

Tyramine modulates electrical
properties of *Drosophila* olfactory
sensilla

People who contributed to this work:

Zainulabeuddin Syed measured the single sensillum recordings and did the spike sorting.

Daniela Pelz did the preparation for the antennal imaging and assisted with the analysis.

All other work was done by me.

Introduction

Biogenic Amines

Biogenic amines (BAs) are known as neurotransmitters, neuromodulators and neurohormones for vertebrates and invertebrates. They regulate a variety of biological processes such as endocrine secretion, circadian rhythms, the cardiovascular system, emotions, learning and memory. In vertebrates many of them are thought to play a role in CNS disorders like depression, attention deficit syndrome (Natynczuk *et al.*, 1989), schizophrenia, Parkinson syndrome (Branchek and Blackburn, 2003) and their receptors are potential targets for synthetic drugs of abuse (Miller *et al.*, 2005). Among the most important biogenic amines of insects are octopamine, tyramine, dopamine, serotonin and histamine that are further described below.

Octopamine

Octopamine (OA) was first identified 50 years ago in octopus, and is structurally related to noradrenalin. It is found in neuronal as well as in non-neuronal tissues of most insects. OA modulates properties of peripheral organs, sense organs as well as processes in the CNS, as desensitization, learning and memory or the “mood” (Nicolson and Isaacson, 1987; Beyenbach, 1984; Scheiner *et al.*, 2002). It is involved in the regulation of photoperiod, behavior and stress (David and Coulon, 1985; Evans, 1985).

OA plays a very important role in olfactory learning in insects as it was shown for honeybees and *Drosophila*. In honeybees it mediates olfactory reward conditioning by the octopaminergic VUMmx1 neuron (ventral unpaired median cell of maxillary neuromere1). VUMmx1 is localized in the subesophageal ganglion and innervates the antennal lobe, the lateral protocerebrum and the mushroom body calyces. Its depolarization can substitute the sugar reward in olfactory PER conditioning

(Hammer, 1993; (Hammer, 1997). Injection of OA into the mushroom body or the antennal lobe is also able to replace the sugar reward during olfactory learning (Hammer and Menzel, 1998). These experiments show that OA is important for the association of odorants with appetitive stimuli. In *Drosophila* it has been suggested that octopaminergic input into the mushroom body Kenyon cells is necessary to mediate appetitive olfactory conditioning, whereas dopaminergic input into the same cells is required for aversive conditioning (Schwaerzel *et al.*, 2003).

Tyramine

For a long time, tyramine (TA) was considered to be just a precursor molecule for octopamine without a neurological role with distinct effects. Meanwhile we know that many species (primates, fruit flies, mollusks, honeybees, moths and nematodes) have receptors specific for TA (Saudou *et al.*, 1990b;Gerhardt *et al.*, 1997a;Blenau *et al.*, 2000;Rex and Komuniecki, 2002;Ohta *et al.*, 2003;Miller *et al.*, 2005). It is believed that TA and OA in invertebrates can have antagonistic effects similar to adrenalin and noradrenalin in vertebrates, for example on the neuromuscular junction in *Drosophila* larvae (Nagaya *et al.*, 2002) and in foraging honeybees (Schulz and Robinson, 2001).

TA is known to be involved in cocaine sensitization (McClung and Hirsh, 1999) and in the regulation of hyperactivity in response to ethanol (Schwaerzel *et al.*, 2003). TA was shown to modulate transepithelial chloride conductance in the malpighian tubules of *Drosophila* in a calcium dependent manner (Blumenthal, 2003). It induces not only a higher transepithelial potential (TEP) but also slow oscillations in the TEP. These have also been found in the malpighian tubules of other insect species (Nicolson and Isaacson, 1987; Beyenbach, 1984) and most importantly in moth pheromone sensilla (Zack, 1979). TA also increases the gustatory and visual sensitivity (Scheiner, 2002; Erber not published, 2005).

Dopamine

Dopamine (DA) for example plays a role in development of sensory tissue (Neckameyer *et al.*, 2001), stimulates locomotion and grooming and leads to hyperactive behaviour in flies (Yellman *et al.*, 1997). Furthermore the protein release by salivary glands of cockroaches is regulated by serotonergic and dopaminergic neurons (Baumann *et al.*, 2004). DA receptor expression is found throughout the nervous system, in the brain and the periphery of *Drosophila* (Gotzes *et al.*, 1994;Feng *et al.*, 1996). One DA receptor in *Drosophila* (Ledent *et al.*, 1998) is preferentially expressed in mushroom bodies (Han *et al.*, 1996). A role in olfactory processing is suggested by the findings of DA receptor expression in the antenna and/or legs of *Drosophila* (Feng *et al.*, 1996). It has also been shown, that DA inhibits inward currents in isolated rat olfactory neurons (Okada *et al.*, 2003).

Serotonin

The functions of serotonin (5-HT = 5-hydroxytryptamine) are numerous and involve aggression in lobsters, feeding and learning in snails and nematodes, locomotion in lampreys, acquisition and retrieval of learned behaviors, as well as sleep, appetite and mood in mammals (Blenau and Baumann, 2001). 5-HT also plays a role in early development of *Drosophila* (Colas *et al.*, 1999). Most serotonergic neurons in insects are interneurons in the brain and the ventral cord with projections to most parts of the nervous system (Valles and White, 1988). In honeybees the receptor density is very high in the mushroom bodies and low in the antennal lobes (Page *et al.*, 1998).

Histamine

The first identified histamine (HA) receptors in invertebrates are two putative HA-gated chloride channels. One of them is localized to the lamina of the eye (Gisselmann *et al.*, 2002), where HA is the major neurotransmitter released from photoreceptors (Hardie, 1989). *Drosophila* mutants devoid of HA are viable but blind (Buchner *et al.*, 1993). HA is also expressed in a rather small number of neurons in

the brain that innervate several distinct neuropil regions (Nassel, 1999). In vertebrates HA plays a major role in allergic reactions and inflammation, but is also synthesized by some neurons in the brain.

Signal transduction by biogenic amines

Biogenic amines act both locally restricted as neuromodulator and neurotransmitter and systemic as neurohormone. Firstly they can be secreted by neurons into the synaptic cleft and secondly they can be distributed via hemolymph and blood respectively. They induce activation, inhibition or modification of proteins via specific metabotropic G-protein coupled receptors, or they directly regulate ion channels, in neurons as well as in non-neuronal cells.

Signal transduction takes place via three main mechanisms (Fig. 1). The seven-transmembrane-receptor is coupled to a trimeric G-protein. Depending on its identity the G protein stimulates the phospholipase C (PLC) to cleave phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (Adams *et al.*, 2000). IP₃ leads to Ca²⁺ release from the endoplasmatic reticulum into the cytoplasm, whereas DAG activates the calcium dependent protein kinase C (Bruch *et al.*, 1997). Alternatively the G protein activates or inhibits the adenylyl cyclase (AC) that catalyzes the conversion of ATP to cAMP. As the intracellular cAMP level increases, the protein kinase A (PKA) is activated and phosphorylates different target proteins.

By sequence analysis around 20 biogenic amine receptors were found in the genome of *Drosophila* (Table 1). Some of them have been characterized pharmacologically and molecularly (Arakawa *et al.*, 1990; Saudou *et al.*, 1990b; Kutsukake *et al.*, 2000; Han *et al.*, 1996; Reale *et al.*, 1997; Blenau and Baumann, 2001).

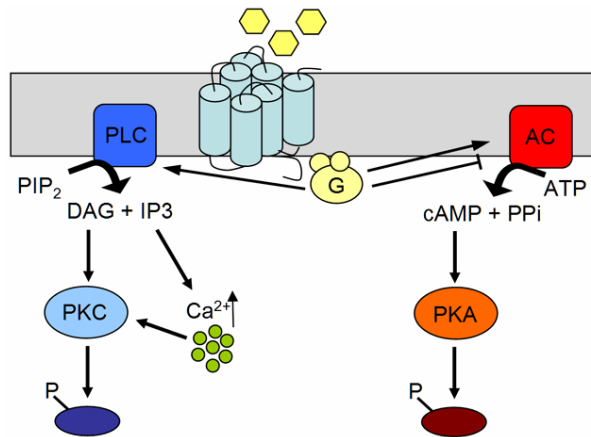


Figure 1: Signal transduction pathways of G-protein coupled receptors via activation of adenylyl cyclase leading to activation of PKA by cAMP and phosphorylation of target proteins, or inhibition of adenylyl cyclase reducing phosphorylation of proteins and via activation of PLC leading to activation of PKC and phosphorylation of proteins and additionally Ca^{2+} release into the cell.

Table 1: The biogenic amine receptors of *Drosophila* and their ligands.

Biogenic amine	Receptor	Biogenic amine	Receptor
Dopamine	DopR	Octopamine	Ocr1
	DopR2/DAMB		Oamb
	K13sc		CG16766
	DD2R		CG6989
Serotonin	5-HT1A	Unknown	CG12796
	5-HT1B		CG18314
	5-HT2		CG6919
	5-HT7		CG7078
	CG8007		CG7432
Tyramine	TyrR		CG7994

Some receptors were identified by cloning and named others were found by sequence analysis. The prefix CG (Computed Gene) followed by a number is used for genes identified during the annotation of the whole genome sequence.

Biosynthesis and inactivation of biogenic amines

BA synthesis takes place in the nervous system as well as in non-neuronal cells. They are synthesized by specific enzymes from amino acids (Fig. 2). For some of these enzymes mutant *Drosophila* strains or expression driver lines are available (Table 2).

- TA is produced from tyrosine by the enzyme tyrosine decarboxylase (Tdc). Two genes code for this enzyme, one of which is expressed in non-neuronal cells (*tdc1*), the other one in neurons (*tdc2*) (Cole *et al.*, 2005). *Tdc1* is expressed throughout the body, including the gut musculature, rectal papillae and malpighian tubules, *tdc2* is expressed in the brain and nerve cord and the female reproductive tract. Flies with a mutation in the *tdc2* gene lack neuronal tyramine and octopamine and are female sterile, whereas a null mutation in the *tdc1* gene is lethal (Cole *et al.*, 2005).
- Flies lacking tyramine β hydroxylase (T β H) fail to synthesize OA from TA and have a 10-fold increase in TA levels in the brain. They were described to be vital and having a normal external morphology but females are sterile because they retain the fully developed eggs (Monastirioti *et al.*, 1996).
- Dopa decarboxylase (Ddc) catalyzes 5-HT biosynthesis from 5-hydroxytryptophan and DA biosynthesis from l-dopa. It is expressed in the adult brain, hindgut, and thorax and in larval epidermis, frontal ganglion and glial cells (True *et al.*, 1999; Hodgetts and O'keefe, 2005).
- *Drosophila* tyrosine hydroxylase (DTH) converts tyrosine to L-Dopa the precursor of DA. Homozygous mutant flies (Neckameyer *et al.*, 2001) are embryonic lethal (Neckameyer and Quinn, 1989).
- The tryptophan hydroxylase (TRH) is important for 5-HT synthesis from tryptophan and also hydroxylases phenylalanine. It is ubiquitously expressed (Neckameyer and White, 1992).

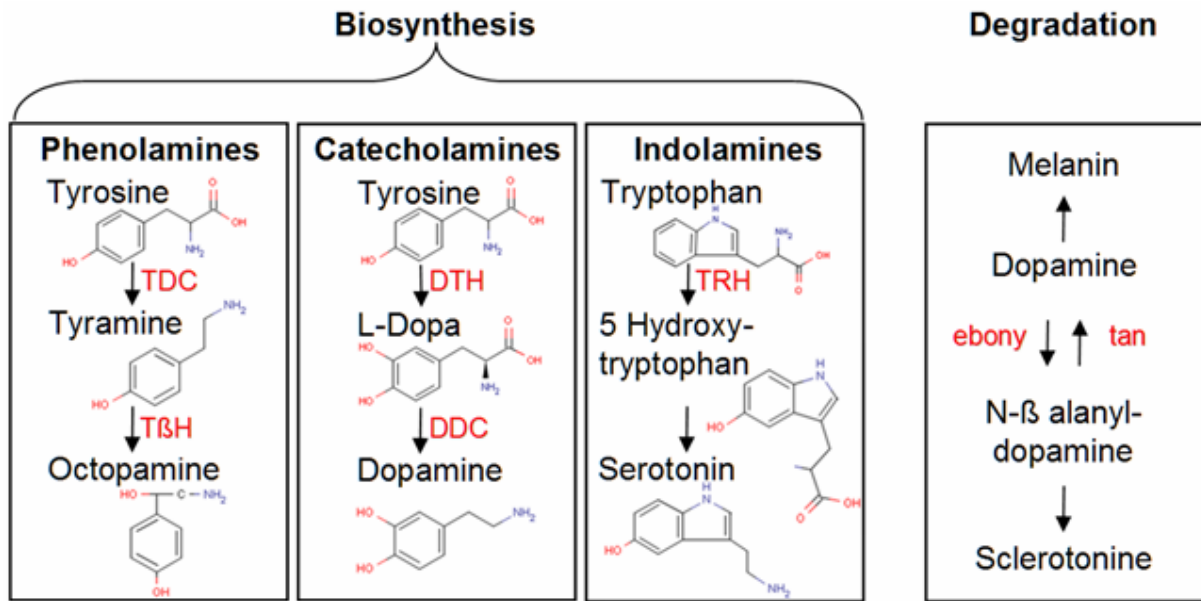


Figure 2: The biosynthesis and inactivation of biogenic amines. Phenolamines and catecholamines derive from tyrosine, whereas indolamines derive from tryptophan. Degradation and recycling is performed by the gene products of *ebony* and *tan*.

- Dopamine is degraded and recycled by the enzyme β -alanyl-dopamine-synthase encoded by the *ebony* gene. Mutant *ebony* flies are deficient for the activity of β -alanyl-dopamine-synthase, they are unable to synthesize β -alanyl-dopamine (Perez *et al.*, 1997), while dopamine levels are elevated approximately twofold (Walter *et al.*, 1996). But *ebony* is also degrading the other biogenic amines and therefore thought to be particularly important (Richardt *et al.*, 2003). Mutant *ebony* flies show darker cuticle pigmentation than wildtype flies and deficits in the regulation of circadian rhythms. A phenotype is also seen in reduced responses in electroretinograms as effect of impaired recycling of histamine (Borycz *et al.*, 2002).

Table 3: Overview of fly strains used for the functional investigation of biogenic amines.

Gene	Protein function	Allele and phenotype
<i>TβH</i>	Tyramin-β-Hydroxylase, enzyme for synthesis octopamine from tyramine	<i>TβH</i> mutant: Impaired synthesis of octopamine => octopamine deficiency, accumulation of tyramine (Monastiriotti <i>et al.</i> , 1996)
<i>ebony</i>	β-Alanyl-Dopaminsynthase, degrades biogenic amines	Ebony mutant: Putatively accumulation of biogenic amines
<i>TyrR</i>	Tyramine receptor	<i>Hono</i> mutant: defective regulation of gene expression => 70% less receptor protein (Kutsukake <i>et al.</i> , 2000)
<i>Tdc1</i>	Tyrosine decarboxylase, enzyme for synthesis of tyramine from tyrosine, in non-neuronal cells	Tdc1-Gal4: construct for expression of tyramine in non-neuronal cells (Cole <i>et al.</i> , 2005)
<i>Tdc2</i>	Tyrosine decarboxylase, enzyme for synthesis of tyramine from tyrosine, in neurons	Tdc2-Gal4: construct for expression of tyramine in neurons (Cole <i>et al.</i> , 2005)

Olfactory sensilla of *Drosophila melanogaster*

Olfactory sensilla of *Drosophila* house 1-4 olfactory receptor neurons (ORN) and are distributed on the surface of two olfactory organs, the antenna and the maxillary palp. We distinguish three main morphological types of sensilla (basiconic, coeloconic and trichoid), and many physiological sensillum classes according to the odor response spectra of their neurons (de Bruyne *et al.*, 1999; de Bruyne *et al.*, 2001a; Yao *et al.*, 2005). The different sensillum types and classes show a fixed localization on the antenna (Shanbhag *et al.*, 1999; de Bruyne *et al.*, 2001a).

The internal anatomy of the different sensillum types is similar. Figure 3 shows the organization of a large basiconic sensillum as example. The ORNs are part of the olfactory epithelium and send their dendrites into the sensillum. They are surrounded not only by glia but also by three thin glia-like accessory cells. The thecogen cell is wrapped around the outer dendrites, whereas the other two cells,

trichogen and tormogen cell, ensheath the inner dendrite. The three accessory cells are connected by septate junctions and separate the outer sensillum cavity, thus the sensillum lymph, from the antennal lymph. They determine the composition of the sensillum lymph as they secrete ions and proteins (e.g. Ca^{2+} and odor binding proteins) and therefore show many characteristics of secretory and transport cells, like vesicles and microvilli and large mitochondria. The thecogen cell borders the outer sensillum cavity with hemidesmosomes (Shanbhag et al., 2000). In moths it was shown that olfactory accessory cells generate a transepithelial potential (TEP) that has been assigned to contribute to the receptor potential, thus increasing the sensitivity of ORNs (Thurm, 1965).

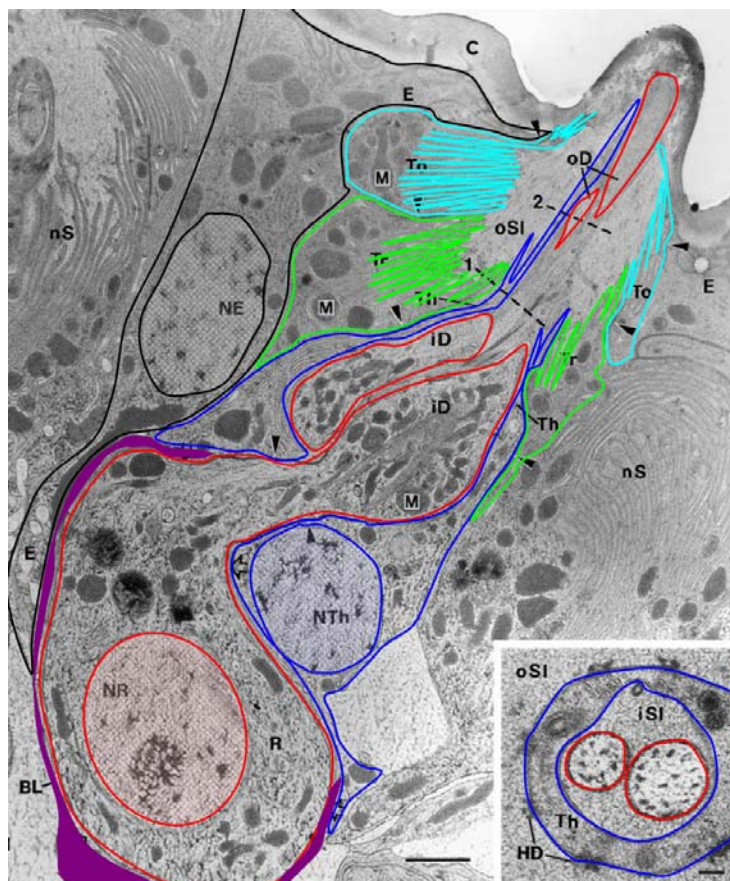


Figure 3: Internal organization of a large basiconic sensillum in *Drosophila*. Modified from Shanbhag et al., 2000. Shown in red is the ORN (R) with outer dendrite (oD), inner dendrite (iD) and nucleus (NR). In blue, green and cyan are shown the thecogen (Th), the trichogen (Tr) and the tormogen (To) cell. The black line outlines an epidermis cell (E) and the thin glial sheath wrapped around the soma of the ORN is shown in purple. The inset shows the outer dendritic segments in cross-section (indicated by the dashed line 2). Two dendrites are enclosed by a ring-shaped profile of the thecogen cell.

iSI inner sensillum lymph cavity, oSI outer sensillum lymph cavity, C cuticle, nS neighbouring sensillum, M mitochondria, N nucleus, HD hemidesmosome-like structures, BL basal lamina, septate junctions are marked by arrowheads.

Scale bar $1\mu\text{m}$, inset 100nm .

Biogenic amines in olfactory sensilla

There is strong evidence that BAs modulate the periphery of the olfactory system. Experiments with various moth species showed that injection of OA increases nerve

impulse responses of pheromone receptor neurons, but not the receptor potentials, nor the TEP (Pophof, 2000;Pophof, 2002;Grosmaître et al., 2001a). In honeybees bred for nonhygienic behavior, OA significantly increases the EAG response to diseased brood odor (Spivak *et al.*, 2003). Moreover a putative OA receptor has been cloned from the antenna of the moth species *Bombyx* and *Heliothis* and localized to cells at the base of olfactory sensilla (von Nickisch-Roseneck *et al.*, 1996). In accordance to that finding Dolzer *et al.* (Dolzer et al., 2001b) shows that octopamine affects the accessory cell dependent TEP but not the spontaneous activity of ORNs. One possible source for OA in the antenna can be secretion into the antennal heart that pumps hemolymph into the antenna. Most species possess an accessory heart at the base of the antenna and neurosecretory terminals can be found in cockroach antennal hearts that seem to release octopamine directly into the antenna (Pass *et al.*, 1988). *Drosophila* also possesses an antennal heart (de Bruyne, personal communication). A direct innervation of ORNs in the antenna has been described in mosquitoes. Neuroendocrine cells in the antenna extend an axon via the antennal nerve to the antennal lobe and project collaterals to the periphery of the antenna by forming synapses with the dendrites of the sensory cells (Meola and Sittertz-Bhatkar, 2002). Additional evidence comes from *Drosophila* where the tyramine receptor gene *hono*, cloned by Saudou *et al.* (Saudou et al., 1990a;Dolzer et al., 2001b), seems to be expressed in the antenna (Kutsukake *et al.*, 2000). The *hono* mutant (Japanese: honoka = faint smell), lacking about 70% of tyramine receptor expression, is described to show impaired avoidance behavior towards certain odors in T-Maze experiments (Kutsukake *et al.*, 2000).

Aims of this study

The observations mentioned above lead us to the suggestion that BAs modulate properties of olfactory receptor neurons.

In order to narrow the choice of candidate biogenic amines down, I established the expression profile of biogenic amine receptors in the *Drosophila* antenna by RT-PCR. Then I screened many *Drosophila* mutants concerning specific biogenic amines for a phenotype in the peripheral olfactory system by measuring their odor response profiles with electroantennograms. For the one that showed an altered odor response profile single sensillum recordings were performed. Further experiments to explain the effects found by electrophysiology were done with the aid of expression driver lines for enzymes involved in the biosynthesis of biogenic amines. I did anatomical analysis by confocal microscopy to identify the cells that synthesize tyramine and functional analysis by electroantennograms and calcium imaging.