While this exemplary combination of studies yields important new insights on DIP/Dpr function in the developing nervous system, a central remaining question has not been answered: How does the network of DIP/Dpr interactions relate to the structures of the neural networks they help to pattern? While this remains unclear, the in-depth biophysical characterization of DIP/Dpr interactions presented here provides a firm basis to address this question in future studies.

ACKNOWLEDGMENTS

This work was supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 795180 (to M.A.) and by the Wellcome Trust (202827/Z/16/Z) to E.S.

REFERENCES

Brasch, J., Katsamba, P.S., Harrison, O.J., Ahlsén, G., Troyanovsky, R.B., Indra, I., Kaczynska, A., Kaeser, B., Troyanovsky, S., Honig, B., and Shapiro, L. (2018). Homophilic and heterophilic interactions of type II cadherins identify specificity groups underlying cell-adhesive behavior. Cell Rep. *23*, 1840–1852.

Carrillo, R.A., Özkan, E., Menon, K.P., Nagarkar-Jaiswal, S., Lee, P.-T., Jeon, M., Birnbaum, M.E., Bellen, H.J., Garcia, K.C., and Zinn, K. (2015). Control of synaptic connectivity by a network of Drosophila IgSF cell surface proteins. Cell *163*, 1770–1782.

Cosmanescu, F., Katsamba, P.S., Sergeeva, A.P., Ahlsen, G., Patel, S.D., Brewer, J.J., Tan, L., Xu, S., Xiao, Q., Nagarkar-Jaiswal, S., et al. (2018). Neuron-subtype-specific expression, interaction affinities, and specificity determinants of DIP/Dpr cell recognition proteins. Neuron *100*, this issue, 1385–1400.

Luo, D., Liqun, T., Lee, T., Nardine, B., and Null, J.R. (1999). Using the MARCM system to positively mark mosaic clones in Drosophila. Drosoph. Inf. Serv. *82*, 102–105.

Özkan, E., Carrillo, R.A., Eastman, C.L., Weiszmann, R., Waghray, D., Johnson, K.G., Zinn, K., Celniker, S.E., and Garcia, K.C. (2013). An extracellular interactome of immunoglobulin and LRR proteins reveals receptor-ligand networks. Cell *154*, 228–239.

Seiradake, E., Jones, E.Y., and Klein, R. (2016). Structural perspectives on axon guidance. Annu. Rev. Cell Dev. Biol. *32*, 577–608.

Tan, L., Zhang, K.X., Pecot, M.Y., Nagarkar-Jaiswal, S., Lee, P.-T., Takemura, S.Y., McEwen, J.M., Nern, A., Xu, S., Tadros, W., et al. (2015). Ig superfamily ligand and receptor pairs expressed in synaptic partners in Drosophila. Cell *163*, 1756–1769.

Venken, K.J.T., Schulze, K.L., Haelterman, N.A., Pan, H., He, Y., Evans-Holm, M., Carlson, J.W., Levis, R.W., Spradling, A.C., Hoskins, R.A., and Bellen, H.J. (2011). MiMIC: a highly versatile transposon insertion resource for engineering Drosophila melanogaster genes. Nat. Methods *8*, 737–743.

Xu, S., Xiao, Q., Cosmanescu, F., Sergeeva, A.P., Yoo, J., Lin, Y., Katsamba, P.S., Ahlsen, G., Kaufman, J., Linaval, N.T., et al. (2018). Interactions between the Ig-superfamily proteins DIP-α and Dpr6/10 regulate assembly of neural circuits. Neuron *100*, this issue, 1369–1384.

Zhang, K.X., Tan, L., Pellegrini, M., Zipursky, S.L., and McEwen, J.M. (2016). Rapid changes in the translatome during the conversion of growth cones to synaptic terminals. Cell Rep. *14*, 1258–1271.

Synaptic Communication upon Gentle Touch

Sylvia Fechner[1] and Miriam B. Goodman[1,*]
[1]Department of Molecular and Cellular Physiology, Stanford, CA 94305, USA
*Correspondence: mbgoodman@stanford.edu
https://doi.org/10.1016/j.neuron.2018.12.001

Gentle touch sensation in mammals depends on synaptic transmission from primary sensory cells (Merkel cells) to secondary sensory neurons. Hoffman et al. (2018) identify norepinephrine and β_2-adrendergic receptors as the neurotransmitter-receptor pair responsible for sustained touch responses. The findings may deepen understanding of how drugs affect touch and pain sensation.

Merkel cells skim the border zone between skin and body. They cluster together with keratinocytes, rising up in touch domes and encircling hairs that extend touch above the skin's surface in rodents. In both touch-sensing organs, the Merkel cells are innervated by sensory neurons (Aβ afferents) and contain dense-core vesicles and structures resembling presynapses. Their purpose in touch sensation was obscure for nearly a century but has been clarified recently—Merkel cells are epithelial-derived mechanosensory cells. They rely upon Piezo2 channels to sense mechanical stimuli, they fire action potentials, and their stimulation is sufficient to activate sensory afferent neurons that enable the sense of touch (Figures 1A and 1C) (Ikeda and Gu, 2014; Maksimovic et al., 2014; Woo et al., 2014). Merkel cells are thought to use chemical synaptic transmission to communicate with Aβ afferents (Iggo and Muir, 1969) and comprise a subset of the low-threshold mechanoreceptors that account for touch sensation. The identity of the neurotransmitters and postsynaptic receptors responsible for this communication is debated. Using a powerful and unusual combination of classical and modern techniques, Hoffman et al. (2018) demonstrate that, following mechanical stimulation, Merkel cells release norepinephrine (NE) and their afferents rely upon a β_2-adrenergic receptor (β_2AR) expressed in the sensory afferent to complete the information transfer.

To reach these conclusions, the team sought to establish that vesicular release from Merkel cells was required to activate sensory afferents, overexpressing tetanus neurotoxin light chain subunit (TeNT) in epithelial cells to suppress SNARE-dependent vesicular release. As

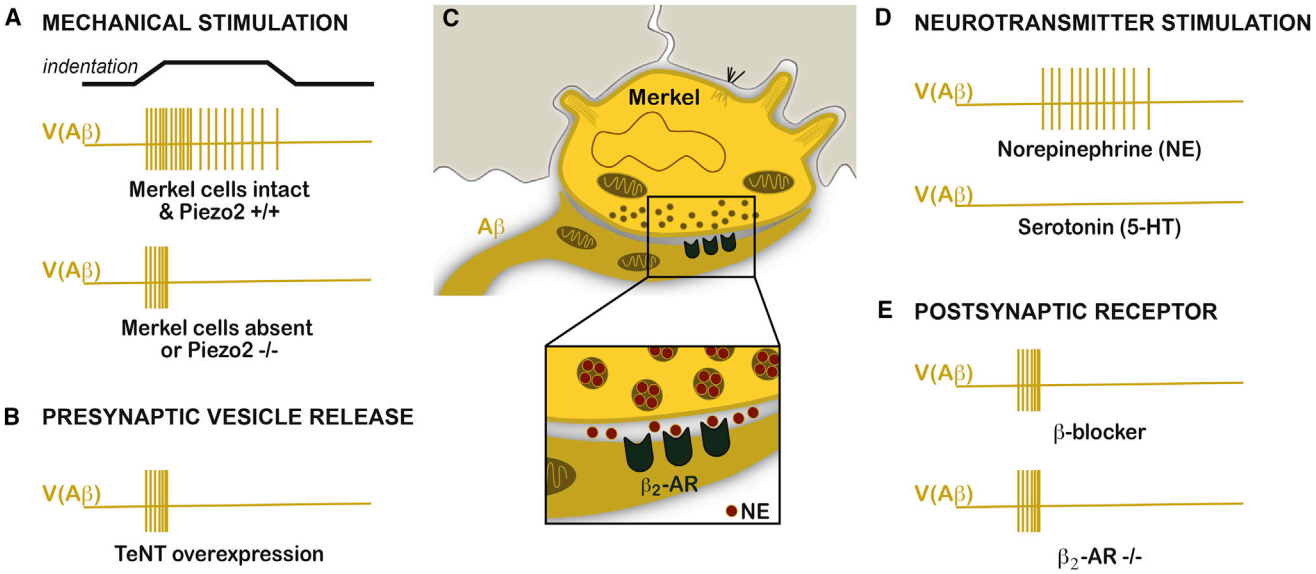

Figure 1. Model for Sensory Signaling and Synaptic Transmission in Touch Dome Afferents

Upon mechanical stimulation, Aβ afferents respond according to the two-receptor model with a dynamic component (mainly induced by Aβ afferents) and a static component (mainly induced by Merkel cells). The static component is absent in mice lacking Merkel cells or lacking Piezo2 ion channels in epidermal cells, including Merkel cells (A), and in mice overexpressing tetanus neurotoxin light chain subunit (TeNT) to inhibit presynaptic vesicle release (B). (C) illustrates the synapse between the Merkel cell and its sensory afferent. Exogenous application of norepinephrine (NE), but not serotonin (5-HT), elicits static firing (D), and mice deficient in the function postsynaptic β_2-adrenergic receptors due to drug application or gene knockout lack static firing (E).

expected for the loss of Merkel cell-derived signaling, the response properties of the Aβ afferents innervating touch domes were severely disrupted (Figure 1B) and resembled those of mice lacking Merkel cells entirely and mice lacking Piezo2 in epidermal cells (Maksimovic et al., 2014; Woo et al., 2014). These results support the long-standing inference that the dense-core vesicles seen along the basal surface of Merkel cells mediate epithelial cell-neuron synaptic transmission and affirm that the first synapse activated during touch is a Merkel cell-neurite synapse.

To discover the proteins expressed by presynaptic Merkel cells and enabling touch-induced neurotransmitter release, the team also analyzed the set of transcripts expressed by Merkel cells. This dataset, which will support further studies of the development and physiology of Merkel cells, includes transcripts encoding proteins essential for presynaptic release of neurotransmitters via vesicle fusion. It also includes the enzymes that synthesize monoamine neurotransmitters (e.g., dopamine, epinephrine, NE), degrade them (monoamine oxidase), and load them into vesicles (VMAT-2). The authors verified that Merkel cells express tyrosine hydroxylase (TH), a key enzyme in the synthesis of catecholamine neurotransmitters, in three ways: immunofluorescence, TH-GFP reporter mice, and *in situ* hybridization. Collectively, these findings support the idea that Merkel cells are catecholaminergic, but do not uniquely identify the neurotransmitter responsible for transmitting information from Merkel cells.

To identify the relevant neurotransmitter in an unbiased manner, Hoffman et al. (2018) used HPLC coupled to electrochemical detection to enhance sensitivity to compare whisker follicles isolated from animals that lacked or retained Merkel cells due to the loss of Atoh1 gene function (Maksimovic et al., 2014). This innovative approach, combining genetic dissection with analytical chemistry, identified NE as the most likely neurotransmitter and suggested that serotonin (5-HT) is not involved. Using a fluorescent catecholamine analog, FFN206, they showed that catecholamines are released in response to elevated external potassium levels and mechanical stimulation. Such evoked release is inhibited by the voltage-gated calcium channel antagonists nimodipine and ω-conotoxin MVII-C, linking their release to Merkel cell stimulation. Additional work is needed to firmly connect synaptic transmission to the dense-core vesicles present in Merkel cells. Nonetheless, a model that is emerging from these data is that the dense-core vesicles long known to be present in Merkel cells are filled with NE, which is released in response to mechanical stimulation and depolarization via SNARE- and calcium channel-dependent vesicle release.

After identifying NE as the dominant neurotransmitter released from the Merkel cells, Hoffman et al. (2018) turned their focus to the Aβ afferents and asked whether NE or other candidate monoamines can directly activate postsynaptic cells. Applying exogenous NE, but not dopamine or 5HT, to the receptive fields of Merkel cell afferents in *ex vivo* skin-saphenous nerve recordings evoked Aβ afferent firing in the absence of touch stimuli (Figure 1D). Given that NE signals through metabotropic adrenergic receptors, Hoffman et al. (2018) hypothesized that β_2ARs mediate Aβ responses. To test this hypothesis, they evaluated the expression of this receptor in the Aβ afferents and determined whether or not Aβ responses were sensitive to β-blockers or the expression of β_2AR in the Aβ

afferents, making use of a conditional knockout strategy to specifically block expression of the β_2AR in neural crest-derived somatosensory neurons. The response to mechanical stimulation in the β_2AR conditional knockout mice (Figure 1E) resembled that reported for animals lacking functional synaptic vesicle release machinery (Figure 1B) and those reported previously in mice lacking Merkel cells or having Merkel cells deficient in the expression of the Piezo2 channel (Figure 1A). Collectively, these findings establish that the Merkel cell-neurite junction is the first touch synapse and reveal that this synapse relies on NE and β_2AR to enable Merkel cells to properly excite somatosensory afferents in touch domes.

What does it mean for clinical practice that NE and β_2ARs are critical for touch sensation? Drugs targeting adrenergic receptors are widely used to treat cardiovascular diseases. Paresthesia (numbness) is reported by patients receiving these drugs, as is skin irritation and pruritus (itch). The crucial role of β_2ARs in touch might underlie these side effects. Whereas β_2AR antagonists suppress Merkel cell-Aβ afferent signaling, tricyclic antidepressants (TCAs) enhance these signals by blocking NE reuptake. TCAs are common treatments for neuropathic pain and generally thought to affect descending pathways controlling pain. The present study and prior work linking neuropathic pain to expression of β_2ARs (Yalcin et al., 2009) raise the possibility that TCAs may function in the skin. Although the extent to which Merkel cell-afferent signaling contributes to neuropathic and spontaneous pain sensation is far from clear, a recent study has connected loss of Merkel cell signaling to itch (Feng et al., 2018). Thus, the central role of NE and β_2ARs revealed in this study may influence understanding of how drugs in common use affect sensory function in the skin.

The Merkel cell-neurite complex is an unusual sensory structure. Both the epithelial Merkel cells and their Aβ afferents are activated by mechanical stimulation and by synaptic signals delivered from the Merkel cells. Thus, the sensory afferent that communicates with the central nervous system appears to function as both as a primary sensory neuron directly responding to mechanical stimulation and a secondary sensory neuron responding to synaptic signals from an upstream sensory cell. Together, the two cells account for slowly adapting responses to mechanical stimulation. The afferent responds to dynamic stimuli with a brief burst of action potentials and Merkel cells account for the sustained firing observed during static indentations (Figure 1). What is the origin of the static firing? First, the release of NE from the Merkel cell could be sustained for the duration of the stimulus. A second possibility is that an initial burst of NE and postsynaptic activation of β_2ARs triggers sustained afferent firing. This mechanism might involve second messenger cascades that modulate ion channel activity to increase firing and that are terminated slowly. With the identification of NE as the primary neurotransmitter responsible for communication between Merkel cells and Aβ afferents, the present study now makes it possible to investigate the mechanism of how Merkel cells evoked sustained firing in their afferents.

Merkel cells now join a group of modified epithelial cells that function as first responders in peripheral sensory organs and communicate directly with the nervous system through chemical synaptic transmission. This group includes other mechanoreceptors such as the hair cells of the inner ear and lateral lines responsible for vertebrate hearing, balance, and the sensation of water flow as well as chemoreceptors like the receptor cells that mediate vertebrate taste sensation. All of these epithelial cells detect physical or chemical stimuli and make use of chemical synaptic transmission to communicate with sensory afferents. External sensory organs are not the only tissues that rely on modified epithelial cells to detect physical stimuli. The epithelia that line the digestive tract also harbor modified cells that function as chemoreceptors (Bellono et al., 2017) and mechanoreceptors (Wang et al., 2017). More broadly, epithelial cells assemble into sheets that define tissue boundaries, and animals make use of specialized epithelial cells to transmit information across this border. Given that synaptic proteins are present in the genomes of animals that lack neurons (Burkhardt and Sprecher, 2017), perhaps such modified epithelial cell sensors predate the development of nervous systems and their persistence suggests the senses that rely on epithelial cells are likewise ancient.

ACKNOWLEDGMENTS

Work supported by NIH grant R35NS105092 to M.B.G.; S.F. was supported by a DFG postdoctoral fellowship (313913559).

REFERENCES

Bellono, N.W., Bayrer, J.R., Leitch, D.B., Castro, J., Zhang, C., O'Donnell, T.A., Brierley, S.M., Ingraham, H.A., and Julius, D. (2017). Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell *170*, 185–198.e16.

Burkhardt, P., and Sprecher, S.G. (2017). Evolutionary origin of synapses and neurons - bridging the gap. BioEssays *39*, https://doi.org/10.1002/bies.201700024.

Feng, J., Luo, J., Yang, P., Du, J., Kim, B.S., and Hu, H. (2018). Piezo2 channel-Merkel cell signaling modulates the conversion of touch to itch. Science *360*, 530–533.

Hoffman, B.U., Baba, Y., Griffith, T.N., Mosharov, E.V., Woo, S.-H., Roybal, D.D., Karsenty, G., Patapoutian, A., Sulzer, D., and Lumpkin, E.A. (2018). Merkel cells activate sensory neural pathways through adrenergic synapses. Neuron *100*, this issue, 1401–1413.

Iggo, A., and Muir, A.R. (1969). The structure and function of a slowly adapting touch corpuscle in hairy skin. J. Physiol. *200*, 763–796.

Ikeda, R., and Gu, J.G. (2014). Piezo2 channel conductance and localization domains in Merkel cells of rat whisker hair follicles. Neurosci. Lett. *583*, 210–215.

Maksimovic, S., Nakatani, M., Baba, Y., Nelson, A.M., Marshall, K.L., Wellnitz, S.A., Firozi, P., Woo, S.-H., Ranade, S., Patapoutian, A., and Lumpkin, E.A. (2014). Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. Nature *509*, 617–621.

Wang, F., Knutson, K., Alcaino, C., Linden, D.R., Gibbons, S.J., Kashyap, P., Grover, M., Oeckler, R., Gottlieb, P.A., Li, H.J., et al. (2017). Mechanosensitive ion channel Piezo2 is important for enterochromaffin cell response to mechanical forces. J. Physiol. *595*, 79–91.

Woo, S.-H., Ranade, S., Weyer, A.D., Dubin, A.E., Baba, Y., Qiu, Z., Petrus, M., Miyamoto, T., Reddy, K., Lumpkin, E.A., et al. (2014). Piezo2 is required for Merkel-cell mechanotransduction. Nature *509*, 622–626.

Yalcin, I., Choucair-Jaafar, N., Benbouzid, M., Tessier, L.-H., Muller, A., Hein, L., Freund-Mercier, M.-J., and Barrot, M. (2009). β(2)-adrenoceptors are critical for antidepressant treatment of neuropathic pain. Ann. Neurol. *65*, 218–225.