OSCA1.2,[1] suggesting that TMEM63A and TMEM63B are less sensitive to mechanical stimuli than OSCA1.2. This led to further investigations on how oligomerization states influence the mechanosensitivity in the OSCA/TMEM63 family members. Zheng et al. found that mutations on IL2 of OSCA1.2 abolish dimerization and also result in a higher P_{50} value.[1] Single-channel recordings revealed that, compared with the monomeric mutant of OSCA1.2, dimerization has minimal influence on the conductance of the pore but increases the duration of pore opening.[1] These data suggest that dimerization could stabilize the open state of OSCA1.2 and confer enhanced mechanosensitivity.

Taken together, the structure of TMEM63 reported by Zheng et al. and other research groups along with their functional characterization provide valuable insights into the monomeric architecture of TMEM63 family mechanosensitive channels. These findings serve as a foundation for further comprehensive investigations into the mechanisms by which these channels perceive mechanical stimuli and open their pores.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Engineering mechanoreceptor feature selectivity

Joriene C. de Nooij[1,*]
[1]Department of Neurology and Columbia University Motor Neuron Center, Columbia University Irving Medical Center, New York, NY, USA
*Correspondence: sd382@cumc.columbia.edu
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Touch and proprioception rely on the discriminative abilities of distinct classes of mechanosensory neurons. In this issue of *Neuron*, two studies[1,2] provide evidence that biomechanical mechanisms and ultrastructural cellular specializations are key contributors in defining mechanoreceptor stimulus threshold and selectivity.

Most of our daily activities are meticulously guided by somatic mechanosensory signals. Proprioceptive signals from muscle stretch receptors, for instance, coordinate basic movements such as reaching for a cup of coffee, while dynamic touch receptors in skin can sense micro-slippage from the texture of the coffee cup's surface to help gauge the appropriate grip force.[3,4] The ability to discriminate between these various mechanical stimuli depends on dedicated classes of proprioceptive and mechanosensory neuron subtypes, which differ in stimulus selectivity, activation threshold, and adaptation properties.[5–7] In many species, the peripheral sensory terminals of these neurons are associated with non-myelinating terminal Schwann cells, lamellar cells, or other specialized fibroblasts and combine into elaborate sensory end organs.[6,8] Historically, these morphologically unique structures have been used as a means to identify the distinct proprioceptive and mechanoreceptive neurons. In recent years, this mode of identification has partly been supplanted by next-generation sequencing technologies, which not only revealed the molecular signatures through which to distinguish these neurons from one another but also provided insight into their physiological characteristics.[9,10] Despite increased molecular insights, an aspect

that has continued to elude this field is how external forces activate individual mechanoreceptors to yield selective tuning properties to tissue stretch, pressure, or vibration. In particular, it has remained uncertain whether activation threshold and feature selectivity are primarily a consequence of distinctions in the molecular makeup of the sensory neurons (e.g., transduction or ion-channel composition) or whether this is based on other cellular or tissue environmental aspects. In this issue of *Neuron*, two studies[1,2] provide evidence for the latter.

In the first of these two articles, Tuthill and colleagues set out to understand the mechanisms that control feature selectivity of the femoral chordotonal organ (FeCO) sensory neurons in *Drosophila*, which relay proprioceptive feedback from the femoral-tibial joint in the fly front leg.[1] The FeCO contains three classes of sensory neurons—claw, hook, and club—which, akin to proprioceptive muscle spindle afferents in tetrapods, detect various kinesthetic features: claw neurons report static tibia position, hook neurons sense directional movement, and club neurons encode bidirectional movement and low-amplitude vibration in the high-frequency range.[11] Claw and hook neurons are further defined with respect to their "direction selectivity" (flexion or extension). Using single-nucleus sequencing, the authors show that these FeCO sensory subtypes are remarkably similar with respect to the mechanotransduction- and voltage-gated ion channels they express, suggesting that kinematic feature selectivity may primarily be rooted in aspects other than molecular distinctions. To investigate this, Mamiya et al. performed detailed anatomical reconstructions of claw, hook, and club neurons within the FeCO, relying on an X-ray holographic nano-tomography dataset and new genetic reporters for each of the subtypes.[1,12] These analyses revealed that claw, hook, and club neuron cell bodies are grouped by subtype into three spatial compartments and indirectly connect to the tibia through separate medial (claw and hook) and lateral (club) tendons that both attach to the arculum—a structure with semblance to a tuning fork that is flexibly attached to both the distal femur and tibia. By imaging and modeling arculum dynamics, along with claw and club neuron tendon movements, Mamiya et al. noted that small (<1 μm) vibratory stimuli to the tibia (but not larger tibia flexion/extension movements) cause arculum rotational displacements that selectively strain the lateral club cell-associated tendon. These observations suggested that a force transformation by the arculum renders club FeCO neurons selectively responsive to high-frequency vibratory stimuli.

Mamiya et al. also offer insight into the mechanism through which individual claw neurons (responsible for position sense) are tuned to specific joint angles. The above-mentioned imaging analyses revealed that along with the arculum, many claw and hook extension neurons (but not club or hook flexion neurons) move during tibia flexion and extension. This suggested that individual classes of FeCO neurons and their tendons are subject to different strain levels at different tibia angles. To investigate this, the authors precisely measured claw cell translocations during tibia movement, used these observations to construct a model of claw cell dynamics, and then tested the predictions derived from this model using a new volumetric *in vivo* calcium imaging approach. Collectively, the data demonstrated that claw flexion neurons (which are positioned in an array along the proximal-distal axis of the femur) translate distally during tibia flexion and proximally during tibia extension. These translocations are not the same for all claw cells; for a given joint angle, claw cells in the distal-most position show larger movements than those located more proximally. Modeling this spatial gradient of claw cell movements led to the prediction that the geometry and stiffness of the fibrils that attach claw neuron dendrites to the medial tendon, as well as material properties of their surrounding connective tissue, can result in a gradient of mechanical strain for claw-flexion and claw-extension selective neurons. *In vivo* calcium imaging of claw neurons during flexion and extension matched the predicted graded activity levels, thus supporting the existence of a joint angle or "goniotopic" claw neuron activity map. A similar map for vibration frequency selectivity was uncovered for club neurons, but the biomechanical mechanisms that may underlie this tonotopic map remain to be resolved. Thus, these studies demonstrated that *Drosophila* proprioceptor feature selectivity may have biomechanical origins.

The second study, from the Ginty lab, takes aim at the mechanism through which mechanical forces acting on skin or deep connective tissue activate mechanoreceptors that relay dynamic touch.[2] Sensory afferents that form longitudinal lanceolate endings associated with guard hair follicles and afferents that innervate Meissner or Pacinian corpuscles are all fast-conducting (Aβ-range) rapidly adapting low-threshold mechanoreceptors (RA-LTMRs) that primarily rely on the Piezo2 mechanosensitive cation channel for their activation.[6,13] However, these neurons differ in activation thresholds and tuning properties: lanceolate and Meissner endings have relatively higher thresholds and show a preference for low-frequency stimuli (40–100 Hz), while Pacinian afferents exhibit extremely low thresholds and are tuned to high-frequency vibration (>200 Hz). These differences in force thresholds and tuning properties were initially attributed to their divergent sensory end organs, but this clarification became less satisfactory with the observation that some end organs can be innervated by multiple RA-LTMR afferents with different activation thresholds. In an effort to resolve this issue, Handler et al. developed a new fluorescent FLAG-tagged *Piezo2* mouse model to permit a better assessment of the localization of Piezo2 within RA-LTMR sensory terminals.[2] Using these *Piezo2*$^{smFP\text{-}FLAG}$ animals, they were able to confirm that Piezo2 protein is membrane bound, strictly localized to the region of the sensory terminals that are embedded within the end organs, and is absent from any end-organ non-neuronal accessory cells.

The structural analyses of the sensory end organs further revealed that Piezo2 also localizes to small finger-like axonal protrusions that emanate from the main sensory terminal neuronal shaft. These structures were described previously; however, advances in electron microscopy techniques (including focal ion beam-scanning electron microscopy or FIB-SEM and transmission electron microscopy or TEM), along with deep-learning approaches, for the first time permitted a detailed survey of the

ultrastructural features of the sensory endings and these finger-like structures in their native form. In a beautiful set of analyses, Handler et al. produced high-resolution 3D renderings of the sensory terminals and end organs of guard hair lanceolate, Meissner, and Pacinian afferents.[2] Across these end organ types, these analyses uncovered numerous new neuronal and non-neuronal components and structural features unique for individual mechanoreceptor end organ types. Importantly, however, these studies also revealed two core features that appear shared by all dynamic touch neurons. First, they showed that all RA-LTMRs possess similar finger-like axonal protrusions on their sensory terminals (including Aδ lanceolate endings). Second, these axonal protrusions form tight associations (resembling adherens junctions) with end-organ support cells that appear to anchor the terminals in place. This structural arrangement suggests that when external forces dislocate the end-organ support cells or the surrounding collagen matrix, it will stretch the anchored axonal protrusions and open the Piezo2 (or other) transduction channels. By extension, larger numbers of anchored axonal protrusions will proportionally increase the stretchable axonal surface area and the number of opened Piezo2 channels. Consistent with this idea, individual RA-LTMR afferents show dramatic differences in protrusion density, with Pacinian afferents—with the lowest activation threshold and high-frequency vibration selectivity—endowed with thousands of axonal protrusions. Along with offering new mechanistic insight into the tuning properties of RA-LTMR subtypes, these studies provide a rich ground for future explorations. What is the nature of the molecules that make up the adherens junctions, what regulates the density of the axonal protrusions, and to what extent are these axonal protrusions and their molecular contents plastic under conditions of disease or normal aging?

Space limitations of this preview preclude doing justice to the many technical accomplishments in both studies, but together Mamiya et al.[1] and Handler et al.[2] offer important insights on how biomechanical features such as anatomical structure and local variations in tissue elasticity serve to augment the discriminative capacity of mechanoreceptive sensory systems. In these "omic" times, these studies also wonderfully exemplify the continued relevance of anatomical exploration in neuroscience.

DECLARATION OF INTERESTS

The author declares no competing interests.

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