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Sensational channels

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This year's Nobel Prize in Physiology or Medicine was awarded to David Julius and Ardem Patapoutian for "explaining the molecular basis for sensing heat, cold and mechanical force." Their findings capped off a scientific quest to identify the mechanisms within the somatosensory system mediating the detection of internal and external environments.

> Where perception is, there also are pain and pleasure, and where these are, there, of necessity, is desire.—Aristotle (*On The Soul*)

Like all animals, people rely on detection of the physical world through the skin. Through touch we navigate in our environment, manipulate tools and objects, determine their shape and temperature, and sense physical pain or pleasure. Philosophers have pondered the nature of these processes since ancient times. Aristotle wrote that "…we do not perceive what is equally hot and cold or hard and soft, but only excesses, the sense itself being a sort of mean between the opposites that characterize the objects of perception" (*On the Soul* 423b30–424a10). A present-day physiologist would be tempted to interpret this statement as a hypothesis for the existence of specialized sensory nerves that detect physical stimuli, which are then integrated and interpreted by the central nervous system. What started as a purely philosophical quest changed with the advent of the scientific method, which fast-forwarded our understanding of the world through the collection of evidence via observations and experiments. The 2021 Nobel Prize in Physiology or Medicine celebrates the achievements of David Julius and Ardem Patapoutian in uncovering the receptor molecules that enable us to perceive physical force and temperature.

Scientific efforts to understand sensation began with Jean-Baptiste Lamarck (1744–1829), who posited that sensation occurs when a motion is excited in the fluid of a sensory nerve. Erasmus Darwin, the grandfather of Charles Darwin, was one of the first to provide evidence for the existence of specific nerves that detect heat. Later works by Charles Bell, Johannes Muller, and other physiologists of the late 18th and early 19th centuries solidified the notion of dedicated nerve fibers that specialize in the detection and transmission of specific aspects of touch. Maximillian von Frey (1852–1932) is credited for proposing a model whereby pain and innocuous touch are independent tactile qualities detected by different types of sensory nerves. He suggested that the skin has tactile, cold, warm, and pain spots with distinctive regional variation and sensitivity. To this day, his nominal "von Frey hair test" is used in clinical and scientific settings to probe skin sensitivity.

Our current understanding of the somatosensory system was shaped by anatomical and neurophysiological works in the 20th century, many of which were recognized by the Nobel Prize: Camillo Golgi and Santiago Ramón y Cajal in 1906, Sir Charles Sherrington and Edgar Adrian in 1932, and Joseph Erlanger and Herbert Spencer Gasser in 1944. We now know that physical stimuli are detected by somatosensory neurons. These specialized neurons, which are located in the trigeminal cave and along the spine in the form of clusters called somatosensory ganglia, send their projections to the skin, muscles, joints, and some internal organs. These are the longest cells in our body. The projections, called somatosensory afferents, specialize in the detection of physical stimuli, such as temperature, mechanical pain, or chemical irritants. Some afferents innervate specialized organs in the skin dedicated to the detection of various forms of light touch, such as Merkel cells and Meissner and Pacinian corpuscles, while others can form elaborate structures around hair follicles to detect hair movement. Sensation begins when a physical stimulus generates action potentials—oscillations of electric potential on the plasma membrane that propagate along the afferent to deliver information about the detected stimulus to the integrative centers in the spinal cord and brain.

This view, though far from complete, painted a coherent neurophysiological picture of how the somatosensory system detects physical stimuli. However, a crucial question remained: what molecules allow sensory afferents to detect diverse stimuli like heat, cold, and mechanical force? The existence of such molecules was supported by the observation that action potentials in somatosensory neurons are preceded by an initial depolarization of the plasma membrane known as the receptor potential. The molecular conduits of receptor potentials were known to be ion channels, proteins that form ion-selective pores in the plasma membrane. For decades, the identities of the ion channels creating receptor potentials in somatosensory afferents remained elusive. Moreover, the experiments that could identify such ion channels were out of reach for a long time. David Julius's and Ardem Patapoutian's laboratories ushered the entire field into a new era by developing tools to accomplish this task, using those tools to identify the ion channels, and proving their essential

roles for somatosensation in humans and other animals.

Some like it spicy

Most of us are familiar with the burning sensation we experience in our mouths when we eat hot chili peppers or other spicy foods. However, you may be surprised to learn that this sensation does not come from the taste system but from the same somatosensory neurons that detect heat (thermoreceptors) and various forms of pain (nociceptors). In chilis and other peppers, the chemical responsible for this effect is capsaicin, a hydrophobic vanilloid first extracted and named by the German pharmaceutical chemist Christian Bucholz in 1816. Whether applied to the tongue or rubbed into the skin, capsaicin causes the sensation of burning pain, perceptually similar to a thermal burn. Paradoxically, after prolonged use the burning sensation subsides and is replaced with numbness, or analgesia, enabling the use of capsaicin to relieve various types of pain starting in the mid-19th century. For decades, capsaicin has been used as a potent pharmacological tool to study thermoreceptors and nociceptors. Mounting evidence had pointed at the possibility that capsaicin and heat not only acted on the same type of thermoreceptor neurons but also on the same ion channel that generates the receptor potential in these cells. To reveal the molecular identity of the capsaicin receptor, Michael Caterina, at that time a postdoc in David Julius's laboratory at the University of California, San Francisco, used an experimental strategy called expression cloning.

The expression cloning strategy rests on the assumption that the unknown receptor molecule is represented by a single protein, which is sufficient to endow a cell with the ability to sense a stimulus—in this case, capsaicin. To find the receptor, mRNA is extracted from the source, in this case, somatosensory neurons, and converted into a cDNA plasmid library. The library is subdivided into smaller pools, each of which is tested for the ability to produce sensitivity to the stimulus of choice when transfected into a naive cell. The pool that produces the response is subdivided and tested for several rounds further until a single cDNA clone that encodes the receptor of interest is identified. David Julius had used this strategy to identify the serotonin receptor in 1988 in the laboratory of Richard Axel, a co-recipient of the Nobel Prize in Physiology or Medicine in 2004.

To clone the capsaicin receptor, a cDNA library was synthesized from mRNA extracted from rodent somatosensory ganglia and tested in human embryonic kidney-derived HEK293 cells. Julius's group used the results of this screen to identify a molecule now called transient receptor potential vanilloid 1, or TRPV1 (Caterina et al., 1997). When TRPV1 was expressed in HEK293 or other cell lines that are insensitive to capsaicin and heat, it endowed them with the ability to act like somatosensory neurons that respond to these stimuli. This responsiveness fulfills the criterion of scientific "sufficiency," meaning that TRPV1 can act independently of other molecules to confer heat and capsaicin sensitivity. The Julius laboratory further showed that TRPV1 is necessary for the observed responses, as genomic deletion of TRPV1 abolished sensitivity to capsaicin, suppressed sensitivity to heat, and attenuated behavioral sensitivity of mice to high temperatures (Caterina et al., 2000).

The sensitivity of TRPV1 to capsaicin presents a puzzling biological question. The seeds of chili peppers and other *Capsicum* plants, which contain a lot of capsaicin, are predominantly dispersed by birds. How can the birds tolerate consuming large quantities of these spicy seeds? The Julius group investigated this phenomenon and found that the chicken ortholog of TRPV1 retains all the major properties of mammalian channel, such as sensitivity to heat and acid, except for the sensitivity to capsaicin (Jordt and Julius, 2002). This property has been put to use by manufacturers of bird food, who add capsaicin to deter squirrels from bird feeders. This work also identified binding sites for capsaicin and drugs that influence the activation of TRPV1. Later, collaborative efforts between David Julius's and Yifan Cheng's laboratories determined the first high-resolution structure of TRPV1 using single-particle electron cryomicroscopy (Liao et al., 2013) and in subsequent works revealed the special architecture of the binding sites for capsaicin and other vanilloid compounds.

Because capsaicin has been widely used to study pathways associated with pain in humans and rodents, the discovery of TRPV1 triggered numerous efforts to identify the roles of this channel in nociception at large. The Julius group found that TRPV1 is activated by acidosis, a condition that accompanies various types of inflammatory events (Tominaga et al., 1998), and that, in the absence of the channel, mice exhibit reduced hyperalgesia in response to tissue injury (Caterina et al., 2000). TRPV1 is now known to be expressed in a large group of pain-sensing somatosensory neurons, referred to as polymodal nociceptors, which detect pain associated with heat, tissue injury, inflammation, and cancer. A prominent subgroup of polymodal nociceptors detects chemical irritants. Some of these compounds, like those that endow wasabi, onion, and garlic with their savory qualities, may be enjoyable in small quantities. However, most of them are damaging to proteins and nucleic acids, necessitating the existence of a defense mechanism that detects their presence. The molecular receptor for such compounds, a TRPV1 homolog called TRPA1, was identified by Ardem Patapoutian's group (Story et al., 2003) and then by David Julius's laboratory (Jordt et al., 2004). Since its discovery, TRPA1 has gained notoriety as an integrator of multiple toxic compounds present in tobacco smoke, insect bites, poisonous plants, pollutants, and allergens and as a target of chemotherapy drugs.

The biology of the TRPV1 and TRPA1 channels and associated somatosensory neurons ranges beyond the detection of heat and pain. A fascinating example occurs in some vertebrates, such as vampire bats and pit vipers, that co-opted the somatosensory system for predation, based on the ability to detect infrared radiation emitted by their warm-blooded prey. These animals sense radiant heat via specialized pit organs innervated by somatosensory neurons of trigeminal ganglia. In both bats and vipers, the detection is carried out by TRP channels with modified function: in bats, heat is detected by a splice variant of TRPV1, whereas in pit vipers the infrared detector is TRPA1 (Gracheva et al., 2010). These findings underscore the remarkable plasticity of TRP channels, which can be capitalized upon to serve specific sensory needs.

Cooling it down

As counterintuitive as it may seem, the detection of cold is not the same as detecting the absence of warmth. The existence of dedicated cold-sensing fibers that generate action potentials upon cooling had been known since the 1920s from the works of the German neurologist Alfred Goldscheider. It was also known that menthol, a monoterpenoid present in some *Lamiacea* plants, such as spearmint and peppermint, has the peculiar ability to cause a cooling sensation on the tongue without changing the actual temperature. It was not until the 1950s that Herbert Henzel and Yngve Zotterman discovered that menthol activates cold-sensing neurons. Thus menthol, like capsaicin, presented a unique opportunity to identify the molecular receptor of menthol and cold. An expression cloning screen conducted in the Julius lab resulted in the identification of an ion channel from the same family as TRPV1, called TRPM8, that sensed cold and menthol (McKemy et al., 2002). Concurrently, Ardem Patapoutian's group came to the same conclusion by cloning TRPM8 based on a bioinformatic search for TRP family members in genomic DNA databases (Peier et al., 2002). The two groups later created TRPM8-deficient mice and demonstrated that TRPM8 not only detects cold but is also essential for physiological responses to skin cooling (Bautista et al., 2007; Dhaka et al., 2007). The convergence of the results obtained by independent efforts of the two laboratories left no doubt about the molecular basis of cold detection and initiated a new research direction in the somatosensory field.

A touching response

In *On the Soul*, Aristotle posits that the sensation of touch is vital. He argues that an animal may dispense with hearing, taste, smell, or vision but that losing the sense of touch, or as it is also called, mechanosensitivity, is equivalent to death. Indeed, all organisms, from bacteria to humans, can sense the world through mechanical contact. Multicellular organisms also need to differentiate internal and external forces and distinguish various aspects of touch, like vibration, texture, and stretch. Along with taste and olfaction, the sense of touch in the tongue and oral cavity is essential for the perception of the overall taste of food. Yet compared to the other senses, the sense of touch lagged behind in terms of developing molecular level understanding of how it works.

Like heat and cold, mechanical force was known to be detected by specialized somatosensory afferents. Just as the responses of thermoreceptors to different temperatures implied the existence of molecular sensors of heat and cold, the presence of mechanically evoked action potentials in mechanoreceptor neurons suggested the existence of specialized molecular sensors of mechanical stimuli. However, the absence of an obvious physical ligand to activate mechanical sensors led to suggestions that mechanical force could be detected as microdamage to the plasma membrane of mechanosensory afferents, obviating the need for specific sensory molecules. The mere notion of the existence of mechanically activated ion channels was questionable.

First steps to finding broadly conserved channels came after studies identified relevant receptors restricted to bacteria and plants, and the worm *Caenorhabditis elegans*. Candidates for vertebrate channels, including members of the TRP family, were suggested but did not withstand experimental validation. The tried-and-true expression cloning strategy, which led to the identification of TRPV1, TRPM8, and TRPA1, was inapplicable to touch because of the absence of a known chemical mimetic.

The Patapoutian laboratory solved the conundrum by taking the inverse approach. Whereas expression cloning is based on the strategy of attempting to endow cells with a new property (known as a gain-of-function approach), Bertrand Coste, at the time a postdoc in the Patapoutian laboratory at the Scripps Research Institute, used a loss-of-function strategy. First, he screened a number of immortalized cell lines for one that possessed mechanically activated electrical current, the manifestation of the presence of a mechanically gated ion channel. One such cell line, called N2A, responded to mechanical stimulation similar to somatosensory neurons and as thus presented a cellular platform to perform a functional knockdown screen. Next, they used a microarray analysis to generate a list of proteins that were enriched in N2A cells compared to those cell lines that lacked mechanosensitivity. The importance of each of the candidate molecules for mechanosensitivity of N2A cells was tested using functional knockdown via a small interfering RNA approach with an electrophysiological readout. After screening over 70 candidates, Coste identified the Fam38a gene, which encoded the mechanically gated ion channel in N2A cells (Coste et al., 2010). The team named the ion channel Piezo1, from the Greek word "πίεση" (píesi), meaning "pressure." A subsequent bioinformatic search identified the only homolog of Piezo1, called Piezo2. Further testing by the Patapoutian group revealed Piezo2 to be responsible for mediating a subset of mechanically activated currents in mouse somatosensory neurons that are essential for physiological sensitivity to touch (Ranade et al., 2014) and proprioception (Woo et al., 2015). The Piezos, together or separately, were found to be important for normal function of almost every aspect of mechanosensitivity, from baroreceptor neurons that are sensors of airway stretch and blood pressure to epidermal Merkel cells that contribute to light touch detection. Piezo1 and Piezo2 belong to a family of ion channels conserved from protozoa to humans. The identification of the Piezos initiated a new direction in the field of mechanobiology, prompting many groups to investigate the role of the channels in diverse physiological phenomena across species.

It is difficult to name a physiological process that is not influenced by mechanical force. Even though most cells do not directly participate in somatosensory mechanotransduction, they experience mechanical load and respond to mechanical stimuli one way or another. After the seminal discovery of the Piezos, the Patapoutian group expanded their efforts beyond sensory physiology into a tour-de-force journey that illuminated pivotal roles of Piezo1 and Piezo2 in various facets of mechanobiology. They found that Piezo1 regulates lymphatic valve formation, vascular development, iron metabolism, and red blood cell homeostasis and influences the susceptibility of red blood cells to *Plasmodium* infection. Dysfunction in Piezo1 and Piezo2 were found to be

linked to, respectively, xerocytosis and arthrogryposis, which are severe genetic conditions found in humans. Beyond mammals, the Patapoutian group identified roles for Piezo homologs in the mechanosensitivity of the fruit fly and *Arabidosis* plant, demonstrating that the function of the channel is conserved in evolution.

Though initially found in the somatosensory system, the heat- and cold-sensing TRP and Piezo channels underpin biological phenomena related to temperature and mechanosensitivity in other physiological contexts, including thermoregulation, inflammation, red blood cell physiology, and bone formation. The works by David Julius's and Ardem Patapoutian's laboratories are brilliant examples of how basic science can illuminate and advance various fields of inquiry. The celebration of these achievements by the Nobel Assembly underscores the value and far-reaching potential of fundamental research driven by curiosity.

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