The Insect Olfactory System

1 Introduction

The main dataset at our disposal comes from the locust *Schistocerca americana*. It is available on zenodo. These raw data are a mixture of activites from several neurons and must be processed in order to get the spike trains from individual neurons; that's what Spike Sorting is about. The sorting of the raw data is fully document on a GitHub repository.

2 The insect olfactory system

2.1 Some jargon

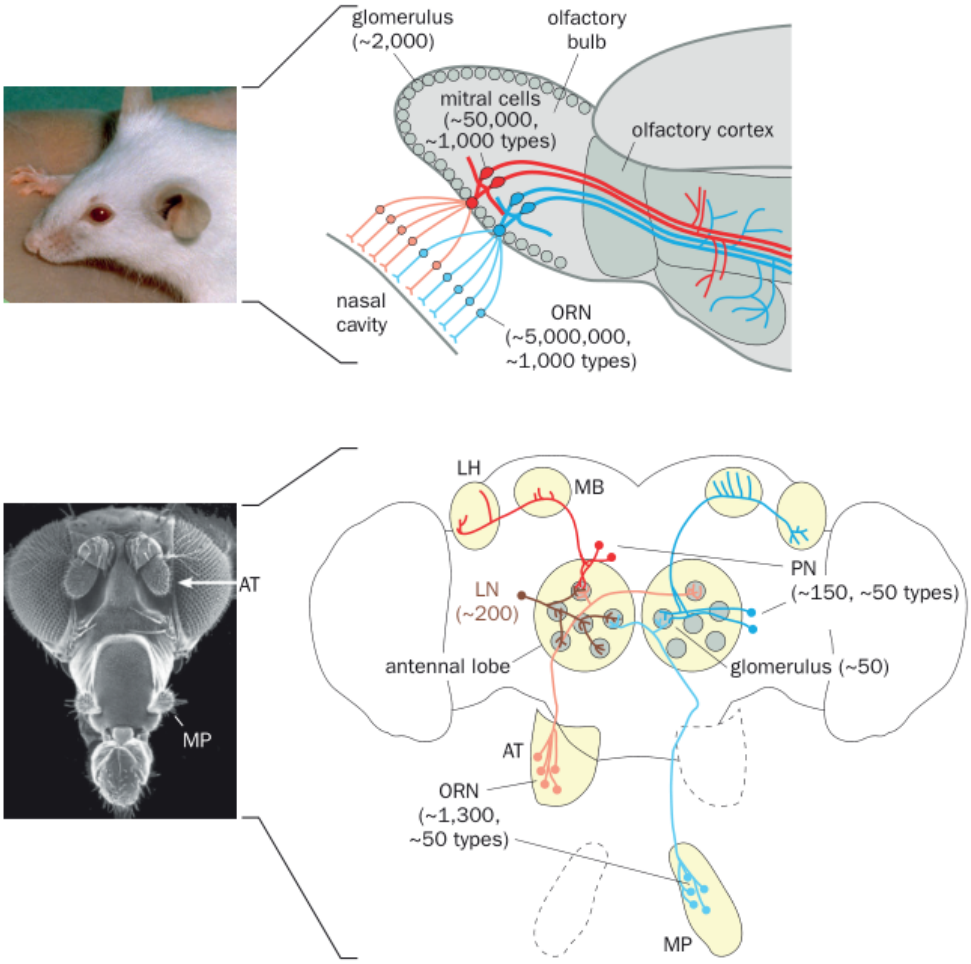
If you want more details on what follows as well as a general reference on neurobiology, I recommend the book of Luo (2020).

Anatomists who were the first to study nervous systems introduced a terminology that is still in use today, although most neuroscientists are not aware of its origin and real meaning. After identifying "brain regions" based on "large scale features" (1 mm is a large scale in that context), the anatomists saw that the *processes* or *neurites*—that is the dendrites and axon—, of some neurons are entirely contained in a given region, while the axon of other neurons make links *between* regions. They introduced the terms *local neuron* or *interneuron* for the first and *principal neuron* or *projection neuron* for the second. Later on, when physiologists developed techniques to record neuronal activity it became clear that neurons come physiologically (or functionally) in two flavors—this is referred to as Dale's principle—: some neurons tend to *increase* the spiking activity of their post-synaptic partners, they are called *excitatory* neurons; while other neurons tend to *decrease* the spiking activity of their post-synaptic partners, they are called *inhibitory* neurons. It turns out that the *anatomical* class of local neurons / interneurons is almost identical with the *functional* class of inhibitory neurons, while the anatomical class of principal neuron / projection neuron is almost identical with the functional class of excitatory neurons—a classical counter example is the principal neuron of the cerebellar cortex, the *Purkinje cell*, that is inhibitory—. Later on, the physiology of the synapse came to be described and "understood". Most synapses are chemical synapses, meaning that when the pre-synaptic action potentials invades the pre-synaptic terminal, it provokes the release of packets (or *quanta*) of a specific molecule, the *neurotrasmitter*. This molecule diffuses in the *synaptic*

cleft and binds to specific postsynaptic receptors leading to a change in the post-synaptic membrane potential. The neurotransmitter is specific to the functional type (excitatory or inhibitory) of the neuron. In both invertebrates (like insects) and vertebrates (like us) the main inhibitory transmitter is γ-Aminobutyric acid (GABA). In the central nervous system (CNS) of *vertebrates*, the main excitatory transmitter is glutamate, while it is acetylcholine in *invertebrates*. We now have techniques based on staining with antibodies directed against the enzymes synthesizing the different transmitters, that allow us to assign a function (excitatory / inhibitory) to neurons without doing physiological recordings.

2.2 Insect and vertebrate olfactory systems

Odors are first "detected" by receptor proteins in the membrane of *olfactory receptor neurons* (ORN). These ORN are located on the antennea (and maxillary palps) of insects and in the nose (the part called the *olfactory epithelium*) of vertebrates. The ORN axons enter the brain (CNS) in the first olfactory relay: the *antennal lobe* (AL or *deutocerebrum*) in insects and the *olfactory bulb* in vertebrates. The ORN axons reach very specific regions, called *glomeruli*, within the first olfactory relay. These glomeruli (singular glomerulus) are little spheres where the synapses between the ORN axons and the olfactory relay neurons are found. Like in any other part of the brain both excitatory and inhibitory neurons are found in the first olfactory relay. In the insect, the excitatory neurons of the AL are called the *projection neurons* (PN), they are cholinergic (they release the transmitter of excitatory neurons, acetylcholine). In vertebrates two types of excitatory neurons are found: the *mitral cells* and the *tufted cells* (but it is common to "neglect" the latter since they can be viewed as "displaced" mitral cells). Both the PN of insects and the mitral/tufted cells of vertebrates are principal neurons: their axon leaves the first olfactory relay and convey information to higher relays: the *mushroom bodies* (MB) and *lateral horn* (LH) in insects and the *olfactory cortex* in vertebrates. The inhibitory neurons of the AL are called *local neurons* (LN), they exhibit a marked variability both in number and in types from one insect species to the next—a single inhibitory (gabaergic) non-spiking type in the locust *Schistocerca americana* and many types mostly inhibitory but some perhaps excitatory, both spiking and non-spiking in the cockroach *Periplaneta american* (Fuscà and Kloppenburg, 2021) and in the domestic bee *Apis mellifera* (Hammer, 1997)—. The inhibitory neurons of the vertebrate olfactory bulb come in two kinds: the *periglomerular cells* and the *granule cells*. Both are inhibitory and (mostly) non-spiking (Luo, 2020, p. 222). The following figure (ibid., Fig. 6-24) illustrates the key elements I just introduced and their spatial relation for a mouse at the top and an insect, the fly at the bottom:



An important property of olfactory systems, in vertebrates and, we think, in insects—for the latter we have "full knowledge" only for the fly *Drosophila megalonaster* and partial knowledge for many moth species—, is illustrated by the use of colors in the vertebrate (top) part of the figure. There are many receptor protein genes—really many in vertebrates like mice where they make up more than 4 % of the whole coding genome (Luo, 2020, p, 217)—, but each olfactory receptor neuron (ORN) expresses only *one receptor protein gene* (leading to a more or less narrow range of odorous molecules able of eliciting a response). A given receptor protein gene is expressed in many ORN (with a random expression pattern as far as we can tell), this is what the blue and red colors are illustrating in the figure. Then every ORN that expresses a given receptor protein gene *projects to the same glomerulus* in the first olfactory relay (or the same group of glomeruli, we think, when ORN axons send branches to many glomeruli like in locusts). Then, in (higher) vertebrates and in most insects species (cockroaches, bees, flies), the principal cells of the first olfactory relay send a single dendrite to a single glomerulus; *they are therefore collecting the activities of many ORN with the same response spectrum*. The case of the locust (discussed in the

next section) is less clear since ORN send branches to many glomeruli and PN also send dendrites to many glomeruli and we do not know if the many glomeruli from which a PN gets its inputs are all innervated by the same ORN type. In other words we do not know if the branching pattern of ORN axon matches the branching pattern of PN dendrites.

Hopefully, what I just wrote will make the long quotation of the next section understandable.

2.3 What was known on the locust olfatory system in 1996

The beginning of the `Box 1` text of Laurent (1996) summarizes what we know:

> Each antenna contains about 50 000 cholinergic and excitatory olfactory receptor neurons (ORNs). Each ORN sends an axon to the ipsilateral antennal lobe (AL), where it terminates in one, two or three of the 1000 glomeruli. A glomerulus thus contains the axonal terminals of 50–150 ORNs. There is good evidence in the pheromonal system of moths that receptor neurons of the same molecular sensitivity converge on to the same glomerulus. However, it is not known (in any insect) whether the non-pheromonal afferents that converge into any one glomerulus express the same olfactory receptor gene(s) (as yet unidentified), as has been demonstrated recently in the vertebrate olfactory bulb. The antennal lobe contains two main types of neuron: the local (LNs) and projection neurons (PNs). The local neurons (about 300 in each lobe) are axonless and send dense projections throughout the entire antennal lobe neuropil[1]. Locust LNs do not generate conventional Na^+ action potentials but rather TTX-resistant[2] (probably Ca^{2+}-dependent) spikelets of variable shapes and amplitudes, which can be recorded from the soma or the dendrites. Spikelets of different shapes and amplitude can often ride on one another, indicating that they can be generated independently at multiple sites within each dendritic tree. LNs are GABAergic and inhibitory. Immunogold histochemistry indicates that GABAergic profiles (presumably belonging to LNs) make output synapses on to both GABAergic and non-GABAergic profiles, and that they receive synaptic inputs from both GABAergic and non-GABAergic profiles. Therefore, LNs probably receive direct synaptic input from ORNs, PNs and from other LNs, and synapse on to PNs, LNs and possibly also on ORN terminals, as supported by data from other insects. The projection neurons (about 830 in each lobe) each arborize in 10–20 glomeruli, and thus resemble the mitral cells of lower vertebrates rather than of mammals.

[1] The neuropil is the region where synapses are located.

[2] TTX, short for Tetrodotoxin, is a molecule (and a poison) that blocks very potently and very specifically the Na^+ channels responsible for the upstroke of the action potential.

References

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