentle touch receptor in the skin that mediates slowly adapting responses of Aβ myelinated sensory fibers to encode fine details of objects. However, the identity of the mechanosensory cell within this complex, as well as the molecular mechanism of mechanotransduction, is unknown. Here we show for the first time that Merkel cells produce touch-sensitive currents in vitro. Piezo2, a mechanically activated cation channel, is expressed in Merkel cells. We engineered mice deficient in Piezo2 in the skin, but not in sensory neurons, and show that Merkel cell mechanosensitivity completely depends on Piezo2. In these mice, Merkel cell–neurite complex–mediated slowly adapting responses in vivo show reduced static firing rates, and moreover, they display moderately decreased behavioral responses to gentle touch. Our results indicate that Piezo2 is the Merkel cell mechanotransduction channel and provide the first line of evidence that Piezos play a physiological role in mechanosensation in mammals. Furthermore, our data present evidence for a two-receptor site model, where both Merkel cells and innervating afferents together as mechanosensors.

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Sponsor: Ardem Patapoutian.

36. Modulation of Ion Channels in Sensory Nodose Ganglia Neurons. JIE ZHANG, ALEXEY EVSEEV, and MARK S. SHAPIRO, *Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX*

Nodose ganglia (NG) neurons are responsible for nociception in many visceral organs. Here, we studied the properties and receptor modulation of several ion channels important for excitability and sensory transduction in NG neurons, using perforated-patch voltage clamp, fura-2 Ca^{2+} imaging, and RT-PCR. We focused on KCNQ ("M-type") K^+, TMEM16A/B (Ano1/2) Cl^-, and acid-sensing (proton-gated) ion channels (ASICs). RT-PCR and immunostaining assays detected message or protein for TMEM16A, ASIC1, 3, and 5 in neonatal rodent NG neurons. We then investigated $G_{q/11}$-coupled receptor modulation of M channels, which play key roles in the regulation of neuronal excitability. Stimulation of $G_{q/11}$-coupled bradykinin (BK), protease-activated (PAR-2), or purinergic P2Y receptors suppressed M-current (I_M) and induced intracellular Ca^{2+} $[Ca^{2+}]_i$ rises, whereas stimulation of muscarinic and angiotensin II receptors only suppressed I_M, but did not induce $[Ca^{2+}]_i$ rises, in both capsaicin-sensitive and -insensitive neurons. In these neurons, using EGFP-PLCδ-PH or CFP/YFP-based FRET probes, we also observed PIP_2 hydrolysis and activation of PKC induced by stimulation of these $G_{q/11}$-coupled receptors. ASIC currents were observed in response to rapid application of a pH 5.5 solution, which was suppressed in the presence of the ENaC/ASIC blocker, amiloride. TMEM16A currents were activated by $[Ca^{2+}]_i$ rises induced by stimulation of $G_{q/11}$-coupled BK receptors, but not by Ca^{2+} influx through depolarization-activated voltage-gated Ca^{2+} channels (VGCC). However, in hippocampal neurons, TMEM16B currents were activated only by opening of VGCCs, but not by stimulation of $G_{q/11}$-coupled glutamate receptors, which induced $[Ca^{2+}]$ rises. Dialysis of NG cells with BAPTA, but not EGTA, totally blocked the TMEM16A current, suggesting the importance of local Ca^{2+} signals in activation of TMEM16A channels. Thus, distinct sources of Ca^{2+} underlie the activation of TMEM16A/B channels in sensory or hippocampal neurons. We are testing for clustering of TMEM16A/B channels with VGCCs or with IP_3 receptors in native NG or hippocampal neurons.

37. Anoctamin Channels in Central Processing Pathways of Sensory Signals. W. ZHANG, S. SCHMELZEISEN, F. NEUREITHER, F. MÖHRLEN, and S. FRINGS, *The Department of Molecular Physiology of Animals, Center for Organismal Studies, University of Heidelberg, Heidelberg, Germany*

Anoctamin proteins can form calcium-activated chloride channels in epithelia, muscle, and neurons. In particular, the isoforms ANO 1 and ANO 2 have been shown to form chloride channels that are gated by cytoplasmic calcium. The two channels differ in their respective calcium sensitivity, their gating properties, their regulation by calmodulin, and their expression patterns. Although ANO 1 is expressed in many types of tissue, ANO 2 expression appears to be limited to neurons. To study the cellular expression and physiological function of ANO 1 and ANO 2, we have raised isoform-specific antisera in guinea pigs and used them on cryosections from rat and mouse. We localized ANO 1 and ANO 2 in the olfactory neuroepithelium, in the retina, and in the spinal cord. To inspect areas of anoctamin expression in the brain, we developed a protocol that yielded reliably immunohistochemical data for ANO 1 and ANO 2 on brain tissue. We found ANO 1 expression in the olfactory bulb where subpopulations of periglomerular cells were immunopositive. ANO 1–positive

interneurons were also found in the following levels of the olfactory tract. Both in the central visual pathway, and in the auditory pathway, ANO 1 and ANO 2 expression was detected in discrete populations of neurons. In the cerebellum, a center of sensory integration and motor control, ANO 1 was expressed in the defined types of GABAergic interneurons as well in the Purkinje cells, the output neurons of the cerebellar cortex. These studies suggest that ANO 1 and ANO 2 serve distinct functions in the circuits that process sensory information. Different subcellular expression of the ANO 1 and ANO 2 channels, together with electrophysiological results, point to involvement in cellular chloride homeostasis and synaptic transmission, respectively.

38. Role of the Voltage-sensing Domain S1–S4 in TRPV1 Channels. JUAN ZHAO and RIKARD BLUNCK, *Groupe d'Étude des Protéines Membranaires (GÉPROM), Department of Physics and Department of Physiology, Université de Montréal, Montréal, Canada*

The transient receptor potential vanilloid-1 (TRPV1) channel is a nonselective cation channel and can be activated by various stimuli such as voltage, capsaicin, acid, and heat. TRPV1 channel is a member of the hexahelical cation channel superfamily such as K^+, Na^+, and Ca^{2+} channels. The mechanisms underlying the activation of TRPV1 channels by heat and agonists have been tightly linked to voltage-dependent gating. In contrast to most voltage-gated ion channels (VGICs), TRPV1 does not feature the highly positively charged S4, which has been shown to be responsible for the voltage sensitivity of VGICs. The origin for TRPV1's voltage sensitivity must lie elsewhere. The objective of this study was to develop an understanding of the role of "classical" S1–S4 voltage sensor, in particular S4, in the TRPV1 channels, and obtain molecular information about the functional coupling between voltage, chemical, and temperature sensors. This study was performed using a combination of cut-open oocyte voltage-clamp and molecular biological (chimeras, point mutations) techniques. To test whether the positive charge is involved in the activation, a series of single point arginine mutations that scanned residues from E536 through T556 in the S4 segment were generated and characterized their response to capsaicin, protons, and heat. To explore the function of S4 segment in TRPV1 activation, we constructed three chimeras in which the S1–S4, S3–S4, or S4 segments of the TRPV1 channel were replaced by the corresponding segments of Shaker K^+ channels. The introduction of positively charged residues in the S4 segment resulted in a steeper activation curve and a defective response to capsaicin and protons. Importantly, several of the mutant channels displayed strong inhibition of hyperpolarization-activated inward currents. Three chimeras gave rise to functional channels that exhibited stronger voltage dependence at positive voltages than TRPV1. The chimeras progressively lost inward current with increasing portions replaced by the corresponding Shaker region. They were activated by protons with higher sensitivity compared with TRPV1. Taken together, these results suggest that with the introduced positive charges we reestablish voltage dependence and can control TRPV1 response to other ligands. Positive charges of TRP channels may be a crucial factor for the gating.

39. RNA Profiling of Major DRG Sensory Neuron Subtypes: A Resource for Identification of Genes Involved in Mechanosensation. YANG ZHENG,[1,2,3] LING BAI,[1,2,3] and DAVID D. GINTY,[2,3] *[1]Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; [2]Department of Neurobiology, Harvard Medical School, Boston, MA 02115; [3]Howard Hughes Medical Institute*

Low threshold mechanoreceptors (LTMRs) are primary somatosensory neurons that respond to innocuous touch of the skin. They are classified into four major classes, Aβ RA-LTMR, Aβ SAI-LTMR, Aδ-LTMR, and C-LTMRs, based on their action potential conduction velocities ((Aβ > 16 m/s), (Aδ 1.6–12 m/s), and (C < 1 m/s)), as well as their rates of adaptation to sustained touch stimuli (rapidly adapting (RA), slowly adapting (SA), and intermediately adapting (IA)). The molecular determinants of LTMR morphologies, mechanical thresholds, and subtype adaptation rates remain largely unknown. To begin to address these and related questions, we have developed a mouse molecular genetic toolbox that enables labeling of each major LTMR subtype. Using these mouse lines, we FACS purified neurons to homogeneity and then performed RNASeq analysis of Aβ RA-LTMR, Aβ SAI-LTMR, Aδ-LTMR, and C-LTMRs, as well as a nonpeptidergic nociceptors subtype (MrgD+ neurons), peptidergic nociceptors, Aβ-nociceptors (WDR), and proprioceptors. Thus, we have generated transcript-based gene expression profiles for each of eight major DRG sensory neuron subtypes. We found a large group of genes differentially expressed across neuronal subtypes, including many genes encoding voltage-gated ion channels, ionotropic and metabotropic neurotransmitter receptors, GPCRs, and Trp channels. These data are currently being used in conjunction with electrophysiological analyses and pharmacological and molecular perturbation experiments to probe the molecular basis of the unique adaption properties of the different LTMR subtypes. Our findings reveal a remarkable molecular heterogeneity among LTMRs and other sensory neuron subtypes, and these data provide a rich resource for identification of genes that underlie morphological, physiological, and functional properties of LTMR subtypes.

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