Linsalata, A.E., He, F., Malik, A.M., Glineburg, M.R., Green, K.M., Natla, S., Flores, B.N., Krans, A., Archbold, H.C., Fedak, S.J., et al. (2019). DDX3X and specific initiation factors modulate FMR1 repeat-associated non-AUG-initiated translation. EMBO Rep. *20*, e47498.

Snijders Blok, L., Madsen, E., Juusola, J., Gilissen, C., Baralle, D., Reijnders, M.R.F., Venselaar, H., Helsmoortel, C., Cho, M.T., Hoischen, A., et al.; DDD Study (2015). Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Effects on Wnt Signaling. Am. J. Hum. Genet. *97*, 343–352.

Tabet, R., Schaeffer, L., Freyermuth, F., Jambeau, M., Workman, M., Lee, C.-Z., Lin, C.-C., Jiang, J., Jansen-West, K., Abou-Hamdan, H., et al. (2018). CUG initiation and frameshifting enable production of dipeptide repeat proteins from ALS/FTD C9ORF72 transcripts. Nat. Commun. *9*, 152.

Yamada, S.B., Gendron, T.F., Niccoli, T., Genuth, N.R., Grosely, R., Shi, Y., Glaria, I., Kramer, N.J., Nakayama, L., Fang, S., et al. (2019). RPS25 is required for efficient RAN translation of C9orf72 and other neurodegenerative disease-associated nucleotide repeats. Nat. Neurosci. *22*, 1383–1388.

Signal Amplification in *Drosophila* Olfactory Receptor Neurons

Byoung Soo Kim[1] and Greg S.B. Suh[1,2,*]
[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea
[2]Skirball Institute of Biomolecular Medicine, Department of Cell Biology, Neuroscience Institute, New York University School of Medicine, New York, NY, USA
*Correspondence: seongbaesuh@kaist.ac.kr
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Olfactory receptor neurons (ORNs) transform scant chemical inputs into significant neural signals. This transformation requires signal amplification. In this issue of *Neuron*, Ng et al. (2019) identified a mechanism by which the signals evoked by pheromones are amplified in the ORNs that selectively promote courtship behavior in *Drosophila*.

In non-primate males, sensing pheromones by chemosensory organs is the essential first step in eliciting sexual arousal and attraction. The sensitivity of chemosensory organs to pheromones is markedly enhanced in older *Drosophila* males, matching periods of increased reproductive maturity (Lin et al., 2016). This sensory enhancement would be also important because a scant quantity of pheromones is released from females. Pheromones emitted by *Drosophila* females are primarily detected by selected populations of olfactory receptor neurons (ORNs) in males, which transmit the converted chemical information to the higher brain centers. It is unclear, however, where and how the enhancement of the sensory signal occurs and how the age of male flies contributes to the signal amplification.

In vertebrates, odorant receptors expressed in chemosensory olfactory neurons are G-protein-coupled receptors (GPCRs) (Buck, 1996). Upon odorant binding, the GPCRs activate the G-protein-coupled transduction cascade and cyclic AMP synthesis that promote the opening of cyclic nucleotide-gated (CNG) channels, thereby stimulating the activity of ORNs. Through the signal transduction cascade, the response to ligands is amplified, and the influx of cations, including calcium, into the cytoplasm of ORNs is elevated, thus generating more substantive neurotransmission. On the other hand, olfactory receptors in *Drosophila* comprise three families: the 7-transmembrane odorant receptors (ORs), gustatory receptors (GRs) (Vosshall and Stocker, 2007), and the P-loop-containing ionotropic glutamate receptor (IRs) (Benton et al., 2009).

While the vertebrate chemosensory receptor system amplifies the signal through the GPCR transduction cascade, it is unclear whether the chemosensory input is amplified in *Drosophila* sensory neurons. The *Drosophila* 7-transmembrane ORs were reported as a ligand-gated ion channel that is activated directly by environmental odorants (Sato et al., 2008; Wicher et al., 2008) but may also promote signal amplification through the G-protein signaling cascade and CNG channels (Wicher et al., 2008). Another study suggested, however, that ORs function strictly as an ion channel and that the synthesis of second messengers from G-protein signaling may not be involved (Sato et al., 2008). The structurally related GRs have been shown to form a ligand-gated ion channel in another insect species (Sato et al., 2011). The P-loop-containing IRs also form a ligand-gated ion channel that confers depolarization in ORNs upon its direct binding to odorants (Benton et al., 2009). The insect chemosensory receptors that are ligand-gated ion channels and may not be coupled to the G-protein cascade have a limited role in augmenting the negligible ligand-evoked signal into substantive neurotransmission. How the sensory signal initiated by the ion channel is amplified in the insect sensory neurons has been a mystery. In this issue of

Neuron, Ng et al. (2019) shed light on this outstanding question by providing a mechanism by which the signal is amplified upon activation of ORs or IRs in *Drosophila* olfactory system.

Ng et al. (2019) focused on the courtship-promoting ORNs. Or47b+ ORNs mediates the detection of the aphrodisiac pheromone that elicits courtship behavior (Lin et al., 2016). Or47b+ ORNs become more sensitive to the pheromone in older males who hold advantages in copulation over younger males (Lin et al., 2016). Another courtship-promoting ORN they examined was the IR84a+ ORN, which mediates the detection of food odorants that also stimulate courtship behavior (Grosjean et al., 2011). Intriguingly, the glomeruli in the antennal lobe (AL) innervated by axonal projections of Or47b+ and Ir84a+ ORNs are located next to each other. Interestingly, the second-order projection neurons (PNs) that are synaptically coupled to these ORNs innervate the region that is highly overlapped with the pheromone-processing area in a higher brain center (Grosjean et al., 2011). Both populations of these ORNs express the channel called Pick-pocket 25 (PPK25) (Starostina et al., 2012). PPK25 channel is a member of the degenerin/epithelial sodium channel family (DEG/ENaC), which is highly conserved from *C. elegans* to humans. Ng et al. (2019) hypothesized that the expression of *ppk25* in these populations of ORNs expressing *Or47b* and *IR84a* may contribute to the pheromone detection and courtship behavior. But how? A stroke of genius emerged from an idea that PPK25 channel might be involved in signal amplification in these pheromone-detecting olfactory neurons of older males.

First, Ng et al. (2019) investigated whether PPK25 is important for mediating the function of Or47b receptor in these neurons by carrying out single sensillum recordings of Or47b+ ORNs. They observed changes in spike frequency in response to the application of palmitoleic acid, which is associated with the aphrodisiac pheromone (Lin et al., 2016). The authors knew that the stimulated activity of OR47b+ ORNs was further magnified when flies became older (Lin et al., 2016). By contrast, older *ppk25* mutant males did not exhibit the magnified response. Ng et al. (2019) next sought to determine whether *ppk25* is required in Or47b+ ORNs for mediating courtship behavior. A knockdown of *ppk25* in Or47b+ ORNs resulted in an impairment in the courtship competition and a delay in the copulation initiation time. To determine a possible mechanism by which *ppk25* contributes to the courtship behavior displayed more intensely by older males, the authors asked whether the expression levels of *ppk25* might be altered in older versus younger males. Using qRT-PCR, they demonstrated that mRNA levels of *ppk25* in 7-day-old males were significantly higher than those in 2-day-old males. Ng et al. (2019) next sought to identify the factor that contributes to the increase in *ppk25* expression in Or47b+ ORNs. In the previous study, a prolonged exposure of young males to juvenile hormone-like compound, methoprene, was shown to enhance the odor-evoked activity of Or47b+ neurons (Lin et al., 2016). Indeed, the authors observed that the treatment of young male flies with methoprene led to a significant increase in *ppk25* expression (Ng et al., 2019).

How would the PPK25 channel be able to amplify the neural signal in Or47b+ neurons? Ng et al. (2019) gained an insight into this by studying the structure of PPK25. The DEG/ENaC channel family to which PPK25 belongs is a two-transmembrane channel that contains an extracellular loop and two cytoplasmic domains. The authors found that the sequence in the N-terminal intracellular domain of PPK25 contains a calmodulin-binding motif (CBM). To determine whether the CBM in PPK25 channel is important for signal amplification, they introduced mutations in the CBM and measured the response to palmitoleic acid in Or47b+ ORNs of flies harboring the mutations. Ng et al. (2019) found through single-sensillum recordings of Or47b+ ORNs that the mutations in CBM, but not in other residues in *ppk25*, resulted in attenuated responses to the odor. This suggests that the signal amplification in Or47b+ ORNs requires the direct interaction between calcium ion and the CBM in PPK25 channel. Another population of olfactory neurons that the authors investigated was Ir84a+ ORNs, which promote courtship and also express PPK25 in these neurons (Starostina et al., 2012). Similar to Or47b+ ORNs, the neural signal was amplified in Ir84a+ ORNs. This signal amplification was also dependent on the functional CBM in PPK25 channel. While these ORNs express two different types of olfactory receptors (ORs and IRs), the mechanism by which the signal is amplified appears identical (Ng et al., 2019).

Finally, Ng et al. (2019) asked whether *ppk25* is required in other *ppk25*-expressing neurons for signal amplification. The authors found that *ppk25* is also expressed in gustatory receptor neurons (GRNs) located in the foreleg that respond to the aphrodisiac pheromone, thereby stimulating the courtship behavior in male flies (Starostina et al., 2012). Recordings of tarsal sensilla that contain PPK25+ neurons in older males demonstrated a discrete neuronal response to a pheromone, 7,11-heptacosadiene, but the mutations in the CBM of *ppk25* blunted the amplified response, similar to the results from olfactory Or47b+ and IR84a+ neurons (Ng et al., 2019). Together, PPK25 appears to mediate a more generalized role in signal amplification regardless of the sensory neuron types expressing ORs, GRs, or IRs.

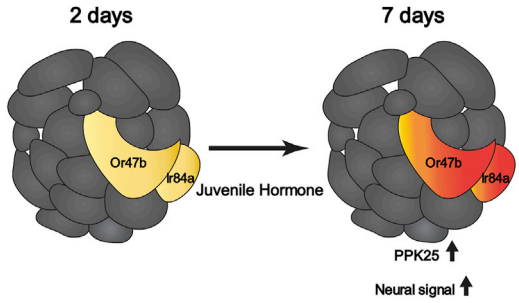

Figure 1. PPK25 Mediates Signal Amplification in Both Or47b+ and Ir84a+ ORNs

Expression levels of *ppk25* in the glomeruli in the antennal lobe innervated by Or47b+ and Ir84a+ ORNs are higher in 7-day-old male than in 2-day-old male *Drosophila*. Juvenile hormone leads to the elevation in *ppk25* expression, which results in signal amplification in these ORNs.

The study by Ng et al. (2019) revealed a mechanism by which the signal elicited by pheromone is amplified in the ORNs that promote courtship behavior (see Figure 1). The mechanism involves PPK25, a member of DEG/ENaC channels that plays a key role in signal amplification and transduction. The CBM in PPK25 channel directly interacts with calmodulin during the rise of the intracellular calcium level, which is spurred by the activation of a ligand-gated OR, eventually generating signal amplification. Interestingly, the CBM is also found in other species, including mouse and human, and the DEG/ENaC channels are expressed in different types of sensory neurons that mediate mechanosensation, baro-reception, proprioception, nociception, and taste reception. This work will provide a foundation for understanding signal amplification in other neuron types.

REFERENCES

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell *136*, 149–162.

Buck, L.B. (1996). Information coding in the vertebrate olfactory system. Annu. Rev. Neurosci. *19*, 517–544.

Grosjean, Y., Rytz, R., Farine, J.P., Abuin, L., Cortot, J., Jefferis, G.S., and Benton, R. (2011). An olfactory receptor for food-derived odours promotes male courtship in Drosophila. Nature *478*, 236–240.

Lin, H.H., Cao, D.S., Sethi, S., Zeng, Z., Chin, J.S.R., Chakraborty, T.S., Shepherd, A.K., Nguyen, C.A., Yew, J.Y., Su, C.Y., and Wang, J.W. (2016). Hormonal Modulation of Pheromone Detection Enhances Male Courtship Success. Neuron *90*, 1272–1285.

Ng, R., Salem, S.S., Wu, S.T., Wu, M., Lin, H.H., Shepherd, A.K., Joiner, W.J., Wang, J.W., and Su, C.Y. (2019). Amplification of Drosophila Olfactory Responses by a DEG/ENaC Channel. Neuron *104*, this issue, 947–959.

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., and Touhara, K. (2008). Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature *452*, 1002–1006.

Sato, K., Tanaka, K., and Touhara, K. (2011). Sugar-regulated cation channel formed by an insect gustatory receptor. Proc. Natl. Acad. Sci. USA *108*, 11680–11685.

Starostina, E., Liu, T., Vijayan, V., Zheng, Z., Siwicki, K.K., and Pikielny, C.W. (2012). A Drosophila DEG/ENaC subunit functions specifically in gustatory neurons required for male courtship behavior. J. Neurosci. *32*, 4665–4674.

Vosshall, L.B., and Stocker, R.F. (2007). Molecular architecture of smell and taste in Drosophila. Annu. Rev. Neurosci. *30*, 505–533.

Wicher, D., Schäfer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature *452*, 1007–1011.

TREK for High-Speed and High-Frequency Conduction through the Axon

Pablo Ávalos Prado[1,2] and Guillaume Sandoz[1,2,*]
[1]Université Cote d'Azur, CNRS, INSERM, iBV, Nice, France
[2]Laboratories of Excellence, Ion Channel Science and Therapeutics, Nice, France
*Correspondence: sandoz@unice.fr
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In this issue of *Neuron*, Kanda et al. (2019) find that the two-pore domain potassium channels TRAAK and TREK1 drive axonal action potential repolarization for high-speed and high-frequency nervous impulses in mammalian myelinated nerves.

An action potential (AP) corresponds to a fast modification of the membrane potential, which rapidly rises and then falls, driven in the soma by the sequential activation of the voltage-dependent Na^+ and the voltage-dependent K^+ channels. In neurons, the main function of the AP is cell-to-cell communication by its propagation along the axon toward synaptic boutons. In non-myelinated axon, the AP travels by regenerating itself (Hodgkin, 1937). The AP induces a sufficient depolarization of the neighbor region inducing the activation of the voltage-dependent Na^+ channels allowing AP regeneration. This AP propagation has a maximum speed of ~1 m/s. Fast AP propagation requires myelin sheaths. Myelin is a multilamellar membrane that enwraps the axon in segments separated by intervals known as nodes of Ranvier (NRs). Myelin presents a high electrical resistance that prevents the AP conduction along the myelinated segments. However, the current carried at near distance, through the axon cable properties, generates a sufficient depolarization at subsequent NRs allowing AP regeneration. This propagation, known as saltatory conduction, allows a propagation of the AP over 100 m/s.

Since Hodgkin and Huxley established the first ionic conductances of the AP in giant squid axon, AP generation and transmission in the nervous system has been a subject of continuous study. Nevertheless, 80 years after their experiments, the molecular identity of the channels underlying AP propagation and regeneration at the NRs in mammals is still a matter of conjecture. Whereas some research suggests that