Notes on Honeybee Sensory Neurobiology

Logan Thrasher Collins

Olfaction

Antennal lobes

- Honeybee antennal lobes (ALs) are composed of about 160 regions called glomeruli in which olfactory receptor neurons from the antennae make synapses on projection neuron cell bodies as well as inhibitory local neurons.
- The projection neurons send cholinergic axons to the mushroom bodies and to the lateral horn (LH) while the GABAergic local neurons facilitate olfactory computations within the antennal lobes.

Mushroom bodies

- The mushroom bodies are paired structures located on either side of the central brain (CB). They are known to facilitate higher sensory integration as well as associative learning processes.
- In honeybees, the mushroom bodies use cup-shaped medial calyces (MCAs) and lateral calyces (LCAs) as their major sensory input regions while using the pedunculi (PEDs) as their major sensory output regions.

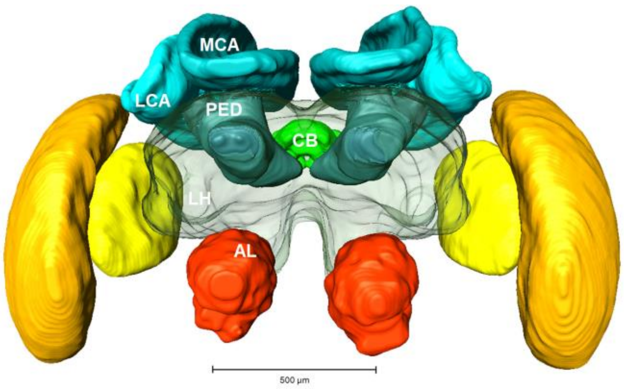
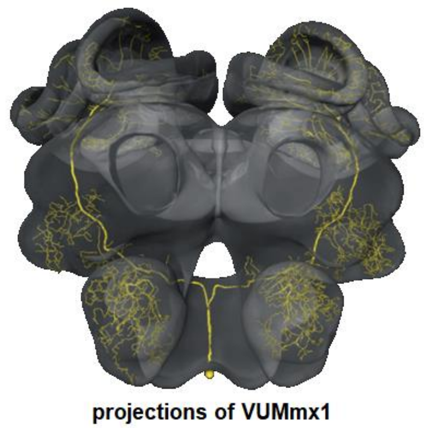


image modified from (Steijven, Spaethe, Steffan-Dewenter, & Härtel, 2017)

- The calyces contain Kenyon cells which receive cholinergic axons from the projection neurons of the antennal lobes and the pedunculi contain the efferent axons of the Kenyon cells.

Associative olfactory learning

- Honeybee associative olfactory learning can occur where the olfactory pathway converges with other pathways.
- Specific odors can serve as conditioned stimuli when they are associated with unconditioned stimuli of appetitive or aversive character.
- Experimental evidence shows that the VUMmx1 neuron is sufficient for olfactory reward learning in bees. Its cell body is located within a region called the subesophageal ganglion and it synapses upon cells in the calyces, the lateral horn, and the antennal lobe.



projections of VUMmx1

image modified from (R. Menzel, 2012)

Vision

Honeybee eyes

- Honeybees possess two frontal compound eyes and three ocelli (simple eyes) located on the top of the head.
- The retinas of honeybee compound eyes are composed of ommatidia, each with nine photoreceptor cells. The types of bee photoreceptor cells include S, M, and L photoreceptors corresponding to UV, blue, and green wavelengths respectively.
- Ocellar retinas are composed of rod cells (note that they do not have ommatidia) and are covered by a lens. However, the focal plane of this lens is behind the actual retina, leading to much lower resolving power than that of the compound eyes. Although the function of ocelli is not entirely understood, they may operate as widefield detectors of illumination changes. In addition, ocellar retinas can be subdivided into dorsal and ventral regions which view the horizon and the sky respectively. Distinct neuronal pathways are associated with these subdivisions.

Optic lobe

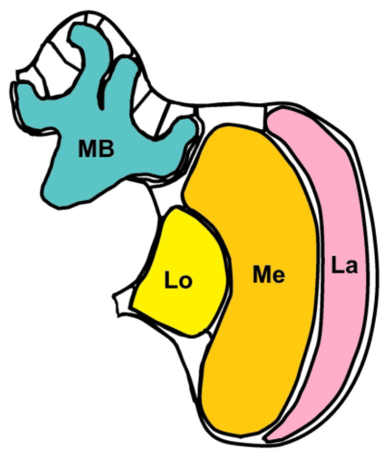


image modified from (Kiya & Kubo, 2011)

- Honeybee vision (associated with the compound eyes) starts with the optic lobe's three regions; the lamina (La), medulla (Me), and lobula (Lo).
- The lamina is positioned directly under the compound eye's photoreceptors. It receives inputs mainly from the L photoreceptors, which are involved in the achromatic pathway and exhibit fast response times. However, some very rough color processing may still occur in the lamina.
- In the medulla, neurons are organized in a columnar retinotopic fashion with eight layers. The columns also possess horizontal connections (unlike the lamina) which likely facilitate color opponency. The medulla's outer layers contain neurons that respond to specific wavelengths and neurons that respond to a broad range of wavelengths while the medulla's inner layers contain color-opponent neurons that compare colors at center and surround regions of receptive fields.
- The lobula consists of six layers. Its outer layers (1-4) are part of the achromatic pathway and exhibit motion sensitivity. Its inner layers (5-6) continue the color processing pathway. Some projections from the inner layers go to the mushroom bodies, possibly facilitating sensory crosstalk and learning.
- Beyond the optic lobe, further visual processing of the achromatic and color pathways occurs in the protocerebrum and central brain.

Audition and antennal somatosensation

Johnston's organ

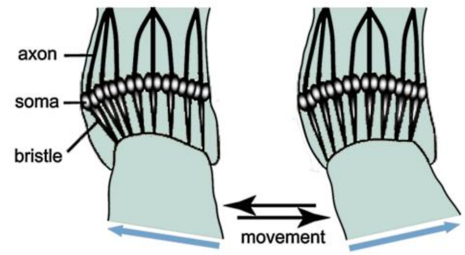
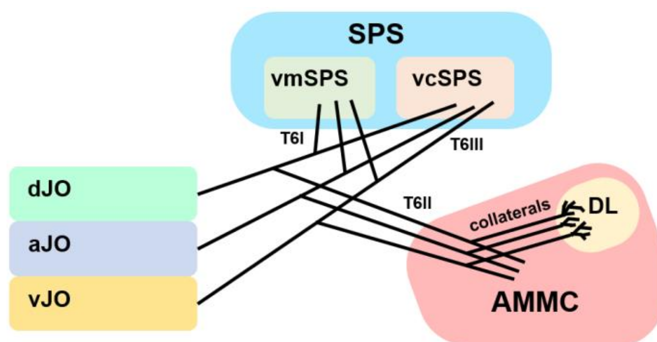


image modified from (Galizia, Eisenhardt, & Giurfa, 2011)

- Honeybees use Johnston's organ as their sensory organ for audition. In bees, audition also acts as a form of somatosensation. Johnston's organ is located on the antennae. It detects vibrations during the waggle dance and air currents during flight.
- Johnston's organ contains about 240 scolopidia, mechanosensory complexes which include bristles that deform and trigger action potentials along efferent axons.
- The soma of neurons within Johnston's organ are divided into dorsal (dJO), ventral (vJO), and anterior groups (aJO).

Projections from Johnston's organ

- The main axons from the soma within Johnston's organ trifurcate into the fascicles called T6I, T6II, and T6III. The T6I axons terminate at the ventro-medial superior posterior slope (vmSPS), the T6II axons terminate at the antennal mechanosensory and motor center (AMMC), and the T6III axons terminate at the ventro-central superior posterior slope (vcSPS).
- In the vmSPS, the axons show some degree of somatotopy arising from the dorsal, ventral, and anterior Johnston's organ regions. Somatotopy is not observed in the AMMC or vcSPS.
- All the sensory axons from Johnston's organ also send small collateral branches to the bee's dorsal lobe (DL).



original image

The AMMC

- The AMMC contains two classes of interneuron, AMMC-Int-1 and AMMC-Int-2.

- AMMC-Int-1 neurons have somas located in the honeybee’s primary auditory center, which is near the central brain. They densely arborize at the AMMC and thinly arborize in the ventral protocerebrum (the protocerebrum is a region of the insect brain that includes the mushroom bodies and central brain as well as several other structures). Their dense arborization in the AMMC runs close to the T6 collaterals at the dorsal lobe.
- AMMC-Int-1 neurons demonstrate spontaneous spiking without sensory input. During exposure to a vibratory stimulus, the spike rate slows slightly. After the stimulus is removed, the spike rate increases to a higher rate than that of the spontaneous spiking, but eventually returns to the basal rate. However, it should be noted that olfactory stimuli and other modulating factors can drastically alter the response properties of AMMC-Int-1 neurons.

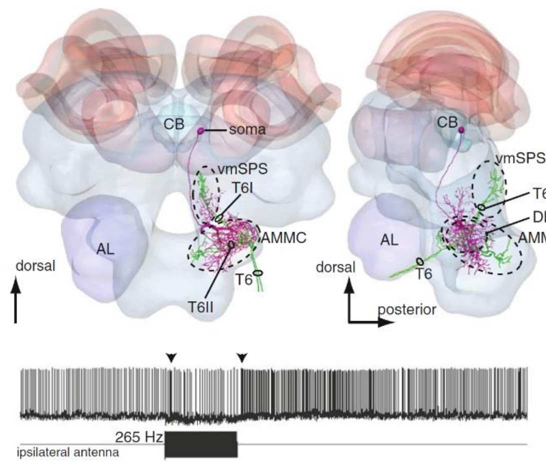


image modified from (Galizia, Eisenhardt, & Giurfa, 2011)

- AMMC-Int-2 neurons have somas located in the dorsal lobe. Their dendrites split into three main branches called x, y, and z. Branch y is the axon while branches x and z are dendritic. It sends a long process to the lateral protocerebrum (LP) and makes synapses. The x arborization represents the densest of the three branches and is located in the AMMC. Branch z passes through the dorsal lobe and into the lateral superior posterior slope (lateral SPS).
- AMMC-Int-2 neurons respond to relatively high vibratory amplitudes, especially those which cause 30 µm (or greater) shifts in antennal position. Their sensitivity reaches a maximum at 265 Hz (a frequency that occurs during the waggle dance), though they also respond to other frequencies.

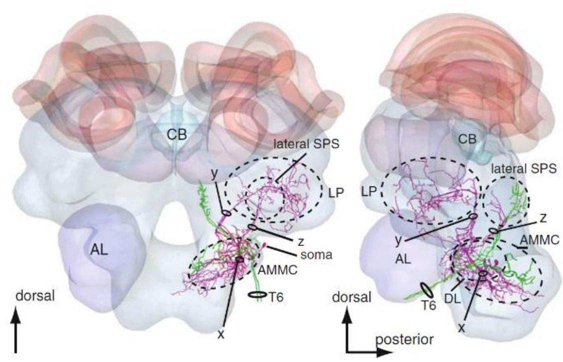


image modified from (Galizia, Eisenhardt, & Giurfa, 2011)

The SPS

- The SPS contains an interneuron known as SPS-D-1 which projects to the ipsilateral and contralateral SPS.
- SPS-D-1 does not respond to 265 Hz alone. However, it responds to long-lasting 265 Hz vibratory stimulation with simultaneous olfactory stimulation at the contralateral antenna.

Gustation

Gustatory sensilla

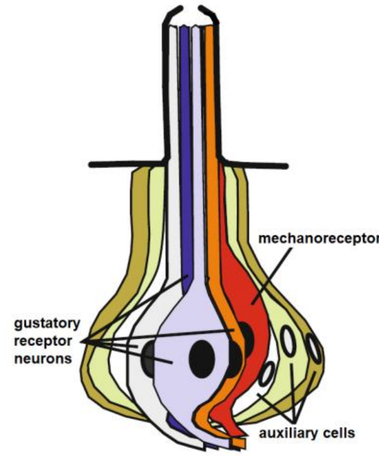


image modified from (Galizia, Eisenhardt, & Giurfa, 2011)

- Gustatory receptor cells are found in sensilla, structures which resemble hairs or pegs. Sensilla are located on the glossa, antennae, labial palps, and several other parts of the bee's body.
- Each sensillum contains 3-5 gustatory receptor neurons that send dendrites up the shaft towards a pore at the sensillum's tip. The somas of the receptor cells (along with a mechanoreceptor cell) are encapsulated by auxiliary cells and bathed in a receptor hemolymph. The gustatory receptor neurons likely use GPCRs to detect various food molecules while the mechanoreceptor facilitates evaluation of the food's position and density.
- Antennal sensilla respond in a dose-dependent and highly sensitive manner to sucrose solutions. In addition, antennal sensilla respond to aqueous NaCl. As the antennal sensilla do not respond to very low concentrations of KCl, they probably do not contain a receptor that responds to water alone (unlike in many other insects). Sensilla on the mouthparts respond to aqueous sucrose, glucose, fructose, LiCl, KCl, and NaCl. They do not respond to $CaCl_2$ or $MgCl_2$. Foreleg sensilla respond to sucrose as well as very low concentrations of KCl, suggesting that these sensilla may contain a receptor that responds to water alone (unlike the bee's other sensilla).

Honeybee central gustatory processing

- Honeybee central gustatory processing takes place primarily in their subesophageal ganglion (SEG). Axons of gustatory neurons and the mechanosensory neurons found in the sensilla project to the SEG's mandibular, maxillary, and labial neuromeres via the mandibular, maxillary, and labial nerves respectively.
- As mentioned, the SEG contains the VUMmx1 neuron, which facilitates pairing of olfactory and gustatory stimuli for reward learning. Other VUM neurons have been identified in the SEG, but their function remains unclear.
- Beyond the SEG, other neurons might be involved in the honeybee's gustatory processing. In the mushroom bodies, the PE1 neuron exhibits increased spiking in response to sucrose gustation. However, PE1 also responds to mechanical and olfactory inputs. Also located in the mushroom bodies are cells dubbed as "feedback neurons" which respond to odors and sucrose as well. In these cases, multisensory integration likely occurs.

References

Dyer, A. G., Paulk, A. C., & Reser, D. H. (2011). Colour processing in complex environments: insights from the visual system of bees. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1707), 952 LP-959. Retrieved from http://rspb.royalsocietypublishing.org/content/278/1707/952.abstract

Galizia, C. G., Eisenhardt, D., & Giurfa, M. (2011). *Honeybee Neurobiology and Behavior: A Tribute to Randolf Menzel*. Springer Netherlands.

Heisenberg, M. (2003). Mushroom body memoir: from maps to models. *Nature Reviews Neuroscience*, *4*, 266. Retrieved from https://doi.org/10.1038/nrn1074

Hung, Y.-S., & Ibbotson, M. (2014). Ocellar structure and neural innervation in the honeybee. *Frontiers in Neuroanatomy*. Retrieved from https://www.frontiersin.org/article/10.3389/fnana.2014.00006

Kiya, T., & Kubo, T. (2011). Dance Type and Flight Parameters Are Associated with Different Mushroom Body Neural Activities in Worker Honeybee Brains. *PLOS ONE*, *6*(4), e19301. Retrieved from https://doi.org/10.1371/journal.pone.0019301

Menzel, R. (2012). The honeybee as a model for understanding the basis of cognition. *Nature Reviews Neuroscience*, *13*, 758. Retrieved from http://dx.doi.org/10.1038/nrn3357

Mota, T., Yamagata, N., Giurfa, M., Gronenberg, W., & Sandoz, J.-C. (2011). Neural Organization and Visual Processing in the Anterior Optic Tubercle of the Honeybee Brain. *The Journal of Neuroscience*, *31*(32), 11443 LP-11456. Retrieved from http://www.jneurosci.org/content/31/32/11443.abstract

Sandoz, J.-C. (2013). Chapter 30 - Neural Correlates of Olfactory Learning in the Primary Olfactory Center of the Honeybee Brain: The Antennal Lobe. In R. Menzel & P. R. B. T.-H. of B. N. Benjamin (Eds.), *Invertebrate Learning and Memory* (Vol. 22, pp. 416–432). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-415823-8.00030-7

Steijven, K., Spaethe, J., Steffan-Dewenter, I., & Härtel, S. (2017). Learning performance and brain structure of artificially-reared honey bees fed with different quantities of food. *PeerJ*, *5*, e3858. https://doi.org/10.7717/peerj.3858