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Montagna Symposium 2016—The Skin: Our Sensory Organ for Itch, Pain, Touch, and Pleasure

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The 65th annual Montagna Symposium on the Biology of the Skin, "The Skin: Our Sensory Organ for Itch, Pain, Touch and Pleasure," was held October 20–24, 2016, in Gleneden Beach, Oregon, USA. Gil Yosipovitch (University of Miami) served as Program Chair with Ethan Lerner (Harvard Medical School/Massachusetts General Hospital), Diana Bautista (University of California, Berkeley), Ellen A. Lumpkin (Columbia University), and Francis McGlone (Liverpool John Moores University) serving as Session Chairs.

Although knowledge gained from research on each class of skin sensory nerve fiber—in health and disease—has been substantial in recent years, these classes have largely been studied separately, and crucial questions remain unanswered regarding the overlap and integration of these parallel cutaneous somatosensory pathways. For example, both itch and pain interact in an antagonistic manner: scratch-induced pain can relieve itch, frequently producing pleasure; opioids can induce itch, and their receptor antagonists have been shown to be effective in its treatment. There are broad overlaps, with evidence of a common mechanism in peripheral sensitization as well as in central sensitization to itch and pain, implicating A-beta and C-fiber nociceptors. Presenting up-to-the-minute research on the multifaceted properties of skin sensory receptors, nerves, and central projections highlighted the often unrecognized strong interactions between them and provided exceptional opportunities for the fertile discussions that followed each talk, touching on the potential to translate across experimental and clinical contexts.

The symposium began with a keynote lecture from Francis McGlone on the role of C-fibers in humans. While C-fibers have a classical role as polymodal nociceptors, pruriceptors, and autonomic efferents, Dr. McGlone emphasized that in humans, and in all mammalian skin, a subset of C-fibers called C-tactile afferents (CT) respond to low force touch. Their discovery in human hairy skin, has led to a view of the skin as a "social organ" as well as a "protective organ."

Dr. McGlone introduced the concept of a "hedonic homunculus" to emphasize that these fibers have a distinct central projection to a para-limbic brain area, the insular cortex, that processes information concerned more with "feeling" than "sensing."

The presentations in the "Itch" session were primarily neurocentric while incorporating the crosstalk with the immune system and the cutaneous environment. The use of state-of-the-art techniques was a feature of this session, including the power of genetics to investigate neurocircuits, optogenetics, *ex vivo* preparations consisting of skin attached to the spinal cord, and imaging. These approaches allowed for detailed information to be communicated with respect to current knowledge regarding mediators, modulators, neurocircuitry, and therapeutic targets.

Session Chair Ethan Lerner set the tone with provocative questions: why do we itch (To remove pathogens? To keep the immune system active?), and what is the sequence of events? (Is it the itch that "rashes" or the rash that itches?) Dr. Lerner then shared work from his laboratory, first focusing on the role of sensory neurons in the development of skin inflammation and itch using *in vivo* imaging in a mouse ear skin allergic hypersensitivity model and then showing that substance P-evoked scratching, which was originally thought to be mediated by the neurokinin-1 receptor (NK1R), is mediated by Mrgpr receptors.

Earl Carstens (University of California, Davis) presented behavioral and expression data suggesting that NK1R is a key component of itch-specific spinothalamic projection neurons. He also highlighted recently developed genetic and induced models of chronic itch (including mouse models of psoriasis and a model with dietary deprivation of polyunsaturated fatty acids that results in skin rash), as well as methods for studying enigmatic neuropathic itch, or alloknesis.

Sonja Ständer (University of Münster) presented findings from clinical studies employing quantitative sensory testing on patients in conjunction with experimental therapies to treat

neuropathic and recalcitrant itch. Of the topical treatments, they have tested an 8% capsaicin patch and found significant relief of chronic itch for months after just a single one-hour application.

Diana Bautista followed up with her lab's recent work on mouse models of acute and chronic itch. She provided behavioral data demonstrating the requirement of certain immune cells, previously implicated in pain hypersensitivity, as playing a dual role in acute and chronic itch. Her group has found that a chemokine associated with human atopic dermatitis (AD) is sufficient to evoke itch behaviors and is correlated with itch in a mouse model of AD, extending her previous study, which exploited natural variation in itch behaviors among genetically distinct mouse strains to identify itch-associated candidate genes in sensory neurons and spinal cord.

Mark Hoon (National Institute of Dental and Craniofacial Research) related his laboratory's recent work on the role of somatostatin-expressing sensory neurons in itch. His group has found that natriuretic peptide precursor B-expressing somatosensory neurons, thought to specifically transmit itch signals, co-express the neurotransmitter somatostatin. Dr. Hoon detailed a variety of optogenetic and genetic tools being utilized 1) to test the hypothesis that somatostatin is an important itch-specific neurotransmitter required for spinal processing of itch via inhibition of inhibitory interneurons and 2) to understand how the release of somatostatin from primary afferents modulates spinal cord processing of itch and response to counter stimuli.

Sarah Ross (University of Pittsburgh) focused on the role of counter stimuli in itch inhibition via a specialized class of spinal cord neurons that her group discovered: the Bhlhb5-expressing, inhibitory neuron (B5-I) and their importance in integrating itch and pain signals. Her group employed a system for *ex vivo* stimulation of the periphery or dorsal root ganglion (DRG) while simultaneously recording from the B5-I population in the dorsal horn of the spinal cord. They have shown an electrophysiological basis for the inhibition of itch via soothing menthol or

painful capsaicin via activation of the B5-I population, which expresses neuronal nitric oxide synthase and galanin, and have also shown a role for dynorphin tone in the spinal cord as a means by which BI-5 activity is modulated and itch is actively suppressed.

The Itch session closed with a talk from Nicole Ward (Case Western Reserve University), who related her lab's work on the interplay of neurons and immune cells in genetic and induced mouse models of psoriasis. Dr. Ward described the dermatome-specific manifestation of psoriasis in humans and the role of skin innervation in the pathogenesis of the *KcTie2* and the imiquimod/Aldara psoriasis mouse models, showing that denervation leads to skin thinning and attenuation of disease phenotype.

Discussion of the relationship and the molecular level between itch and pain, e.g., through release of factors in itch that block pain, provided an intriguing segue to the next session.

The "Pain" session, chaired by Diana Bautista, focused on the molecular and cellular mechanisms underlying acute and chronic pain in the periphery and spinal cord. Presentations and productive discussion introduced cutting edge techniques and novel therapies and centered on three central issues: 1) the use of novel molecular genetic tools to define and manipulate the molecules, cells and circuits of pain; 2) the pros and cons of distinct animal models of pain; and 3) the identification of novel therapeutic targets and approaches and how to move them from the bench to the clinic.

The use of sophisticated tools to define and manipulate the molecules, cells, and circuits that drive pain was a major theme of this session. Cheryl Stucky (Medical College of Wisconsin) shared work on the use of optogenetics to examine the role of calcitonin gene-related peptide - positive DRG neurons in neuropathic, incision, and inflammatory pain. Rebecca Seal (University of Pittsburgh) discussed her study of transgenic mice that express designer receptors exclusively

activated by designer drugs, or DREADDs, in the vesicular glutamate transporter type 3-positive dorsal horn neurons in neuropathic and inflammatory pain models. She described the identification of novel neuronal populations that are unique to distinct pain models. For example, the spared nerve injury model of neuropathic pain and the Complete Freund's Adjuvant model of inflammatory pain engage very different cells and circuits. Finally, Qiufu Ma (Harvard Medical School) presented his studies of cre-recombinase lines that mark distinct populations of spinal neurons, showing that there are many subpopulations that each contribute differentially to itch and/or pain. While these studies share the common goal of defining cell types that promote acute and chronic pain, the parallel approaches yielded unique insights into the underpinnings of pain. The talks triggered a timely discussion on the pros and cons of distinct mouse models and the increasing number of studies showing that constitutive ablation and acute silencing of cells in the pain circuit are not equivalent. Overall, the discussion highlighted the importance of using different approaches and pain models to gain insights into pain circuit function.

Allan Basbaum (University of California, San Francisco) and Daniel Bruce (University of Minnesota) provided information on two therapeutic strategies for combating pain. Dr. Basbaum showed that transplanting medial ganglionic eminence neurons from the cortex of embryonic mice into the dorsal horn of adult dramatically attenuated neuropathic pain or itch. The transplanted neurons integrated into local circuits, restored normal GABA inhibitory signaling in the dorsal horn, and did not affect other sensory modalities. Dr. Bruce showed evidence that dual treatment with peripherally restrictive and selective opioid agonists lopermide and oxymorphinindole attenuates chronic inflammatory pain.

The session ended with Martin Schmelz (University of Heidelberg) discussing the nerve growth factors- and UVB-mediated nociceptor sensitization models in humans. As in mouse

models, evidence suggests that both peripheral and spinal cord modulation may account for spontaneous itch and pain. Dr. Schmelz engendered an exciting discussion regarding pattern theory of itch and pain signaling and argued its applicability to human nociception. All agreed that discussion among clinicians and basic researchers will help ensure that human pain and itch disorders are being examined in mechanistically relevant animals models to facilitate the translation of findings to the clinic.

The "Touch" session, chaired by Ellen A. Lumpkin, highlighted recent findings on the biophysics of mechanotransduction, the role of epidermal cells in touch sensation, and the neural circuitry of touch. Jorg Grandl (Duke University) began with a biophysical presentation on gating mechanisms of Piezo ion channels, which convert mechanical stimuli into electrical signals. Using magnetic nanoparticles and magnetic fields to apply unprecedentedly small forces, his group discovered that the globular domain of the Piezo protein is the most sensitive to small perturbations, making this region a likely candidate for the mechanical gate to open the channel. Further, Dr. Grandl showed that Piezo channels display increasing current with increasing stimulation frequency and suggested that this allows them to transduce repetitive stimuli such as vibrations.

Elena Ezhkova (Icahn School of Medicine at Mt. Sinai) presented work on the development and specification of Merkel cells, which are epithelium-derived mechanosensory cells. During Merkel-cell specification, atonal homolog 1 (Atoh1) and Sox2 are present first, with Insulin gene enhancer protein Isl1, keratin 8, keratin 18, and keratin 20 coming on later. Next, Dr. Ezhkova showed that the polycomb complex regulates transcription factors necessary for Merkel-cell development, as there is an upregulation of Atoh1, Isl1, and Sox2 in polycomb null mice leading

to an increased number of Merkel cells. Finally, Dr. Ezhkova showed that Sonic Hedgehog is both necessary and sufficient for Merkel-cell specification.

Hironobu Fujiwara (RIKEN Center for Developmental Biology) and Ellen Lumpkin focused on key roles of skin cells and epidermal appendages in touch sensation. Dr. Fujiwara described a novel component of hair follicle extracellular matrix (epidermal growth factor-like domain multiple 6) and its role in formation and function of hair follicle-neurite lanceolate complexes, which mediate rapidly adapting responses to touch. Dr. Lumpkin discussed cellular and molecular mechanisms of mechanotransduction in Merkel-cell neurite complexes, which are slowly adapting touch receptors. Dr. Lumpkin summarized recent physiological studies that demonstrate that Merkel cells are mechanosensory receptor cells that are both necessary and sufficient to produce prolonged firing in touch-dome afferents. Finally, Dr. Lumpkin presented data findings on the Merkel cell's presynaptic release machinery, spurring discussion on the identities of neurotransmitters at the Merkel cell-neurite synapse.

Victoria Abraira (Harvard Medical School) concluded the Touch session with a presentation on the architecture of mechanosensory circuits in the deep dorsal horn. Dr. Abraira summarized recent findings on the organization of inputs from low-threshold mechanosensory neurons (LTMRs) into the spinal cord and the identity of neurons downstream of LTMRs, showing that the LTMR recipient zone is 250 μm below lamina II in the dorsal horn, and 60% of the postsynaptic neurons in this zone are excitatory. Eleven interneuron subtypes were distinguished by electrophysiology and morphology, most of which participate in feed-forward inhibition, but some are capable of pre-synaptic inhibition. Additionally, Dr. Abraira showed that the somatosensory cortex provides top-down modulation to the LTMR recipient zone, suggesting that modulation from the cortex might enable the LTMR recipient zone to modulate sensation.

Returning to themes presented in Francis McGlone's talk, biological anthropologist Nina G. Jablonski (The Pennsylvania State University) presented a second keynote at the Saturday banquet on the evolution of human skin as a sensory organ, focusing on two aspects of affiliative touch: highly sensitive discriminative touch and pleasurable, socially reinforcing touch. Social cohesion in primate groups is based on alliances maintained through these affiliative touch mechanisms, and affiliative touch is essential to human psychological development and can have profound effects on interpersonal interactions.

The final session, "Pleasure," was chaired by Francis McGlone and explored evidence for what distinguishes touch perceived as pleasant on the skin from other sensation, such as mechanical pressure or pain. Understanding these distinctions has implications for compliance with topical treatments or disease, as well as with soothing touch to address chronic pain or itch. Talks in this session revealed that field area, stroking velocity, and temperature all factored in this type of touch, beginning with Helena Wasling's (University of Gothenburg) presentation describing single unit microneurography to characterize the receptive fields and firing properties of human CTs. Subsequent psychophysical studies using a robotic tactile stimulator found that these velocities of ~ 5 cm/sec were reported as the most pleasant compared with slower of faster velocities, a finding quantified by the use of facial electromyography. The maximal activation of CTs by stroking stimuli delivered at skin temperature reinforced the hypothesis that CTs are the neurobiological substrate driving affective and affiliative nurturing touch.

Håkan Olausson (Linköping University) continued the discussion of touch afferents, first by presenting a case study in which patients had selective degeneration of large myelinated afferents but intact C afferents and therefore CTs. These patients reported an absence of tickle but reported a pleasant sensation when stroked on hairy skin with a soft brush. When touched on

glabrous skin detection of touch was absent. Dr. Olausson further characterized CTs by fMRI, showing activation of the insula and deactivation of primary somatosensory cortex (S1) with CT stimulation. Transcranial magnetic stimulation in S1 changed the intensity of the stimulation but not the pleasantness, suggesting that affective touch is processed in a distinct cortical region.

Susannah Walker (Liverpool John Moores University) continued the discussion on CTs with her presentation on the affective value of touch. Dr. Walker showed that heart rate and EMG recordings from "smile" and "frown" facial muscles can be used to evaluate the pleasantness of a stimulus, and using these methods, brushing stimuli are rated as most pleasant when delivered to the arm at CT preferred velocities. Additionally, Dr. Walker showed that pairing neutral faces with stimuli that maximally activate CT afferents increased the approachability ratings of the neutral faces. Finally, Dr. Walker showed that dopamine is released in the nucleus accumbens with stroking in rats, and oxytocin is released with massage, suggesting that these systems might be recruited with CT stimulation in humans.

Gil Yosipovitch, in the closing talk, synthesized symposium topics by expounding upon the pleasure and reward associated with scratching an itch. Chronic itch patients, particularly AD patients, often cite the pleasure evoked by scratching. Examination of this sensation with fMRI by his lab has implicated both reward and addiction centers, such as the striatum and the prefrontal cortex, in scratching in healthy and chronic itch patients. Dr. Yosipovitch touched on the "contagious" nature of itch and scratching, hypothesizing that higher order brain regions activated by itch may contribute to this complex phenomenon.

Dr. Yosipovitch moderated a final interactive session, titled "Future Directions: Applying New Approaches to Skin Sensation Research," addressing treatments, funding strategies, unmet needs, and obstacles encountered in developing therapies and establishing relationships between

government, academia, and industry. NIH panelists Preeti Hans (National Institute of Neurological Disorders and Stroke) and Hung Tseng (National Institute of Arthritis and Musculoskeletal and Skin Disease) discussed funding strategies. Industry panelist Thomas Sciascia (Trevi Therapeutics) discussed the challenges and roadblocks to drug development. Frank Liebel (AVON Products, Inc.) discussed the relevance of itch and pleasure to compliance with product use. Possible applications of emerging knowledge to autism spectrum disorders and other conditions of the central nervous system were noted, along with potential instructiveness of the NIH-BRAIN initiative.

The confluence of investigators from diverse fields across academic, clinical, and industrial contexts at the symposium illuminated wide-ranging insights on the role of nerve fibers in the skin. The presentations of published and unpublished research set a foundation for discussing novel types of animal models that gave way to future therapeutic modalities. From examining immune cells implicated in neural hypersensitivity to genetic tools and spinal processes that mediated inhibitory neurons came potential realms of treatment in electrophysiology, spinal cord cell transplant, and optogenetics. Certainly, more work needs to be done in identifying the neurotransmitters that facilitate mechanical input and receptors that mediate behavioral patterns, as well as in refining methods of quantitative sensory tests used for patients. With questions of mechanism and disease processes at hand, experimental and clinical inquiry further contributed to articulating neurophysiological frameworks, including gate control theory and pattern theory, that give shape to the skin as a sensory organ.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The Montagna Symposium on the Biology of Skin, directed by Molly F. Kulesz-Martin, Ph.D., is an annual non-profit scientific meeting, inaugurated in 1950 by William Montagna, Ph.D., that gathers leading cutaneous biologists and dermatologists to discuss the latest findings, techniques and goals in skin biology. Podcast interviews of speakers are available at http://www.jidonline.org/content/skinpods, and blog posts from the meeting may be found at https://jidjottings.wordpress.com/category/jid-jottings/.

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