Friedrich Schiller University Jena

Faculty of Biology and Pharmacy

Institute of General Zoology and Animal Physiology

seit 1558

Diploma Thesis

Which effector genes are efficient for neuronal silencing in OSNs and are suitable for behaviour studies?

MAX-PLANCK-GESELLSCHAFT

Accomplished at the

Max Planck Institute for Chemical Ecology

Submitted by

Tom Retzke

Jena, December 12th, 2013

I. List of Content

II.   List of Figures

III.   List of Tables

IV.   List of Abbreviations

2,3- BDN ……………………………………………….………… 2,3-Butanedione

ADP ………………………………………………....… Adenosin diphosphat

AI ……………...………………………………………….. Attraction index

AL ……....…………………………………………………. Antennal lobe

BEA ……………...…………………………………………… Benzaldehyde

BL-No. ……………………...……………………………… Bloomington number

CS ……………………..………………….. *Drosophila melanogaster* Canton S

DTA …………....… Gene *diphtheria toxin* triggering inhibition of protein biosynthesis

esm ……………………..……………………………... extended speedmatrix

ETA …………………..………………………………... Ethyl acetate

GABA ………………………..……………………………… γ-Aminobutyric acid

GR ……………………………………………………….. Gustatory receptor

IR ………………..…………………………………………… Ionotropic receptor

Kir2.1 ……………………..……………… Inward-rectifier potassium ion channel Kir2.1

LH ……………………………………………………………… Lateral horn

LN …………………………………………………………….. Local interneuron

MB ……………………..……………………………… Mushroom bodies

META ………………………………………………………… Methyl acetate

MOL …………………………………………………………... Mineral oil

NAD ………………………………………………….. Nicotinamide adenine dinucleotid

OBP …………………………………………………… Odorant binding protein

OR ………………………………………………………… Olfactory receptor

OrCo …………………..…………………………………... Olfactory receptor co-receptor

OSN ……………………..………………………………… Olfactory sensory neuron

PN ……………………………………………………….… Projection neuron

ROI ……………………..…………………………………… Region of interest

rpr …………………………………………… Gene *reaper* triggering cell apoptosis

shi ………..……… Gene *shibire* triggering temperature sensitive neuronal silencing

SSR …………….…………………………………………... Single sensillum recording

t2H ……………………………………………………………… (E)-2-hexenol

TeTx …................. Gene *tetanus toxin* triggering inhibition neurotransmitter release

TNFR1 ………………..……………………………... Tumor-necrosis factor receptor 1

UAS ………………………..…………………………… Upstream activation sequence

wt ……………………………..………………………………………... Wilde type

1   Abstract

During their life flies come in contact with a huge number of odorants. These odors are recognized by special sensory neurons in the antennae of flies, so-called olfactory sensory neurons (OSNs). An important aim in olfactory neuroethology is to understand the meaning of identified OSN populations for behavior. A common technique to reach this aim in *Drosophila melanogaster* is to genetically silence OSNs and test the manipulated flies in behavioral studies. Therefore, it is important to know which neuronal silencer should be chosen to get meaningful results. In this study, I tested the efficiency of the synaptic output inhibitor *tetanus toxin* (*TeTx*), the protein biosynthesis inhibitor *diphteria toxin* (*DTA*), the apoptosis inducer *reaper* (*rpr*) and the inward-rectifier potassium ion channel *Kir2.1* in three different bio-assays (Trap-Assay, FlyWalk and Fly Arena). The different effector genes differed in their efficiency and none of them abolished odor-guided behavior in every bio-assay. In conclusion, *DTA* and *rpr* are not suitable for olfactory behavioral studies. Furthermore, *Kir2.1* abolishes odor-guided behavior in chemotactic, but not in anemotactic bio-assays. Only *TeTx* showed an impact on experimental flies in all experiments and should be the method of choice for efficient silencing of OSNs.

2   Introduction

2.1   Biology of Drosophila melanogaster

The vinegar fly *Drosophila melanogaster* belongs to the family of *Drosophilidae* in the order of *Diptera*. Wild type flies are yellow-brown with transverse black stripes on the abdomen. Relative to the size of the head, they have very big, red eyes. Furthermore *Drosophila* exhibits a sexual dimorphism. In general the females are slightly bigger than the males, which have a darker black as an additional cue to distinguish them from females.

The period of development of *Drosophila melanogaster* depends, as in many ectothermic species, on the environment, especially on temperature. In the laboratory flies are kept in the temperature range between 18°C and 29°C (Ashburner & Roote, 2007). Beneath or above these borders, flies are negatively affected in their development. The egg hatches within a day and the resulting larva growths for two days, going through three stages: first, second, and third instar larva. Five days after the larva hatched, it pupates. After additional four days of metamorphosis the adult fly emerges. Female flies are at the latest after 48 hours receptive to courting males and close the life-cycle (Manning, 1967).

2.2   The Olfactory System Drosophila melanogaster

During their life flies come in contact with a huge number of odorants. Some of them are important to find food sources and ovipostion sites (Richmond & Gerking, 1979; Joseph *et al.*, 2009; Dweck *et al.*, in press). Others are necessary to distinguish between conspecifics and predators (reviewed in Dahanukar & Ray, 2011). But how does this specific olfactory information get into the fly brain and ultimately lead to appropriate behavior?

The paired antennae and maxillary palps on the fly´s head build the outer part of the olfactory system. The surface of each antenna is covered with different types of little hairs, called sensilla. Each of these sensilla houses dendrites of up to four olfactory sensory neurons (OSNs), depending on the sensilla type (Venkatesh, 1984; Clyne *et al.*, 1997). There are approximately 1300 OSNs per antenna and

additional ~120 per maxillary palp (Couto *et al.*, 2005). OSNs are surrounded by a fluid, sensillum lymph, which is rich in cations. While water-soluble odorants get dissolved in the lymph, it is assumed that solution of water-insoluble odorants is facilitated by carrier proteins, so called *odorant binding proteins* (OBPs). Odorants are recognized by specific odorant receptors (ORs), which are seven-transmembrane domain receptors expressed on the surface of OSNs (de Bruyne *et al.*, 2001). If one considers these neurons, a law of insect olfaction becomes apparent: *one neuron – one receptor*. This means, that one OSN expresses only one type of OR on its surface. 62 different ORs are known today (Couto *et al.*, 2005). In addition to these the fly features additional types of receptors, gustatory receptors (GRs) and ionotropic receptors (IRs) (reviewed in Touhara and Vosshall, 2009). It is known that ORs, in contrast to GRs and IRs, need to dimerize with a co-factor to convert the chemical information of odorants into an electrical signal, which can be transmitted to higher regions. *Or83b* encodes for this co-factor, also known as olfactory receptor co-receptor or OrCo. Furthermore it genetically characterizes OSNs, because no other cell type expresses this gene (Larsson *et al.*, 2004; Wicher *et al.*, 2008). The receptor signal, which is generated by the OR-OrCo dimer, is converted into action potentials and transmitted *via* the OSN`s axon to the antennal lobe (AL). The AL is the first stage of processing in the olfactory system and consists of ~ 50 subunits, called glomeruli (Hallem and Carlson, 2004; Couto *et al.*, 2005). All OSNs expressing the same OR converge onto the same glomerulus (Vosshall *et al.*, 2000). There they synapse to second-order neurons, which are called projection neurons (PNs, figure 1). Each glomerulus contains axons of several OSNs and dendrites of several PNs. A network of local interneurons (LNs) connects glomeruli with each other. This cell type lacks axons, so the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the excitatory acetylcholine are released from its dendrites (reviewed in Wilson, 2013). Consequently glomeruli are the site where these three cell types (OSNs, PNs and LNs) get connected and are able to interact with each other. The axons of PNs project into higher brain centers, lateral horn and mushroom bodies. The latter are known to regulate and process sleep and olfactory learning (Joiner *et al.*, 2006; Busto *et al.*, 2010), whereas the lateral horn is supposed to be responsible for innate behavior (Gupta and Stopfer, 2012).
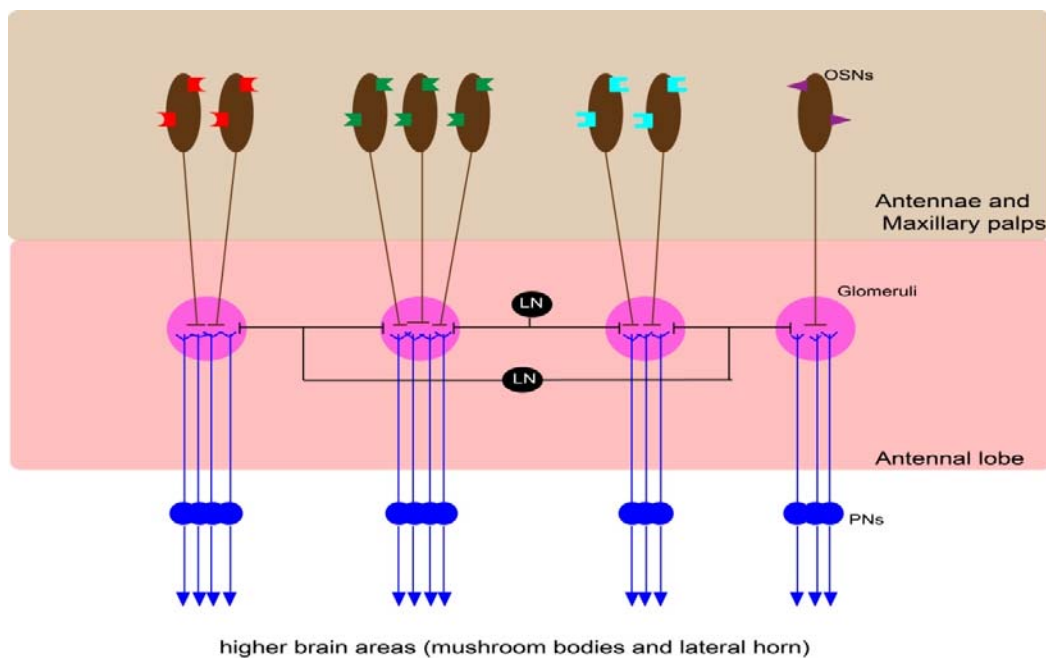
Fig. 1: The olfactory system of Drosophila OR expressing dendrites and cell bodies of olfactory sensory neurons (OSNs) are housed in the peripheral olfactory organs. All OSNs expressing the same type of OR converge to one glomerulus within the antennal lobe. Signals are modified by local neurons (LNs) and relayed to projection neurons (PNs), which send their axons to higher brain areas, mushroom bodies and the lateral horn. (modified from Wilson, 2013)

2.3   Olfaction and Behavior

Everybody can observe the phenomenon, that flies are not attracted by all fruits in the same way. Often there are more flies on grapes than on apples. The question is: Why? What is the mechanism to make the fly prefer something?

Many scientists tried to answer these questions so far (Hallem and Carlson, 2004; Laissue and Vosshall, 2008; Gaudry *et al.*, 2012; Su and Carlson, 2013). Knaden and co-workers 2012 performed an odorant screen with 110 different odors to investigate the valence of these odors to *D. melanogaster*. This was a first step to get a general overview of the meaning of single odors to flies. The next stage is to examine the importance of single ORs. Therefore OR-expressing OSNs have to be silenced. The importance of choosing a suitable silencer was shown by (Thum *et al.*, 2006). They chose neuromuscular end-plates as target to induce paralysis with *shibire^{ts1}*, *tetanus toxin (TeTx)*, *diphtheria toxin (DTA)*, *reaper (rpr)* and an inwardly

rectifying potassium channel *(Kir2.1)*. Furthermore they investigated the efficiency of *TeTx* and *shibire*[ts1] in mushroom bodies of adult flies. In results the work group showed *shibire*[ts1] immobilizes the animals within minutes, while *TeTx* took about 10 hours to induce immobilization. *Kir2.1* worked after 20 hours and *DTA* needed 5 days to paralyze the flies. The apoptosis inducer *rpr* did not work at all. Surprisingly tetanus toxin could not reduce short-term memory when expressed in flies' mushroom bodies. *Shibire*[ts1] showed the expected defect in short-term memory. This indicates the importance of the system where silencers are expressed.

So I took this idea and translated it to olfactory system. Before starting to silence single ORs, it is necessary to proof the efficiency of the single effector genes at all. To realize this I used the targeted gene expression system Gal4/UAS (Brand and Perrimon, 1993). The Gal4 gene encodes for yeast transcription activator protein Gal4, which binds to UAS. UAS is an *upstream activation sequence* and works as an enhancer for gene expression (figure 2). In my diploma thesis I used the *OrCo*-promoter to drive Gal4-expression in OSNs. The Gal4-protein binds to UAS and enables the expression of a downstream lying effector gene (*DTA*, *TeTx*, *rpr* or *Kir2.1*). In this way I could ensure to affect only OSNs. The expressed effector genes *DTA* and *rpr* kill cells in different ways, *TeTx* silences cells by inhibition of the synaptic output and *Kir2.1* lowers the input resistance of the cell (explained in detail in 2.4).

To get a broad overview of the efficiency of these effector genes, I tested the flies in three different Bio-Assays: two-choice Trap-Assay, FlyWalk and no-choice fly arena.
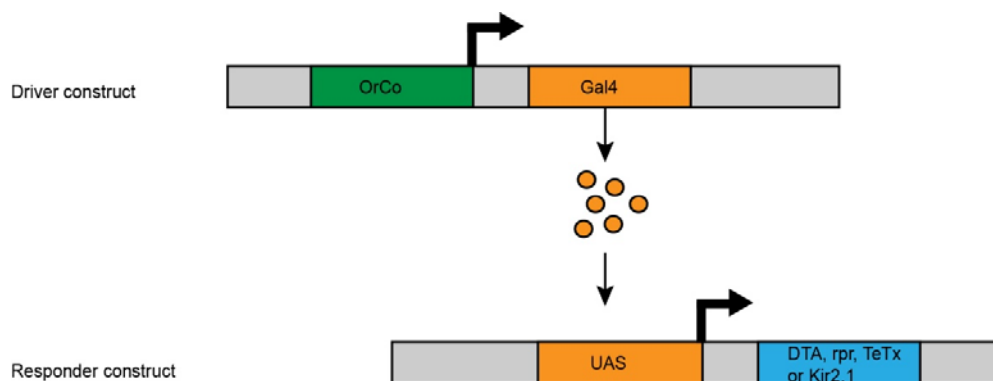

Fig. 2: The UAS7Gal4 targeted gene expression system Transcription of a cell-specific driver, here the OrCo-promoter, leads to expression of the inserted Gal4 sequence in OSNs. By binding to the UAS-sequence in the same cell, Gal4 activates gene expression of the effector genes (DTA, rpr, TeTx or Kir2.1).

2.4   Function of Effector Genes

There are a variety of possibilities to silence olfactory sensory neurons (reviewed in Venken *et al.*, 2011). The most commonly used is the *OrCo^{-/-}* mutant fly, which misses the olfactory co-receptor. These flies are not able to recognize chemical cues *via* their OR-expressing OSNs any longer. But the gustatory receptors and the ionotropic receptors are not affected. Gustatory receptors are also expressed by sensory neurons, not on the antennae of the fly, but on maxillary palps and tarsi (Montell, 2009). Ionotropic receptors, so called ligand-gated ion channels, can be found on both antennae and maxillary palps (Rytz *et al.*, 2013).

In the following I explain the function of the effector genes I used in my thesis in detail.

Tetanus toxin (TeTx)

Tetanus toxin belongs to the clostridial neurotoxins (Simpson, 1986) and inhibits vesicular neurotransmitter release (Williamson *et al.* 1996). This is caused by the cleaving of neuronal synaptobrevin (Martin *et al.*, 2002). This protein is essential for neurotransmitter release, in that it regulates Ca^{++}-dependent fast synaptic vesicle fusion (reviewed in Kidokoro, 2003). But a disadvantage of this silencing protein is the fact, that it only affects chemical synapses, whereas electrical synapses remain unaffected (Phelan & Starich, 2001). Furthermore, the effect of silencing neurons using TeTx can only be seen in neurons postsynaptic to the manipulated cell type, which complicates physiological controls for effector efficiency (Table 1).

Diphtheria toxin (DTA)

Diphtheria toxin is an exotoxin released by the bacterium *Corynebacterium diphtheriae*, which causes diphtheria. In general it gains entry into the cell plasma and inhibits protein biosynthesis (Bell *et al.* 1996). The toxin consists of two subunits: *Diphtheria toxin A* and *Diphtheria toxin B*. The latter contains the domains

T, which triggers the insertion of the toxin into the cell membrane, and R, which is immunoglobulin-like and binds to surface receptors to insert the toxin into the cell plasma. Subunit A contains the catalytic C domain, which is the effector domain (Bennett *et al.* 1994). This domain blocks protein synthesis by transfer of ADP-ribose from NAD to a diphthamide residue. Because of the high toxicity of *Diphtheria toxin*, an attenuated version of subunit A was used, called *DTA*.

Reaper (rpr)

This peptide induces apoptosis by activating the caspase proteolytic cascade that finally leads to DNA fragmentation and chromatin condensation (White *et al.* 1995;Bergmann *et al.* 2003;Hay *et al.* 2004). It contains a conserved sequence called the "death" domain, which is involved in the process of apoptosis. It bears homology to the mammalian regulatory proteins Fas and TNFR1, which are known to lead to programmed cell death, when they are expressed by cells (Golstein *et al.* 1995).

Inward-rectifier potassium ion channel (Kir 2.1)

Inward-rectifier potassium ion channels are a subset of potassium selective ion channels. They are activated by phosphatidylinositol-4,5-bisphosphate (Hansen *et al.* 2011). Their function varies across the different cells in which they are expressed. For example in endothelial cells they are involved in the regulation of nitric oxide synthase or in neurons they are important regulators, modulated by neurotransmitter. The inward rectification is the result of high affinity block by endogenous polyamines and magnesium ions. These ions plug the entry of the ion channel at positive potentials, which results in decreased outward currents. In this the currents direct only in the inward direction (Matsuda 1991). This lowers the input resistance of the neuron, because of this it is not possible to create an action potential and the neuron is silenced.

The first Kir2.0 channel cloned was Kir2.1 (IRK1), which has a single channel conductance of ~22 pS (picoSiemens) and is expressed in the forebrain, heart, and skeletal muscle (Abraham *et al.* 1999).

Table 1: List of used effector genes, their function and physiological ways to assess efficiency

Effector Gene	Function	Physiological Proof
DTA	kills cell	SSR
rpr	kills cell	SSR
Kir2.1	prevents generating of action potentials	SSR
TeTx	inhibts vesicle release	Calcium Imaging of PNs, Patch Clamp of PNs

2.5   Aims of Study

As mentioned before, the meaning of single ORs in behavior is increasingly becoming the focus in Drosophila neuroethology. The best way to find a relation between ORs and behavior is to silence single ORs. However, at the beginning of experiments one has to answer some essential questions:

⚲ Which silencer should be used?

⚲ Is the selected gene suitable in the given assay?

⚲ Does it provoke any behavioral phenotype at all?

With my diploma thesis I want to answer these questions. Although all effectors tested here have been used before in behavioral (Park *et al*., 2002) and physiological studies (Schlief & Wilson, 2007; Faucher *et al.*, 2013), the efficiency and suitability of silencer genes for silencing OSNs for behavioral studies was never investigated in detail before. Therefore I compared flies expressing the different silencer genes with wild type flies (Canton S) and $OrCo^{-/-}$ mutants in three different behavioral assays.

3   Material and Methods

3.1   Fly Lines

All flies were used at an age of 4-6 days. The animals were reared on conventional cornmeal medium at 25°C, 70% relative humidity and 12 hours day-night cycle.

To investigate the function and efficiency of the effector genes several fly strains were needed. First the wild type flies *Drosophila melanogaster Canton S* and the $OrCo^{-/-}$-mutant flies were used for all control experiments. Furthermore fly strains carrying the effector genes *rpr*, *TeTx*, *DTA* and *Kir2.1* (Tab. 2) were tested. To control the gene expression in place the promoter gene *OrCo-Gal4* was also crossed in.

Only female flies were tested during the experiment (except Trap-Assay), because they show a higher response to food-odors (Steck *et al.* 2012).

Table 2: List of the used transgenic fly lines

Fly Line	BL-No.	Genotype	Source
Orco$^{-/-}$	23130	yw; +; orco	Mattias Larsson / Bloomington 23130
UAS-*Kir2.1*	6596	w; P{w[+mC]=UAS-Hsap\KCNJ2.EGFP}1/(CyO); +	BL 6596
UAS-*Diphtheria toxin*	25039	w; P{w[+mC]=UAS-Cbbeta\DT-A.I}18/CyO; +	Bloomington Drosophila Stock Center
UAS-*Tetanus toxin*	28837	w; P{w[+mC]=UAS-TeTxLC.tnt}E2; +	Bloomington Drosophila Stock Center
UAS-*reaper*	5824	w; P{w[+mC]=UAS-rpr.C}14; +	Bloomington Drosophila Stock Cente
OrCo-Gal4 driver Line		yw; +; OrCo->Gal4	Andre Fiala

3.2   Chemicals

In all behavioral experiments odorants were used which are common in *Drosophila*-studies. Mineral oil was used as the solvent for these odors. Ethyl acetate, Benzaldehyde, Methyl acetate, trans-2-Hexenol and 2,3-Butanedione were tested at highest purity commercially available. Furthermore balsamic vinegar was tested, too.

The set of odorants and the used concentrations are shown in table 3.

Table 3: List of the used odorants and their concentrations used in the different Bio-Assays

Odor	chem. Formula	CAS-No.	chem. Class	Company	$C_{FlyWalk}$	$C_{Trap-Assay}$	C_{Arena}
2,3-Butanedione	$C_4H_6O_2$	431-03-8	vicinal diketone	FLUKA	10^{-3}	-	-
Benzaldehyde	C_6H_5CHO	100-52-7	aromatic aldehyde	SIGMA	10^{-1}	-	-
Ethyl acetate	$C_4H_8O_2$	141-78-6	carboxyl ester	Aldrich	10^{-3}	10^{-3}	-
Methyl acetate	$C_3H_6O_2$	79-20-9	carboxyl ester	FLUKA	10^{-3}	-	-
trans-2-Hexenol	$C_6H_{12}O$	928-95-0	aliphatic alcohol	FLUKA	10^{-1}	-	-
Balsamico	-	-	mixture		pure	-	pure

3.3 Behavioural Measurements

3.3.1 Trap-Assay

Preparation

With this two-choice Trap-Assay the odor guided behavior of fruit flies can be quantified. Groups of female and male flies were tested in the Trap-Assay (Fig. 3). Before the experiment the flies were starved in small plastic tubes with humidified cellulose paper for 24 hours at 23°C, 70% relative humidity and 12/12 day-night cycle. Using an aspirator about 15 to 30 flies were transferred into the Trap-Assay where they had to decide between two odor-containing traps.

The Trap-Assay was composed of a big 500 ml yogurt cup, which contains two smaller cups. One of these two cups carrying the tested odor ethyl acetate (concentration: 10^{-3}) and the other one containing mineral oil (i.e. the solvent as a negative control). The odors were presented within the small cups *via* Mµlti®-Ultra Tubes 0.2 ml (ROTH) containing filter paper. The flies were able to get into the traps through cut pipette tips, but were unable to find the way out again. Eleven fly strains were tested: UAS-rpr/+;OrCo-Gal4/+, UAS-TeTx/+;OrCo-Gal4/+, UAS-DTA/+;OrCo-Gal4/+, UAS-Kir2.1/+;OrCo-Gal4/+, UAS-rpr/+;+, UAS-TeTx/+;+,

UAS-DTA/+;+, UAS-Kir2.1/+;+ as well as the control strains wild type (Canton S, CS), OrCo⁻-mutants (OrCo$^{-/-}$), w;+;+ (w^{1118}).

Conditions and Measurement of olfactory response

To measure only the olfactory response Trap-Assays were run in darkness. Therefore a closed, dark climate chamber was used. Using this, the flies could be tested under defined conditions. The experiment run for 24 hours at 23°C, 70% relative humidity and complete darkness.

Afterwards the attraction index AI was calculated by subtracting the number of flies in the control trap from the number of flies in the odor trap divided through the total number of flies in one assay (for more information see 3.3.4)
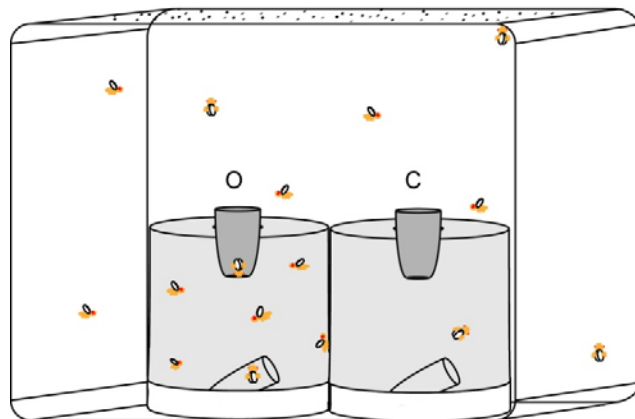

Fig. 3: Trap-Assay Two-choice assay, in which flies have to select between two cups. One carries the odor (O, here ETA [10^{-3}]), the other is a control trap (C) containing mineral oil. The flies were tested for 24 hours at 23 °C and 70% relative humidity.

3.3.2 The FlyWalk

To further examine efficiency of the effector genes I used the FlyWalk (Steck *et al.*, 2012). Using this assay one is able to test individual15 flies at the same time with the same stimulus under identical conditions. The tested flies were 4 to 6 days old and starved for 24 hours before the experiment (see Trap-Assay). Single flies were set in glass tubes with a length of 18 cm and a diameter of 0.8 cm (Fig. 4).

During the whole experiment a constant air flow of 0.3 l/min blew through the individual tubes. Digital flow meters controlled it during the measurement. Relative humidity of about 70 % was guaranteed by intervening water bottles. A constant temperature (20°C) was given by the environment of the assay. Both, temperature and humidity were verified using an additional glass tube without a fly but equipped with a temperature and humidity sensor. To ensure that there was no interference between the animals the whole experiment was running under red-light conditions (LED lights, 625 – 640 nm). Because flies are not able to detect light with a wavelength bigger than 600 nm, external visual stimuli could be neglected (Yamaguchi *et al.*, 2010). Flies could walk freely within the whole tube. Every 90 seconds an odor stimulus was added and the flies were recorded 15 seconds before and after hitting the odor. Only flies that were within the region of interest (ROI, Fig. 4), i.e. not close to the ends of the tubes, were considered for the analysis. Timing of recording and odor presentation was controlled by LabView® and fly coordinates were recorded unsing AnTS® software. Each measurement lasted nine hours and started always at the same time of day. Odors were presented using vials. Each vial contained a single little cup carrying 100 µl of the diluted odor or solvent. The vials were tightly closed to prevent loss of air flow. Furthermore they were connected *via* an one-way valve to the mixing chamber of the FlyWalk. In order to present the odor to the flies, an airflow otherwise, passing through an empty odor vial, was redirected through the odor-containing vial. This way mechanical stimulation due to a change in airflow was minimal.
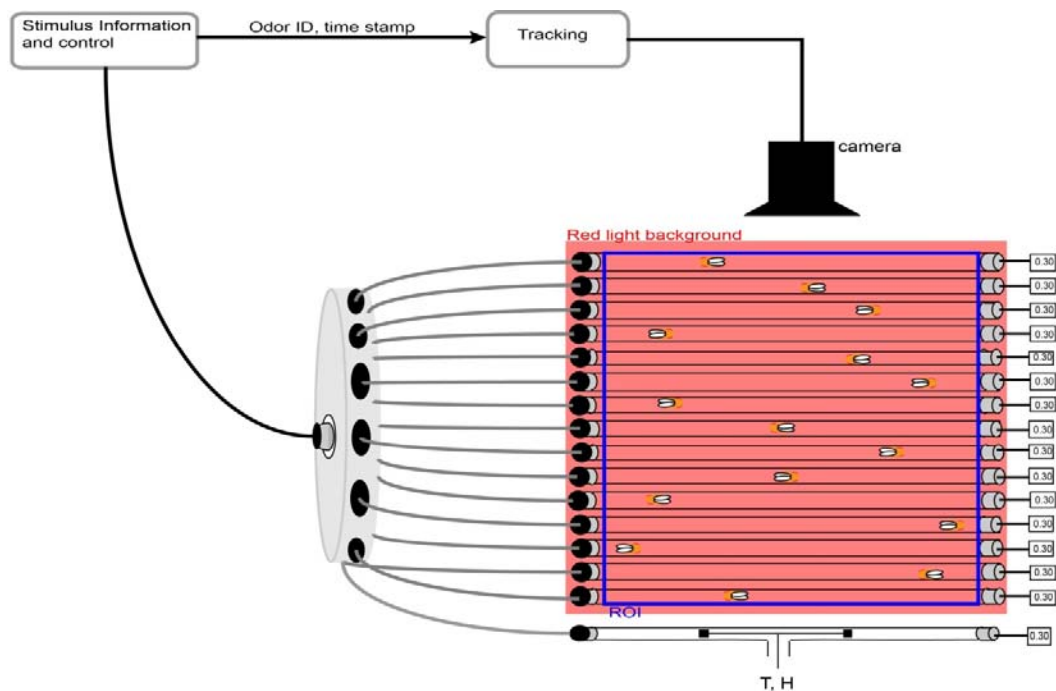
Fig. 4: The FlyWalk Red retroillumination allowed tracking in the dark. The whole experiment run 8 hours and was controlled by two connected programs (LabView® and AnTS). The humidified airflow was controlled by digital flowmeters before and after passing the tubes. In an additional glas tube temperature and relative humidity were measured. The flies were recorded each 90 seconds for 30 seconds. ROI = region of interest. T = temperature, H = relative humidity (modified by STECK et *al.*, 2012)

3.3.3 Fly Arena

Another assay, that was used to investigate the efficiency of the single effector genes, was the fly arena. The assay was performed with a single, freely movable fly for 25 minutes. Thereby the time splits into three parts: five minutes for fly´s acclimatization, ten minutes to test the fly with the solvent and additional ten minutes to test the behavior with the odor presented. The last two parts were recorded, using the *Media Recorder 2* (Noldus). As in the FlyWalk I used red light background (Fig. 5) to exclude visual stimuli from the experiment.

The arena was a square box (125x125x16 mm) made from plastic with a little hole in the lid. This was covered with gauze from the inside for two reasons: First the fly should not have the ability to leave the arena. Second the gauze prevented any direct contact between the fly and the odor source. The latter consisted of a round piece of filter paper (ø 10 mm) and 10 µl of solvent or odor. For my thesis I used

balsamic vinegar as odor, so the solvent was distilled water. Both the filter paper and the whole arena were fixed with stripes of adhesive strip.

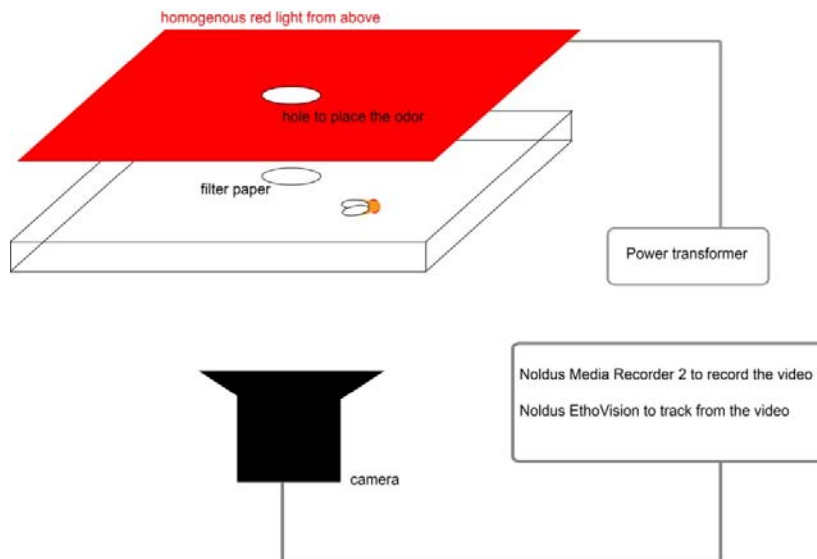The analysis of the recorded videos was performed with *EthoVision®* (Noldus).

Fig. 5: Fly Arena Homogenous red light from above allowed tracking in the dark. Each fly stayed 25 min within the arena (5 min acclimatization, 10 min solvent control and 10 min odor). Only control and odor time were recorded with Media Recorder 2. Analysis was performed with Noldus EthoVision.

3.3.4 Data Analysis and Statistics

The FlyWalk

The Output of the tracking program AnTS® consists of coordinates and corresponding time stamps. This allows an identification of any single fly at anytime. The axis of ordinate offers the possibility to allocate the flies and the axis of abscissas allows a statement about the position of the fly within the glass tube. LabView® (National Instruments) saves the pulse ID and corresponding time stamp. MatLab® (The MathWorks, Inc.) uses all these information to calculate the x-coordinates of the odor and each single fly. With respect to air flow and tube delay, the program calculates the first meeting time of odor and fly. This meeting point is set as time point 0. Afterwards MatLab® interpolates the position of the fly

in 0.1 s intervals and calculates its velocity. All this information is written in a so-called *extended speedmatrix* (esm). This file contains the information about the fly number, the presented odor, the pulse number and the speed (cm/s) of each single fly between 10 sec before and 10 sec after odor hitting (interval = 100 ms). The esm is edited with Microsoft Excel (version 2007) and afterwards statistically analyzed with custom-written functions in R (version 2.15.0, www.r-project.org). R sorts the data with respect to different aspects. To get a speed overview for each tested odor, I am only interested in the time 1 sec before to 7 sec after the meeting point. If a fly is not tracked for any given odor during this time it is excluded from the analysis. The same is true for calculating the response of the flies (distance covered from 0 to 4s). R calculates the speed overview for each odor by computing the median for each fly. Afterwards it calculates the median of flies` medians and plots it. If there is no single tracking event for a fly for an odor at all, the fly is excluded for all other odors, too. For significance tests I used the wilcoxon signed-rank test in R.

Trap-Assay

In order to calculate the attraction index AI (-1≤ AI≤ 1,) the difference in numbers of flies between the odor trap and the control trap was determined and then divided by the total number of flies:

$$AI = \frac{(number\ of\ flies\ in\ the\ odor\ trap - number\ of\ flies\ in\ the\ control\ trap)}{total\ number\ of\ flies}$$

If all flies are in the control trap the AI = -1. If the flies are all in the odor trap AI = 1. All other compositions are in-between.

Afterwards the data was statistically analyzed *via* R (version 2.15.0) using the wilcoxon rank sum test and the Anova test. The graphics were also plotted with R.

Fly Arena

EthoVision® is a tracking program for animal and human behavior. First of all one has to determine the size of the arena and the origin of the coordinate. Because I am interested in the distance of the fly from the odor source, I used the odor source in the middle of the arena as origin. Furthermore I had to fix the size of the sample and the way the program works. I chose *dynamic subtraction* to compare a background picture with each single picture in the video. If the fly is detected the program marks it and saves coordinates in a table. After finishing the analysis, the raw data was exported to Microsoft Excel (version 2007) and edited. Only time and coordinates were used for further analysis. The distance of the fly from the odor source was calculated using the Pythagorean Theorem. To standardize the probability distribution of the flies, I divided their frequency within the single sectors by the mean area of a circle. In doing so I subtracted the smaller inner circle from the great circle.

Statistical analysis was made with R (version 2.15.0).

4   Results

To investigate the meaning of single ORs to flies, it is a common method to silence OSNs and test the animals in physiological or behavioral studies. Previous work (Thum *et al.*, 2006) showed a great variance in efficiency of silencer genes in the fly´s motor system. Because of the popular use of neuronal silencing in sensory systems, it is necessary to investigate their efficiencies and suitability in different Bio-Assays.

4.1. Trap – Assay

First, to get an overview, I tested about 200 animals of both sexes and each genotype in the Trap-Assay. The flies could chose between mineral oil (MOL, control) and ethyl acetate (ETA, c = 10^{-3}). As expected, wild type flies (Canton S) showed a high response to ETA, which is indicated by a median attraction index (AI) of 0.45 (Fig. 6). When I tested flies that lacked the olfactory coreceptor OrCo there was no observable attraction to odor or control, i.e. the attraction index (AI) was 0. Furthermore I investigated white-eye flies (w;+;+), because they build the background for parental crossings. With an AI of 0.95 they showed significantly higher attraction to the presented odor than wild type flies did (p = 0.00004, n =10, wilcoxon rank sum test). Although the OrCo-Gal4 parental control seemed to be more attracted, too, there is no significant difference against wild type flies (p = 0.23, n = 10). Because the result for OrCo-Gal4 parental control is true for all tested effector genes, the data shown here was also used in all following plots.

Both, *diphtheria toxin* (*DTA*) and *reaper* (*rpr*), kill cells through protein biosynthesis inhibition or apoptosis induction, respectively. Therefore I tested, whether these two effector genes would affect the flies' behavior like the *OrCo* mutation. The parental controls for UAS-DTA and OrCo-Gal4 did not differ from each other (p = 0.75, n =10, Fig. 7a). However, also the flies expressing *DTA* in the OR-expressing neurons showed a high response to ETA. Although they differed from OrCo-Gal4

flies (p = 0.04, n =10), there was no significant difference to UAS-DTA control (p = 0.27, n = 10). A test against 0 indicated statistically significant attraction for all three genotypes (p < 0.05). The same happened to *rpr* flies. The affected flies showed the weakest response in comparison with control, but the AI distinguishes only from OrCo-Gal4 (p = 0.005, n = 10, Fig. 7b). Because of an AI of 0.27 there is a significant difference to 0 (p < 0.01). To sum up results so far, both fly lines expressing DTA and rpr in OSNs display decreased attraction compared to OrCo-Gal4 parental controls. However, this observation cannot be unambiguously attributed to a loss of OSN input, because in both cases attraction does not differ from the UAS-effector control.
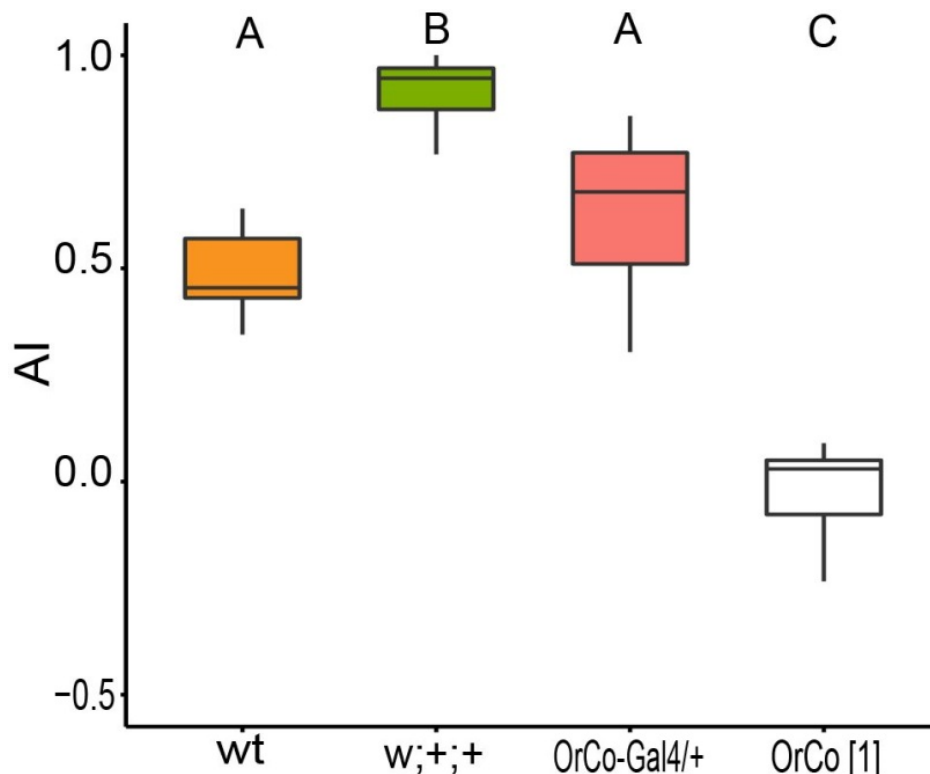

Fig. 6: Attraction index of control flies Wild type flies (orange), white-eye flies (green), OrCo-Gal4 parental control (red) and OrCo$^{-/-}$ mutants (no fill) were tested with ethyl acetate [10^{-3}]. Different letters indicate significant differences between groups (p < 0.05, n = 10, wilcoxon rank sum test). If the test against 0 was not significant, boxes are not colored (wilcoxon rank sum test).
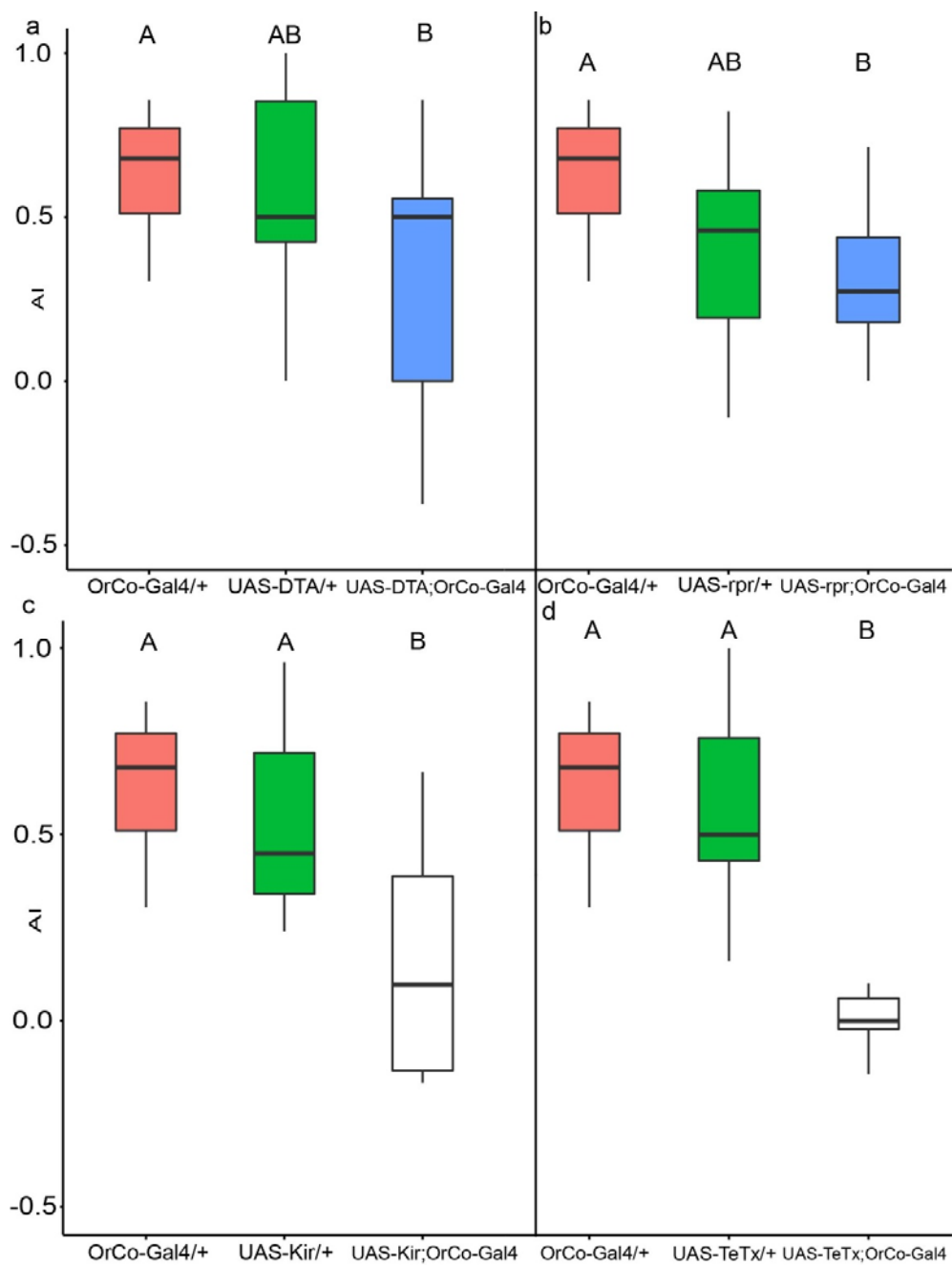
Fig. 7: Attraction indexes of *diphtheria toxin* (a), *reaper* (b), *Kir2.1* (c), *tetanus toxin* (d) and their parental controls Parental controls and experimental flies were tested with ethyl acetate (c = 10^{-3}, diluted with mineral oil). Different letters indicate significant differences between tested groups (p<0.05, n = 10, wilcoxon rank sum test). Colored boxes show a significant difference from 0 (p<0.05, wilcoxon rank sum test). red = OrCo-Gal4 parental control, green = UAS-effector parental control, blue = affected flies, AI = attraction index

In general *Kir2.1* silences neurons by inhibition of action potential creation. So, in contrast to *reaper* and *diphtheria toxin*, the neurons are still alive. As in the former experiments I compared the affected flies with parental controls (Fig. 7c). There was no difference between UAS-Kir and OrCo-Gal4 in attraction to ETA ($p = 0.31$, $n = 10$). The flies expressing Kir2.1 in OSNs showed less attraction and there was a significant difference to both parental controls ($p < 0.01$, $n = 10$). Moreover, attraction to ETA was abolished in those flies (test against 0, $p = 0.20$). *Tetanus toxin* also does not kill cells. In contrast to *Kir2.1* the neuron can generate action potentials, but the synaptic output is blocked. The UAS-TeTx parental control showed a high response to ETA (AI = 0.5, Fig. 7d). There was no significant difference between the parental controls ($p = 0.55$, $n = 10$). However, expressing of *tetanus toxin* in the flies' OR–expressing neurons abolished attraction towards ETA. This result is confirmed by a high significantly difference to parental controls ($p < 0.001$, $n = 10$).

In summary I observed, that *DTA* and *rpr* did not specifically affect odor-guided behavior, whereas *Kir2.1* and *TeTx* abolished behavioral responses to ETA. In this bio-assay many animals were tested at the same time. To exclude any social aspects I further examined the behavior of single flies using another assay, the FlyWalk.

4.2 The FlyWalk

Having shown the efficiency of the effector genes in the Trap-Assay with many flies, I now wanted to investigate their efficiency in the FlyWalk with single flies. Using this assay I could examine the response of flies to six different odors in one experiment. As in the Trap-Assay mineral oil was used as negative control. Ethyl acetate, methyl acetate, 2,3-butanedione and balsamic vinegar are four attractive odorants, while benzaldehyde has been shown to be repellent (Steck *et al.*, 2012). E2-Hexenol was indicated as neutral to the flies, with a slight tendency to attractiveness (Knaden *et al.*, 2012). First of all I ran control experiments to confirm these previous results (Fig. 8). In three different independent experiments I investigated the behavior of 15 wild type flies, 15 OrCo$^{-/-}$ mutants and 15 OrCo-

Gal4 parental control flies (Fig. 9). Wild type flies were attracted to most of the odors. While the repellency of benzhaldehyde was missing, the insignificant response to E2-hexenol confirmed previous data (p = 0.62, wilcoxon signed-rank test). The OrCo-Gal4 parental control responded similarly to the wildtype. Only for ethyl acetate (p = 0.01) and E2-hexenol (p = 0.01) a significant difference to the wild type was observed. OrCo$^{-/-}$ mutants were slightly attracted to balsamic vinegar and strongly attracted to E2-hexanol. All other odor responses were not significantly different from mineral oil response (p > 0.05). When testing experimental flies, I always tested the UAS-parental control and wild type in the same experiment. *Diphtheria toxin* was tested as first effector gene in this assay. While comparing responses to methyl acetate of all three genotypes, it is noticeable that the experimental flies differ significantly from the parental control (p = 0.004, Fig. 10). But if one compares these flies with wild type, a significant difference is missing (p = 0.19). The other way arround could be detected for E2-hexanol. While control flies differ from each other (p = 0.04), *DTA* expressing flies still responded like wilde type (p < 0.05). In general I could not detect a response reduction of UAS-DTA;OrCo-Gal4 flies, compared to UAS-DTA parental control and wild type.
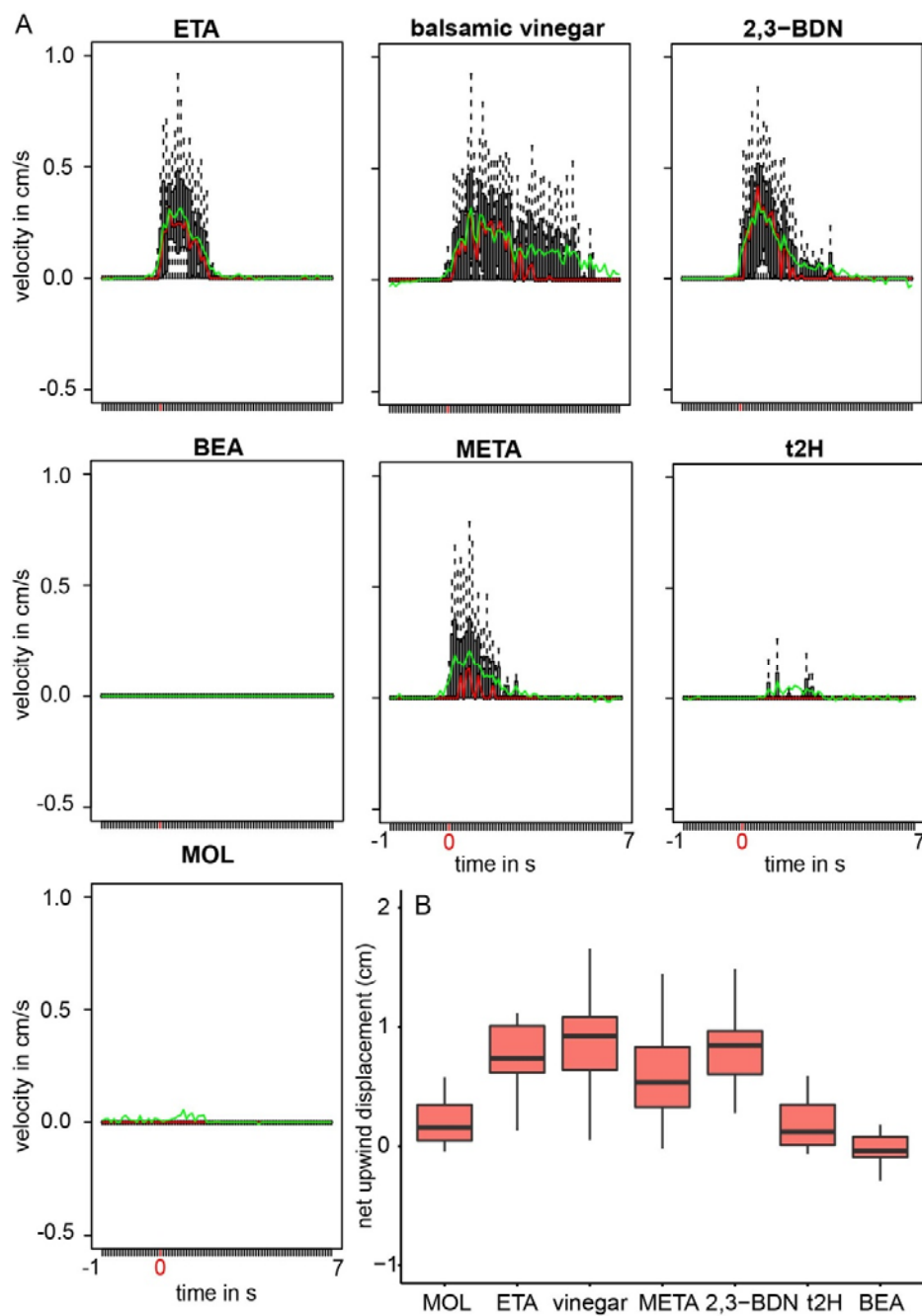
Fig. 8: A Speed overview of wild type flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}] **B response of wild type flies to each tested odor** median response of 15 single flies
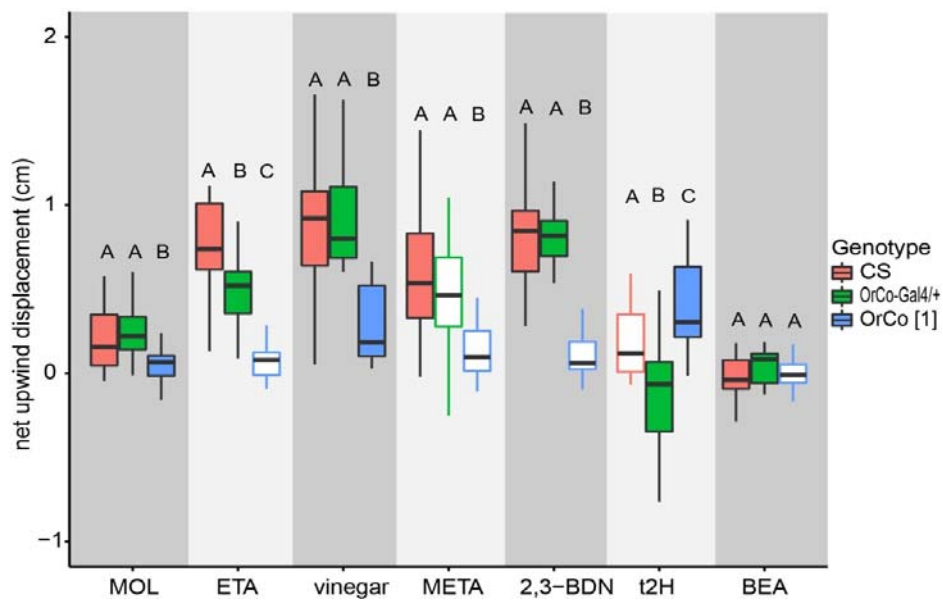
Fig. 9: Odor responses of control flies Median of the response median of 15 animals per genotype. Statistical tests were performed for genotypes within each single odor (p < 0.05, wilcoxon signed-rank test). Significant differences between control responses (MOL) and tested odors are colored, lacking difference are depicted by white boxes (p < 0.05, wilcoxon signed-rank test).
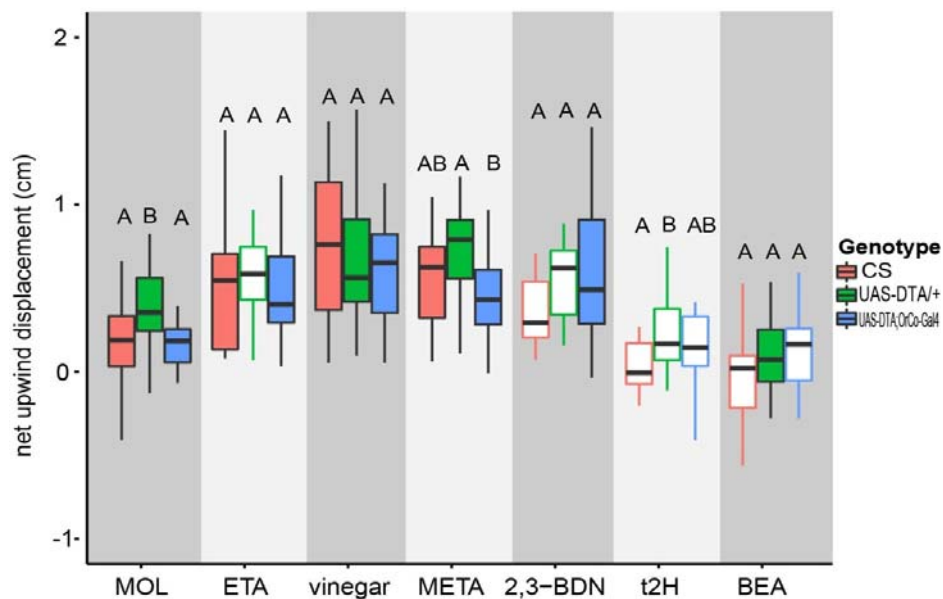

Fig. 10: Odor responses of flies expressing *DTA* Median of the response median of 15 animals per genotype. Statistical tests were performed for genotypes within each single odor (p < 0.05, wilcoxon signed-rank test). Significant differences between control responses (MOL) and tested odors are colored, lacking difference are depicted by white boxes (p < 0.05, wilcoxon signed-rank test).

I next tested the silence effiency of *rpr* in the FlyWalk (Fig. 11). Although for ETA a significant difference between experimental flies and the two control strains could be detected ($p < 0.05$), there was no noticeable difference to mineral oil response ($p = 0.95$), i.e. the lack of significant responses towards ETA was not attributable to the specific genetic manipulation of OSNs as lack of responses was already observed in the parental UAS-control. Balsamic vinegar was attractive for all three genotypes. The same could be observed for META and 2,3-BDN. The experimental flies were slightly repelled by t2H, which in contrast is an OrCo$^{-/-}$ attractant. Furthermore there was no significant difference between wild type, UAS-rpr control and UAS-rpr;OrCo-Gal4 flies, int their reaction to benzaldehyde ($p > 0.05$).

To sum up the results for the FlyWalk up to this point, both effector genes *DTA* and *rpr* were not working for this bio-assay. I found some significant differences to control flies, but mostly only to one of the two controls. So in general I can conclude, that no consistent decrease in odor responses could be detected.

Therefore, I went further in my experiments and tested *Kir2.1* in the FlyWalk. Flies expressing this inward-rectifier potassium ion channel displayed reduced response to ETA in the Trap-Assay. However, I did not find any significant modulation in the response of experimental flies to ETA, when being to the same compound in the FlyWalk ($p > 0.05$, Fig. 12). The same was true for balsamic vinegar, E2-hexanol and methyl acetate. Only for the attractive odor 2,3-BDN ($p = 0.01$) and the repellant BEA ($p = 0.008$), a significant decrease in responses could be observed. In conclusion, there is a general trend towards reduction of attractant responses in Kir-expressing flies, but attractant responses were never completely abolished. However, responses to the repellent BEA were affected in the expected direction.
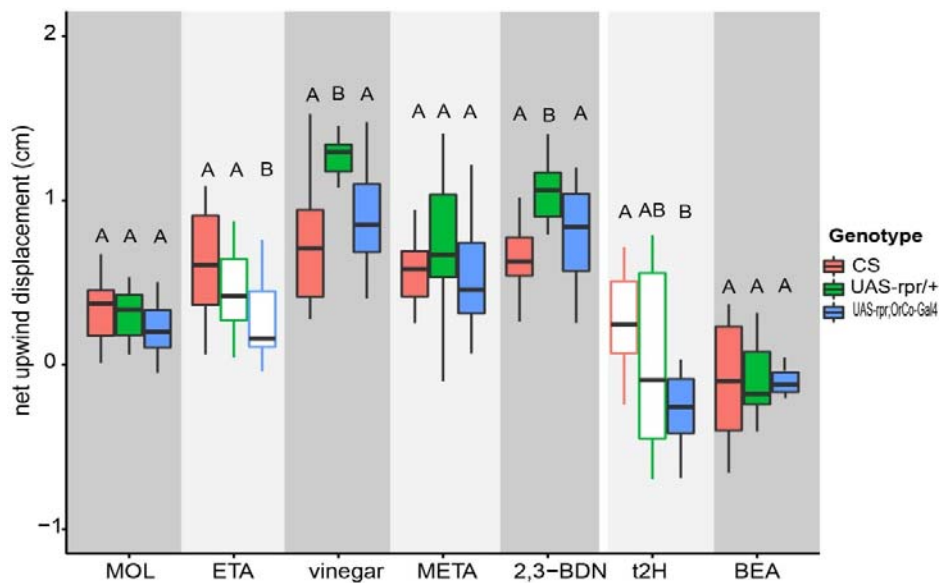
Fig. 11: Odor responses of flies expressing *rpr* Median of the response median of 15 animals per genotype. Signigicant differences were calculated for genotypes within each single odor (p < 0.05, wilcoxon signed-rank test). To show significant differences between control responses (MOL) and tested odors, the boxes are colored, blank if there is no difference (p < 0.05, wilcoxon signed-rank test).
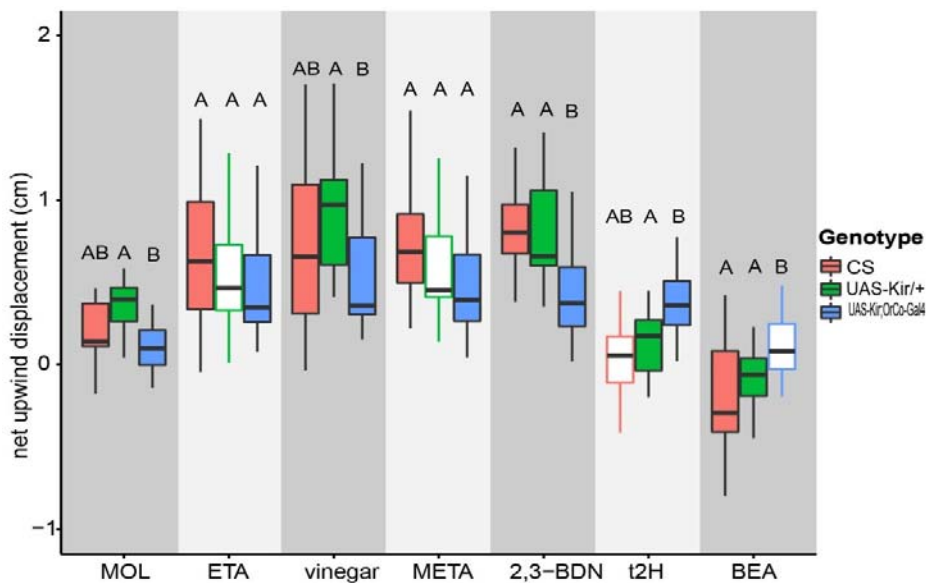

Fig. 12: Odor response of flies expressing *Kir2.1* Median of the response median of 15 animals per genotype. Signigicant differences were calculated for genotypes within each single odor (p < 0.05, wilcoxon signed-rank test). To show significant differences between control responses (MOL) and tested odors, the boxes are colored, blank if there is no difference (p < 0.05, wilcoxon signed-rank test).

Finally I used the FlyWalk to investigate the effect of expressing *tetanus toxin* (Fig. 13), which before had resulted in the most significant results when flies were tested in the Trap-Assay. Using this effector gene I could observe a reduction in their

response in general, but no silencing at all. While the responses of experimental flies to ethyl acetate were significantly different from the responses of the other two genotypes (p < 0.05), there is still a little significant difference from mineral oil response (p = 0.045). Furthermore the response of UAS-TeTx;OrCo-Gal4 flies to META is not significant different from their MOL response (p = 0.87). However, the significant difference to the control strains is attenuated by the fact, that these genotypes did not significantly respond to META in this dataset (p > 0.05). Moreover the response of flies expressing *TeTx* to balsamic vinegar seems to be reduced, but a significance test could not show a significant difference between them and control flies (p > 0.05). The same is true for 2,3-butanedione, E2-hexanol and benzaldehyde. Although there is no significant difference to MOL response for the last two odors (p = 0.44, p = 0.46), the experimental flies do not differ from the wild type and parental control, as well.
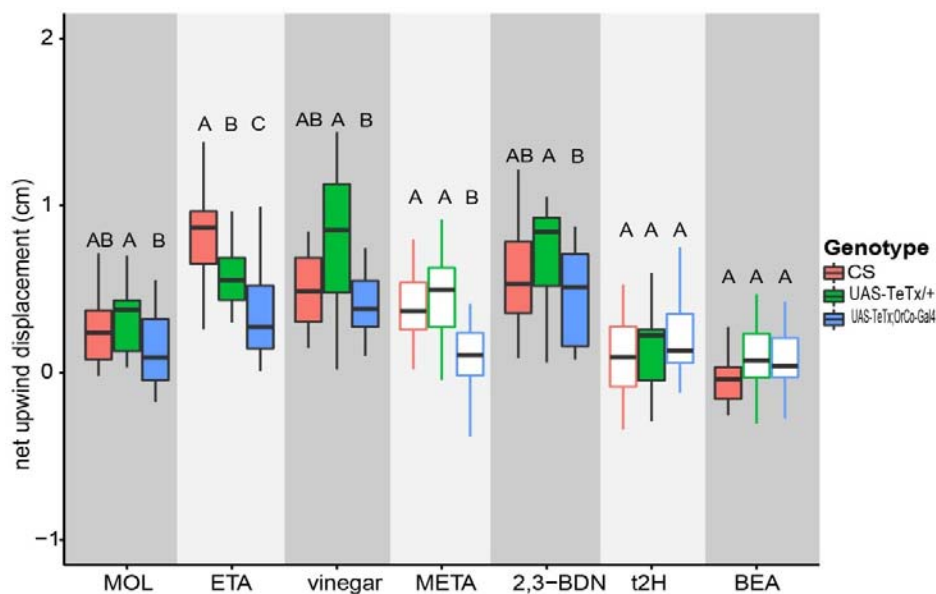

Fig. 13: Odor response of flies expressing *TeTx* Median of the response median of 15 animals per genotype. Signigicant differences were calculated for genotypes within each single odor (p < 0.05, wilcoxon signed-rank test). To show significant differences between control responses (MOL) and tested odors, the boxes are colored, blank if there is no difference (p < 0.05, wilcoxon signed-rank test).

In summary the observed effects are similar to those I got in the Trap-Assay. While *DTA* and *rpr* did not show any impact on flies´ behavior, there was a consistent decrease in odor response of *Kir2.1* – and *TeTx*-expressing flies, even if its occurrence was not as strong as in the Trap-Assay.

These big differences in efficiency of effector genes reveal many questions. Some of those I wanted to answer using an additional bio-assay, the Fly Arena. This assay combines key aspects of the two previous used assays. Similar to the Trap-Assay it works with an odor gradient, but like in the FlyWalk single animals are tested.

4.3   Fly Arena

The Fly Arena is a gradient based bio-assay, which offers the opportunity to investigate behavior of single flies. An odor is presented in the middle of the arena. To avoid stimulation of contact chemoreceptors, there is no direct contact between odor source and animal. I examined the behavior of flies of each genotype while presenting the odor blend balsamic vinegar to them. To ensure that there are no other cues leading animals to the middle of the arena, the same flies were tested with distilled water before odor presentation. As expected, wild type flies were not attracted by the control, which is indicated by an equal distribution over the whole area of the arena (S11A). When vinegar was presented, the animals were attracted and their walks were concentrated around the odor source (S11A). By comparing mean distances from the odor source in control and experiment, I could confirm this observation (Fig.14). On average, the distribution of flies was significantly shifted towards the center of the arena, when balsamic vinegar was presented as an odor source. The probability distribution per area fits in these observations, too. Up to 2.5 cm distance from the odor source the probability distribution for an animal smelling the odor is significantly higher than for animals tested with water ($p < 0.05$). In contrast OrCo$^{-/-}$ mutants were expected not to be attracted to vinegar. Due to the fact that all animals were tested under the same conditions, the results for these mutants were surprising. Like the wild type, OrCo$^{-/-}$ flies did not respond to distilled water. However, also like wild type these flies were attracted to balsamic

vinegar (S11C). Their distance from the odor source is not significantly different from wild type (Fig. 15, p = 0.11). Furthermore mutants spent significantly more time closer than 0.5 cm around the odor source, than wild type flies did (Fig. 16, p = 0.006). The remaining distribution is similar to that of wild type flies (p > 0.05). Like in all previous experiments OrCo-Gal4 flies are one of my parental controls. Their behavior is comparable to that of wild type flies. They did not respond to distilled water (S11B), but showed attraction to balsamic vinegar (S11B). Although there is a significant difference between balsamic vinegar presentation and control (p = 7*10^{-5}), there is no difference to the mean distance of wild types (Fig. 15, p = 0.6). Furthermore their probability distribution correlates with wild type´s, too (Fig. 16, p < 0.05).
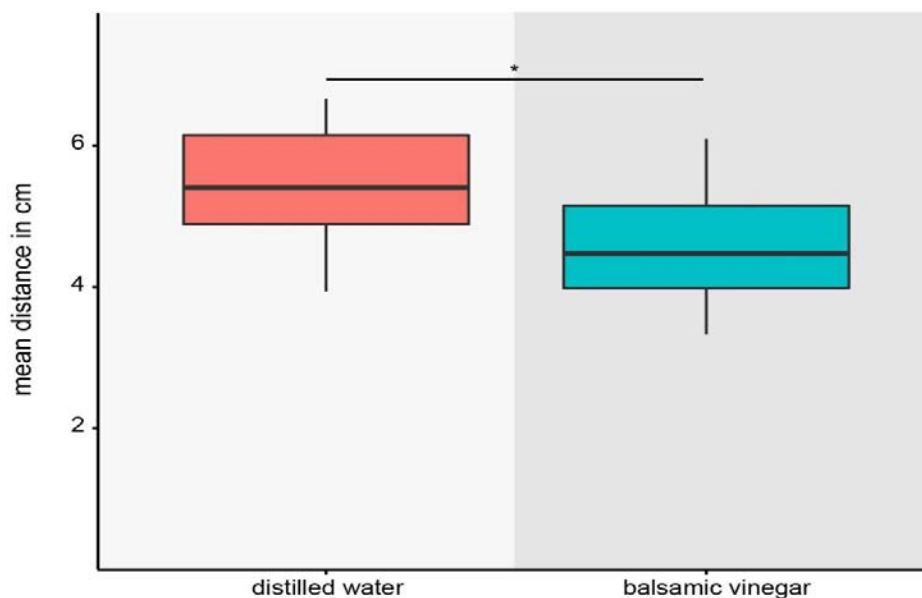

Fig. 14: Mean distance of wild type flies from odor source Drosophila melanogaster wild type (Canton S) were tested in the Fly Arena with distilled water (control) and balsamic vinegar. When presenting odor, their distance from odor source was significantly shorter than in control experiments (p = 0.002, wilcoxon rank-sum test).
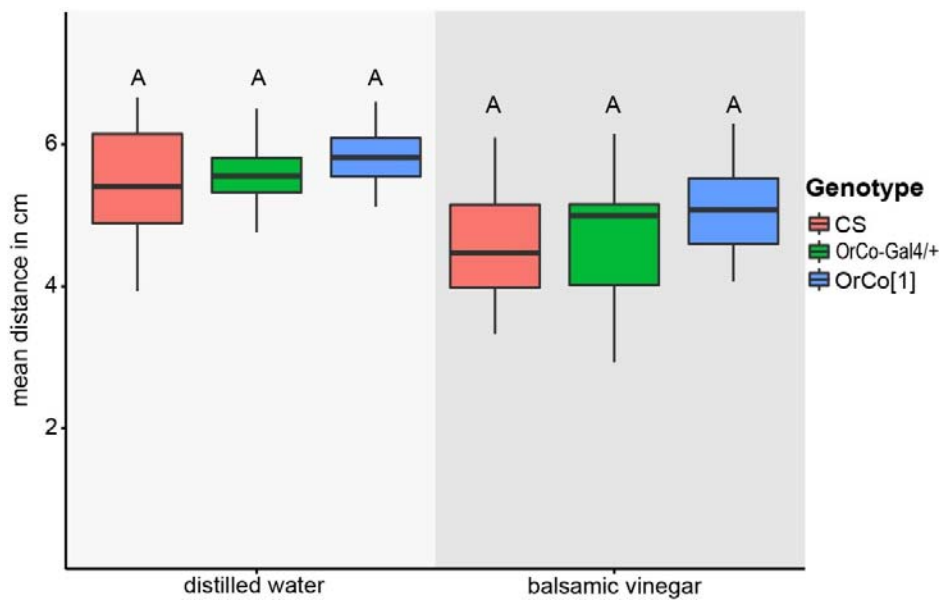
Fig. 15: Mean distance from odor source for all control strains OrCo-Gal4 parental control and OrCo^{-/-} mutants were tested with distilled water (control) and balsamic vinegar. In comparison with wild type both showed a similar behavior. While significant difference between response to water and balsamic vinegar is indicated by colored boxes, the letters above the boxes show differences between the genotypes (p < 0.05, wilcoxon rank-sum test).
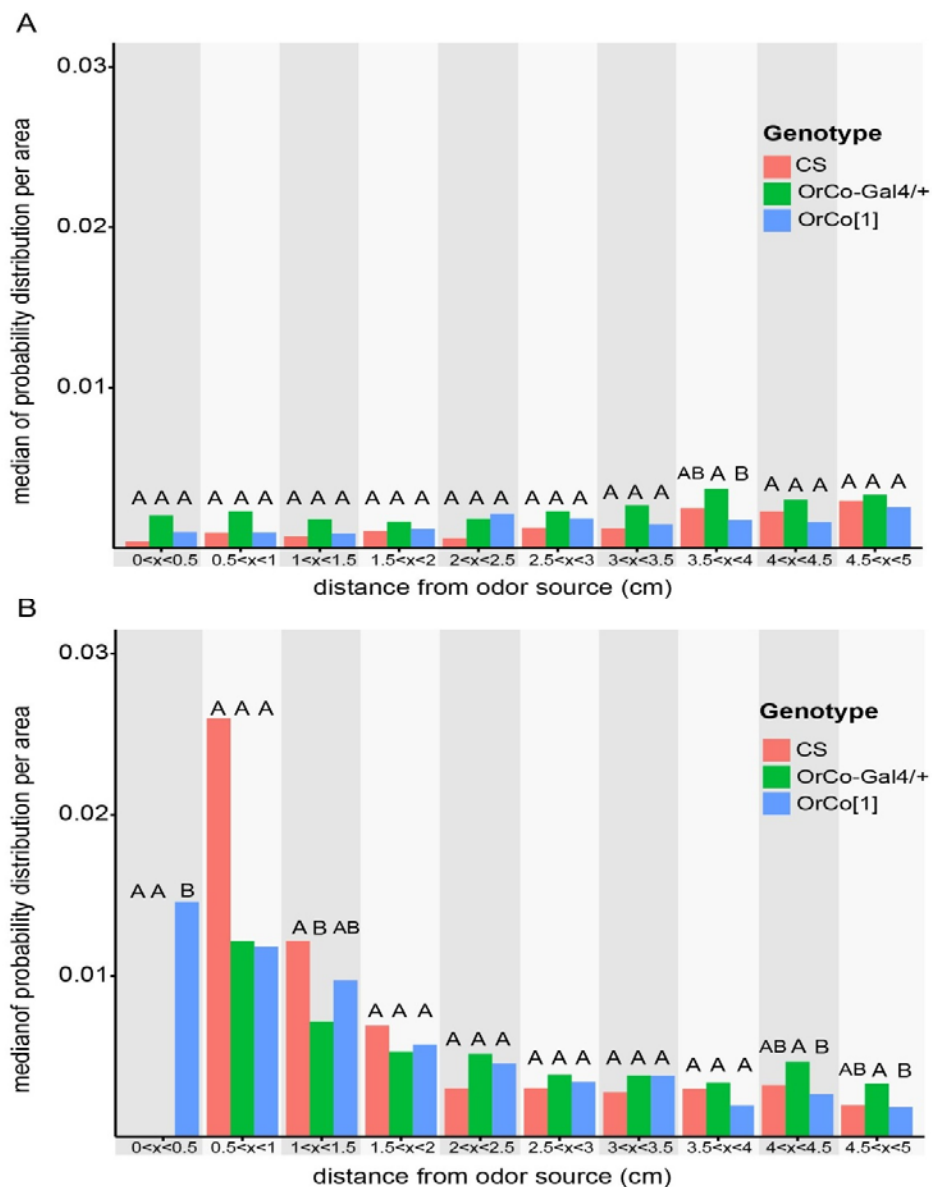
Fig. 16: (A) Probability distribution of controls when tested with water (B) Probability distribution of controls when tested with balsamic vinegar The median over 20 flies per genotype. The statistics were made for each single bin (p < 0.05, wilcoxon rank-sum test).

Although *DTA* and *rpr* did not show any impact in previous assays, I examined their effects on behavior in the Fly Arena, too. During experiments with UAS-DTA parental control flies, I could observe an equal distribution over the whole arena when presenting distilled water only (S12A). When presenting balsamic vinegar, the flies were attracted by the odor like wild type flies (S12A). The investigation of mean distances showed no significant difference between parental controls during

odor presentation (p = 0.95). Furthermore there is no difference in the probability distribution of UAS-DTA and OrCo-Gal4 parental controls (Fig. 18, p > 0.05). However, like in the previously tested assays, the experimental flies expressing *DTA* again were not affected in their behavior. Although there is a significant difference to OrCo-Gal4 parental control flies (p = 0.004), these flies are still responding to balsamic vinegar in a highly significant way (Fig.17, p = $2*10^{-5}$).
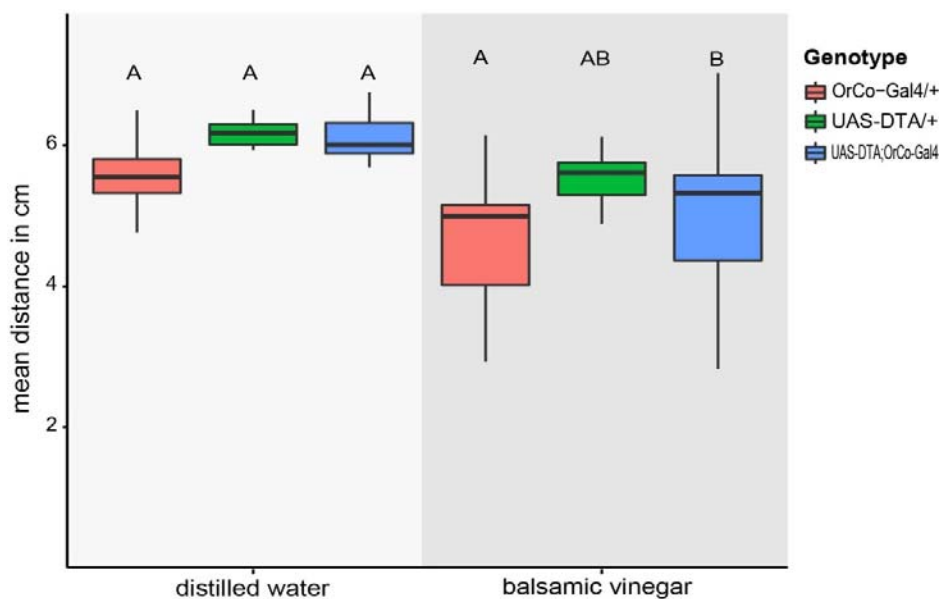

Fig. 17: Mean distance from odor source of *DTA*-expressing flies UAS-DTA parental control and UAS-DTA;OrCo-Gal4 flies were tested with distilled water (control) and balsamic vinegar. In comparison with OrCo-Gal4 parental flies, both showed a similar behavior. While significant difference between response to water and balsamic vinegar is indicated by colored boxes, the letters above the boxes show differences between the genotypes (p < 0.05, wilcoxon rank-sum test).
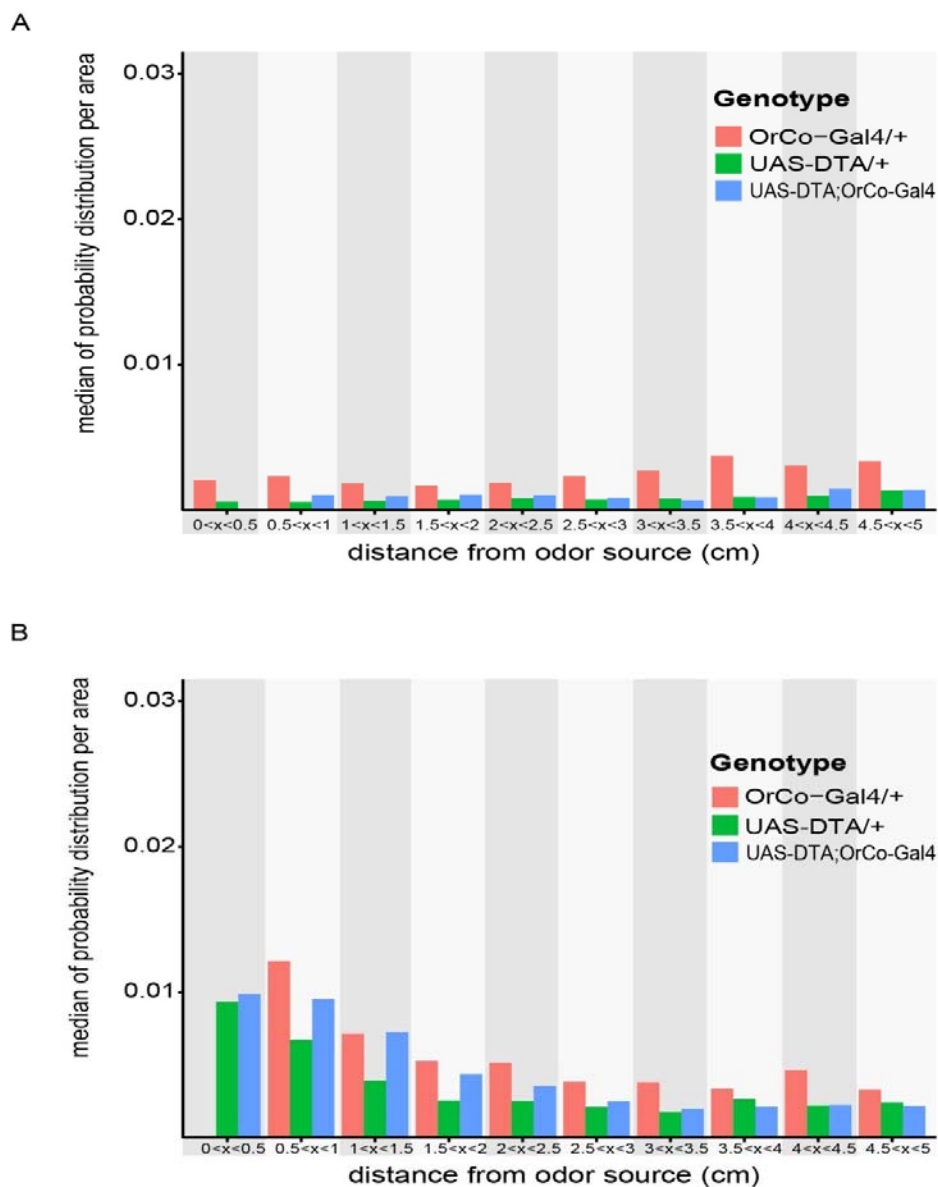
A

B

Fig. 18: A) Probability distribution of flies expressing *DTA* when tested with water (B) Probability distribution of flies expressing *DTA* when tested with balsamic vinegar The median over 20 flies per genotype. The statistics were made for each single bin (p < 0.05, wilcoxon rank-sum test).

Their probability distribution confirms this by the fact, that there is no significant difference in distribution of control flies and experimental flies (Fig.18, p > 0.05). Because of its similarity in efficiency to *DTA* in Trap-Assay and FlyWalk, I examined *rpr* subsequently. Correlating to *DTA*-expressing flies, *rpr*-expressing flies did not show any attraction to distilled water, but to odor (S13B). So their mean distances in both experiments, control and odor presentation, are not

significantly different from those of parental controls (Fig. 19, p > 0.05). Furthermore their attraction to balsamic vinegar is highly significant (p = 0.0002). Taking a look at the probability distribution of parental controls and *rpr*-expressing flies confirms this, too. Although it is not significant (p = 0.25), experimental flies seemed to spent more time closer than 0.5 cm from odor source than UAS-rpr and OrCo-Gal4 parental controls did (Fig. 20).
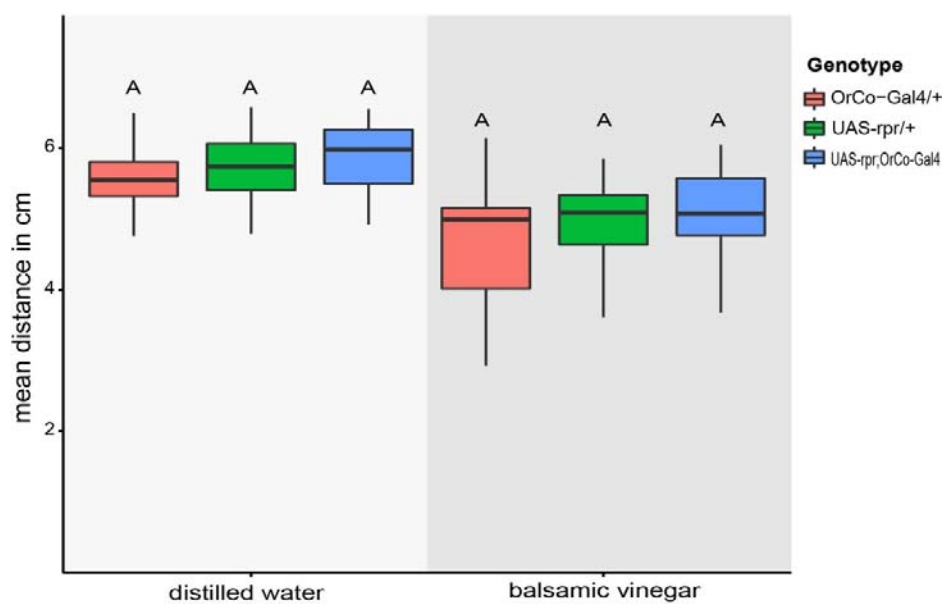

Fig. 19: Mean distance from odor source of *rpr*-expressing flies UAS-rpr parental control and UAS-rpr;OrCo-Gal4 flies were tested with distilled water (control) and balsamic vinegar. In comparison with OrCo-Gal4 parental flies, both showed a similar behavior. While significant difference between response to water and balsamic vinegar is indicated by colored boxes, the letters above the boxes show differences between the genotypes (p < 0.05, wilcoxon rank-sum test).
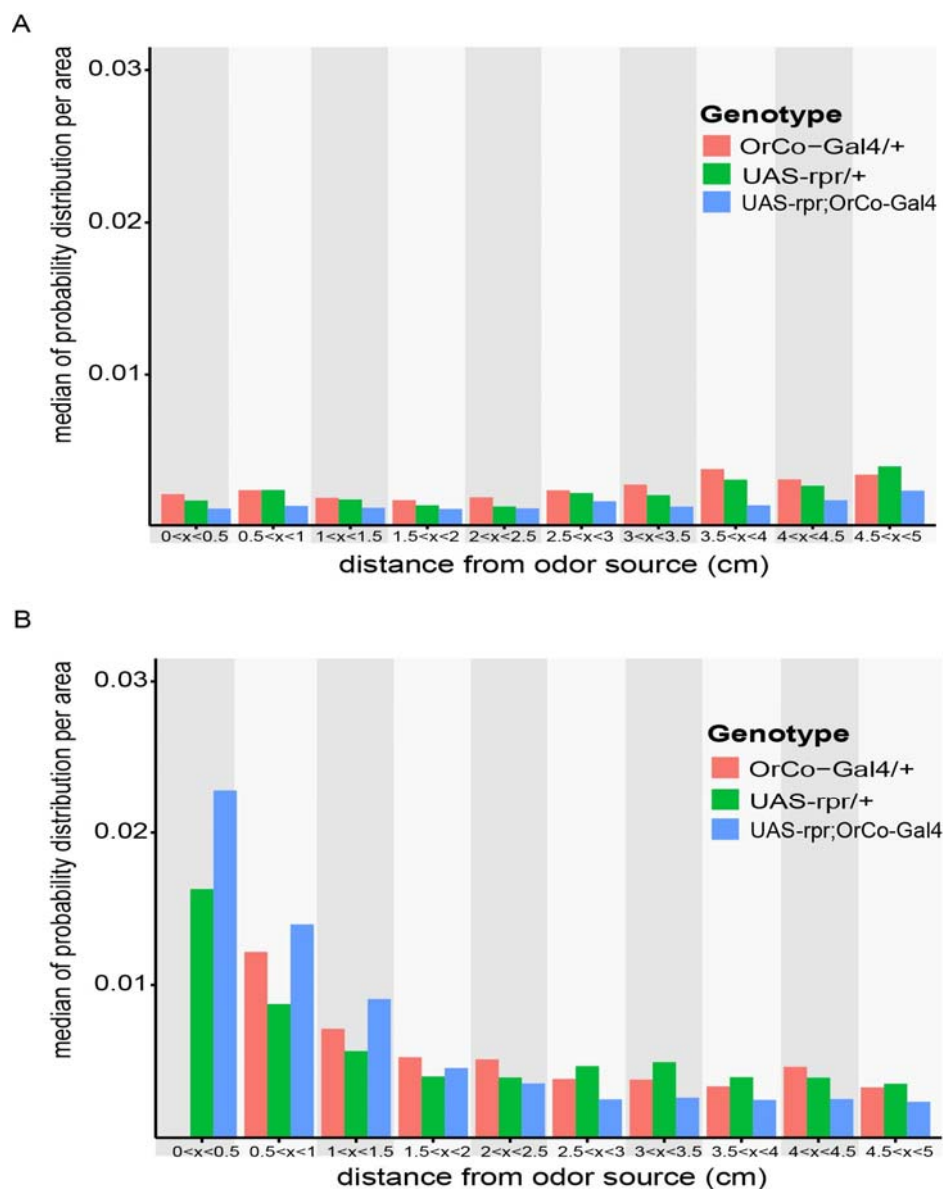
Fig. 20: A) Probability distribution of flies expressing *rpr* when tested with water (B) Probability distribution of flies expressing *rpr* when tested with balsamic vinegar The median over 20 flies per genotype. The statistics were made for each single bin (p < 0.05, wilcoxon rank-sum test).

However, to sum up data from the Fly Arena up to here, I conclude that *DTA* and *rpr* are as inefficient in this third assay as in the Trap-Assay and FlyWalk.

In the next step I examined *Kir2.1*-expressing flies. While this silencer worked in the Trap-Assay very well, there was no visible effect on flies tested in the FlyWalk. Now it was interesting to investigate their behavior in the Fly Arena. As expected, the UAS-Kir2.1 parental control behavior is indistinguishable from those of OrCo-

Gal4 controls and wild type (S14A). The observation could be confirmed by analyzing their mean distance and probability distribution. Both showed no significant differences from the other parental control, neither in control nor in odor testing (Fig. 19, Fig 20, p > 0.05). While *Kir2.1* expressing flies did not respond to vinegar in the Trap-Assay, they apparently recognized its odor, when tested in the Fly Arena, which is indicated by a high density of walks over the mid of the arena (S14B). Although their mean distance is not significantly different from those of parental controls (p > 0.05), the significance test did not display a difference between their response to distilled water and vinegar (Fig. 21, p = 0.05). According to my observations and in contrast to mean distance statistics, the probability distribution of UAS-Kir2.1;OrCo-Gal4 flies is equal to parental controls (Fig. 22, p > 0.05).
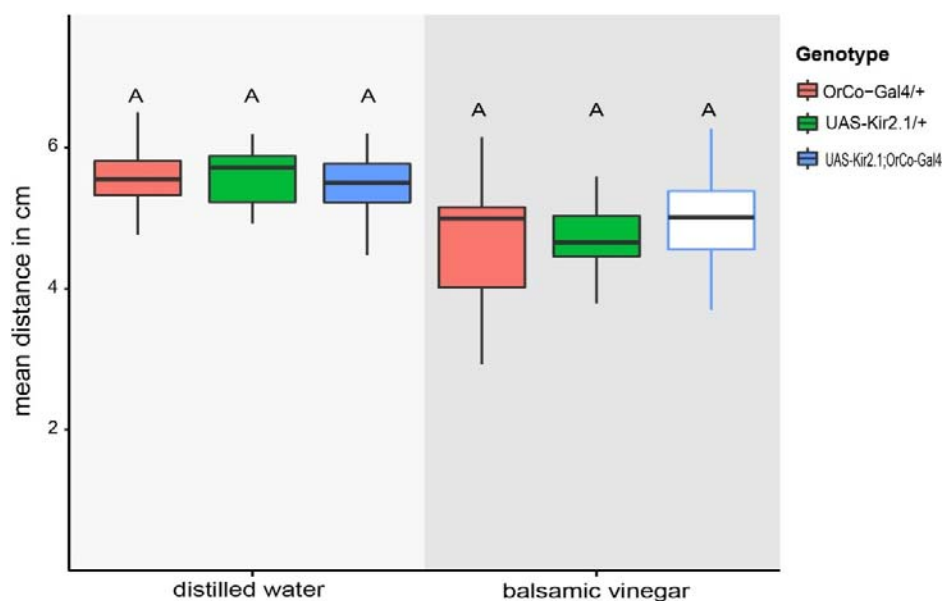

Fig. 21: Mean distance from odor source of *Kir2.1*-expressing flies UAS-Kir parental control and UAS-Kir;OrCo-Gal4 flies were tested with distilled water (control) and balsamic vinegar. Although there is no significant difference between the genotypes, the response of experimental flies to vinegar does differs not from their response to water (p = 0.0524, wilcoxon rank-sum test). While significant difference between response to water and balsamic vinegar is indicated by colored boxes, the letters above the boxes show differences between the genotypes (p < 0.05, wilcoxon rank-sum test).
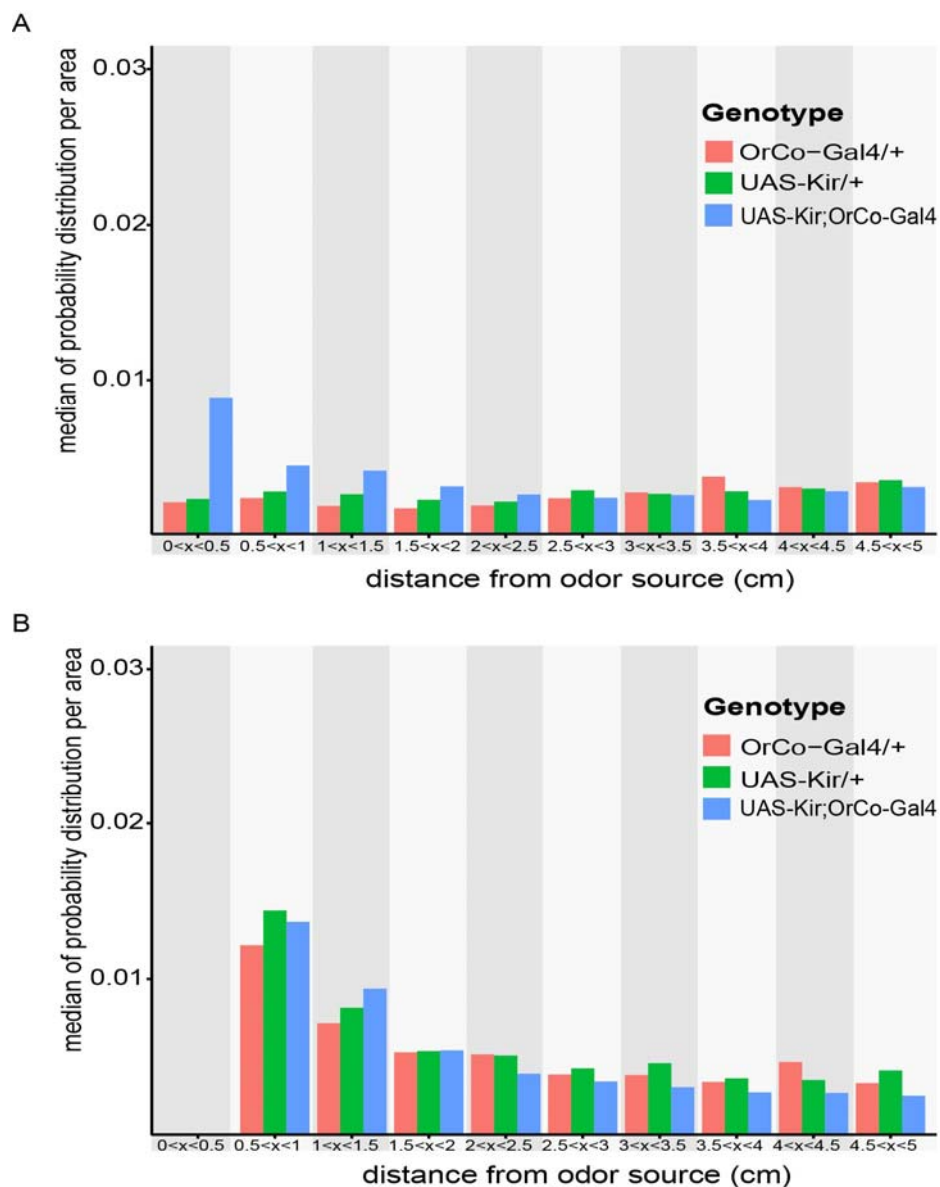
Fig. 22: A) Probability distribution of flies expressing *Kir2.1* when tested with water (B) Probability distribution of flies expressing *Kir2.1* when tested with balsamic vinegar. The median over 20 flies per genotype. The statistics were made for each single bin (p < 0.05, wilcoxon rank-sum test).

Tetanus toxin is the only silencer, that worked in the Trap-Assay and showed an effect on flies in the FlyWalk. The UAS-TeTx parental control responded to balsamic vinegar like all other controls, while they were not responding in the control experiments (S15A). This is also represented in the analysis of the mean distance from odor source (Fig. 23). While their median distance in control

experiments is at 5.5 cm, the flies are 0.8 mm closer when presenting vinegar (median distance = 4.7 cm, p = 3*10^{-7}). The probability distribution of UAS-TeTx control flies supports the previous results, too. Neither for distilled water, nor for balsamic vinegar a significant difference from OrCo-Gal4 parental control could be detected (p > 0.05, Fig. 24). However, as in the Trap-Assay, the experimental flies did not respond to vinegar at all (S15B). While their mean distance in control experiments does not differ from that of parental controls (p > 0.05), their inability to find the odor source is significant against both, OrCo-Gal4 (p = 0.004) and UAS-TeTx (p = 0.001, Fig. 23). Furthermore, the response of UAS-TeTx;OrCo-Gal4 flies to the odor is not significantly different from their response in control experiments (p = 0.25). These results are also reflected in the analysis of the probability distribution (Fig. 24). Their probability distribution up to an area of 5 cm around the odor source is consistently lower than 1 %. While the probability distribution of control flies in this area is 1 % and higher, differences with the experimental flies are significant up to 3 cm around the odor source (p < 0.05).
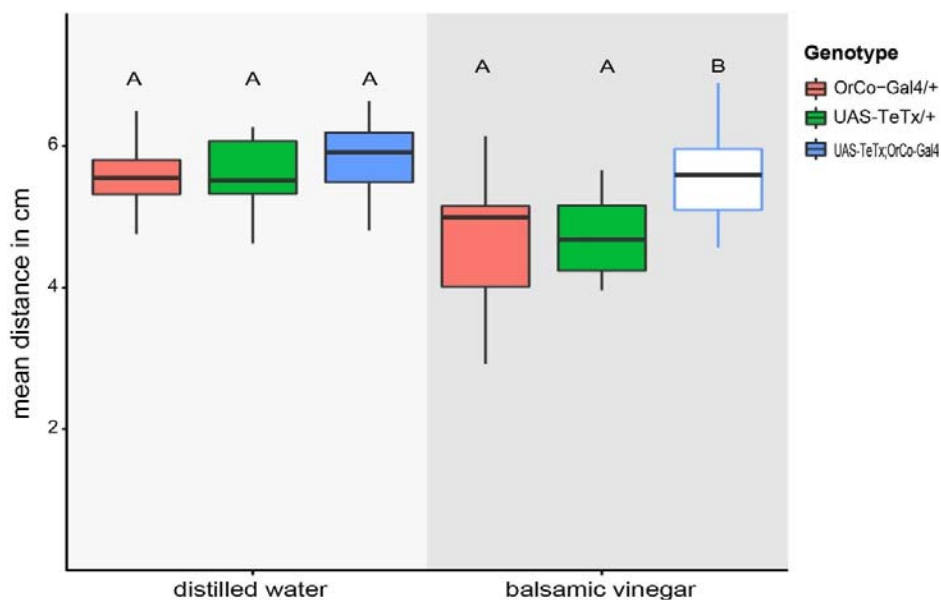

Fig. 23: Mean distance from odor source of *TeTx*-expressing flies UAS-TeTx parental control and UAS-TeTx;OrCo-Gal4 flies were tested with distilled water (control) and balsamic vinegar. There are no differences between genotypes when testing the control (p < 0.05, wilcoxon rank-sum test). The experimental flies did not show a significant response to balsamic vinegar (p = 0.25). While significant difference between response to water and balsamic vinegar is indicated by colored boxes, the letters above the boxes show differences between the genotypes (p < 0.05, wilcoxon rank-sum test).
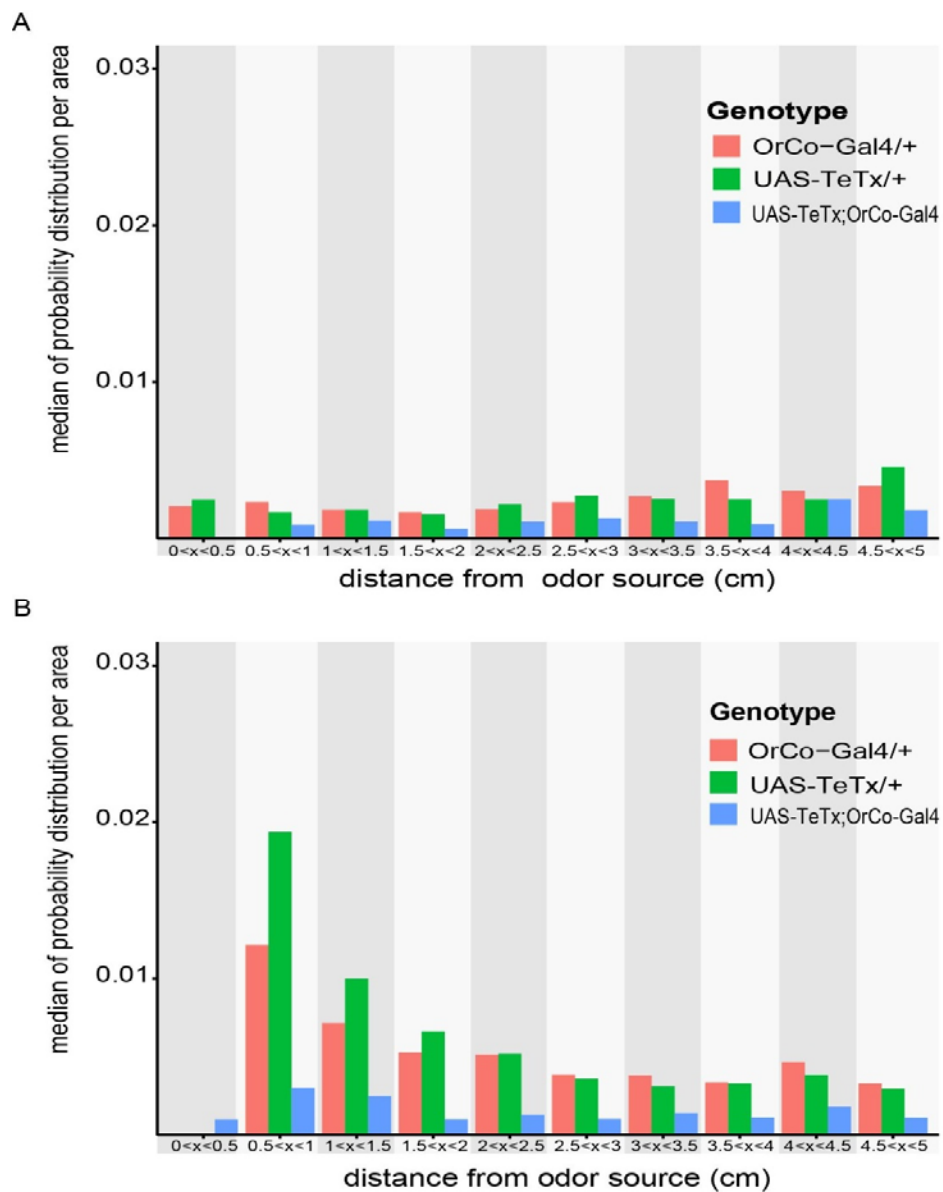
Fig. 24: A) Probability distribution of flies expressing *TeTx* when tested with water (B) Probability distribution of flies expressing *TeTx* when tested with balsamic vinegar The median over 20 flies per genotype. The statistics were made for each single bin ($p < 0.05$, wilcoxon rank-sum test).

5   Discussion

Silencing olfactory sensory neurons (OSNs) is a common technique to investigate their meaning in the olfactory circuit of flies. Popular silencers are *tetanus toxin*, *diphtheria toxin*, *reaper* and *Kir2.1*. This thesis examined their efficiency and suitability using three different bio-assays.

5.1   Efficiency depends on expression level

Taken together, my results show that none of the effector genes is 100% efficient in silencing OSNs expressing olfactory receptors (ORs). The reasons are gene-specific and depend on their mechanism of action. *Diphtheria toxin* was used in attenuated form because of its high toxicity. Therefore, its effect may vary from OSN to OSN, depending on the absolute expression level. *Reaper* (*rpr*) induces apoptosis, which leads in consequence to cell death. This mechanism is very complex and has a huge number of players, some of which (e.g. NF-κB) are not obligatorily expressed in adult tissues (Bergman *et al.*, 2003; Hay *et al.*, 2004). With respect to this, the impact of *rpr* depends strongly on the expression levels of the other peptides and proteins involved, which may not be present in adult OSNs. However, both silencers may kill a subpopulation of OSNs in our experiments, while surviving neurons should be largely unaffected by the genetic manipulations. These surviving OSNs should be physiologically similar to wild-type neurons and could be sufficient to evoke attraction behavior. Instead of killing the OSNs *Kir2.1* reduces the input of neurons by inhibition of action potential generation (Matsuda, 1991). Higher concentrations usually result in increasing OSN activity, which may eventually overcome the inhibitory effect of *Kir2.1*. Therefore, high odor concentrations could still evoke odor-guided behavior in flies expressing the effector gene *Kir2.1* in their OSNs. The physiological phenotype of *tetanus toxin* is similar to *Kir2.1*. Expression of *tetanus toxin* (*TeTx*) inhibits neurotransmitter release (Williamson *et al.*, 1996) (Martin *et al.*, 2002). However, as a higher OSN activity also results in a higher rate of neurotransmitter release and as only one vesicle can be enough to elicit post-synaptic response (Kazama and Wilson, 2008)

OSNs expressing *tetanus toxin* could still detect high odor concentrations. This is especially true for ONS expressing olfactory receptors, which are highly sensitive to single odors, e.g. OR42b for ETA (de Bruyne *et al.*, 2001; Kreher *et al.*, 2008). Regarding to this I would expect that *Kir2.1*- and *TeTx*-expressing flies need more olfactory input to evoke responses in their OSNs, while these in turn are saturated at low activity levels. Future studies therefore should test the behavior of flies to decreasing odor concentrations.

5.2   Different efficiency of silencer genes in different assays

Whenever one uses bioassays to investigate olfactory behavior two questions are raised: (1) Do flies like the odor, i.e. the question of odor identification and evaluation and (2) can flies find the odor, i.e. the odor source localization. During FlyWalk experiments odors are pulsed into a constant airflow. The olfactory system of the tested flies only has to identify and evaluate the presented odor, while mechanoreceptors assess the position of the odor source using wind direction (optomotor anemotaxis, reviewed inJarman, 2002). The information reaching CNS in *Kir2.1*- and *TeTx*-expressing flies is apparently enough to identify and evaluate the presented odor. Especially the attractants are known for strong activation of single ORs (Galizia *et al.*, 2010; http://neuro.uni-konstanz.de/DoOR/default.html), e.g. OR42b (ETA), OR92a (2,3-BDN) and OR59b (META). However, since Trap-Assay and Fly Arena experiments examine olfactory behavior in completely windless environments the olfactory system of tested flies further has to compute the directionality of the odor gradient (Flügge, 1934). This can be achieved using two different strategies: (1) measuring the difference in concentration between two independent sensors, separated in space (e.g. the fly antennae), or (2) moving through the gradient and measuring concentration changes over time (osmotropotaxis, reviewed in Gaudry et al., 2012). Only *Kir2.1* and *TeTx* were able to abolish behavior in both assays. There are two possible explanations: First, the concentration within the gradient was too low to elicit any OSN activity, or second the concentration was sufficient to identify and evaluate the odor, but the OSN throughput was not strong enough to localize the odor source. With respect to Fly

Arena experiments both hypothesis are supported for *Kir2.1* and *TeTx*. While it seems that *Kir2.1*-expressing flies could identify the odor, from a certain point they lost their orientation and searched in a bigger area for the odor source than wild type did. In contrast, *TeTx*-expressing flies seemed to be not able to identify and evaluate the odor in gradient based experiments. They showed no searching behavior in arena experiments.

5.3   Different efficiency for different odors presented in FlyWalk

Steck and co-workers (2012) showed an odor specific behavior of wild type flies in the FlyWalk. The speed overviews of the tested flies revealed that all tested effector genes modified single odor responses that however were still significantly different from mineral oil. I observed two patterns: First the effect of the silencers was global, i.e. the responses to all odors were reduced in flies expressing *Kir2.1*- or *TeTx*. The second pattern, which was observed in flies expressing *DTA* or *rpr*, was the reduction of single odor-specific responses, e.g. ETA in *rpr*-expressing flies and META in *DTA*-expressing flies. These findings could have been due to the impact of additional involved molecular players (see 5.1.). In order to unveil, whether single OSN populations become killed while others are unaffected, one could use physiological methods like single sensillum recording (SSR), to investigate the different OSN populations in more in detail. A further reason for the changes in response patterns is the used odor concentration. Flies are able to detect and behaviorally respond to single odors at a concentration down to a dilution of 10^{-7} in the case of ETA (personal communication with Michael Thoma) which supports the hypothesis, that residual activity reaching the CNS may be sufficient to elicit the obeserved behavior. In addition the different OR-expressing OSNs vary in abundance (Vosshall *et al.*, 2000), i.e. while 20 neurons express OR22a, there are about 50 OSNs expressing OR47b. One could speculate that a loss of 50 % of active neurons in smaller OSN populations could have a higher impact on the flies' behavior than silencing 50 % of a bigger OSN populations, because of the resulting differences in absolute numbers of active neurons.

5.4   Odor responses of *OrCo*^{-/-} mutants

OrCo^{-/-} mutant flies served in my experiments as control to evaluate efficiency of tested effector genes. In general they were believed to be entirely anosmic due to the importance of the OrCo-protein in olfactory signal transduction (Larsson *et al.*, 2004; Wicher *et al.*, 2008). This assumption was rejected by Benton and co-workers (2009) who identified a novel family of ionotropic chemoreceptors involved in olfaction (IRs). During FlyWalk experiments OrCo^{-/-} flies showed attraction to (E)-2-hexenol, the same could be observed for balsamic vinegar in the Fly Arena. Latter is especially interesting since in the FlyWalk experiments *OrCo*^{-/-} mutants showed no response to this odor. One possible explanation could be the impact of IRs. IR-expressing OSNs are independent from *OrCo*-expression in their functionality, i.e. the effector genes coupled to OrCo-Gal4 are not expressed in these neurons. Their vitality is equal to those in wild type flies. Balsamic vinegar is not a pure odor, but an odor blend. It mainly contains acetic acid, which is the most attractant part of it in windtunnel experiments (Becher *et al.*, 2010). In addition Ai and colleagues (2010) described the IR64a to be specifically activated by acids, among others acetic acid. Both findings suggest that the tested *OrCo*^{-/-} mutant flies identified and localized the odor source in arena experiments using information transmitted by their IRs, more specifically by following the acetic acid gradient. However, this acetic acid gradient was also accessible for OrCo-Gal4/UAS-*TeTx* flies (although they did not respond to it). Therefore, other factors must have contributed to the observed difference in the behavioral phenotypes between OrCo mutants and *TeTx*-expressing flies, which should be identical in the information their OSNs transmit to the CNS. An attractive hypothesis would be that *OrCo*^{-/-} mutant flies have evolved a different valence code for odorants, due to their deletion of the *OrCo* gene 10 years ago (Larsson *et al.*, 2004). At least since 2004 these mutant flies are in laboratory use, which in turn equals approximately 250 generations which would correspond to approximately 5000 years of human evolution. On this time-scale the loss of OR signals may have supported an increase in salience of IR signals. Since the *TeTx*-expressing flies lost their functional OR expressing neurons only in the generation that was behaviorally tested, there was no evolutionary meaningful time to change the IR's impact on the odorant valence. For further experiments it would be interesting to investigate the

role of IRs in *OrCo*^{-/-} mutants in more detail. It is possible that signals coming from IRs are suppressed by OR-information, i.e. (E)-2-hexenol is recognized by both IRs and ORs, but OR-signals change the valence of the odor. Furthermore the Fly Arena setup offers the opportunity to examine those changes in valence for single odors, comparing *OrCo*^{-/-} and wild type flies.

6   Acknowledgements

7 References

Ai, M., Min, S., Grosjean, Y., Leblanc, C., Bell, R., Benton, R., & Suh, G. S. (2010). Acid sensing by the Drosophila olfactory system. *Nature*, *468*(7324), 691–695. doi:10.1038/nature09537

Ashburner, M., & Roote, J. (2007). Culture of Drosophila: the laboratory setup. *CSH Protoc*, *2007*, pdb ip34. doi:10.1101/pdb.ip34

Becher, P. G., Bengtsson, M., Hansson, B. S., & Witzgall, P. (2010). Flying the fly: long-range flight behavior of Drosophila melanogaster to attractive odors. *J Chem Ecol*, *36*(6), 599–607. doi:10.1007/s10886-010-9794-2

Benton, R., Vannice, K. S., Gomez-Diaz, C., & Vosshall, L. B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. *Cell*, *136*(1), 149–162. doi:10.1016/j.cell.2008.12.001

Bergmann, A., Yang, A. Y.-P., & Srivastava, M. (2003). Regulators of IAP function: coming to grips with the grim reaper. *Curr Opin Cell Biol*, *15*(6), 717–724. doi:10.1016/j.ceb.2003.10.002

Brand, A. H., & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, *118*(2), 401–415. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8223268

Busto, G. U., Cervantes-Sandoval, I., & Davis, R. L. (2010). Olfactory learning in Drosophila. *Physiology (Bethesda)*, *25*(6), 338–346. doi:10.1152/physiol.00026.2010

Clyne, P., Grant, A., O'Connell, R., & Carlson, J. R. (1997). Odorant response of individual sensilla on the Drosophila antenna. *Invert Neurosci*, *3*(2-3), 127–135. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9783438

Couto, A., Alenius, M., & Dickson, B. J. (2005). Molecular, anatomical, and functional organization of the Drosophila olfactory system. *Curr Biol*, *15*(17), 1535–1547. doi:10.1016/j.cub.2005.07.034

Dahanukar, A., & Ray, A. (2011). Courtship, aggression and avoidance: pheromones, receptors and neurons for social behaviors in Drosophila. *Fly (Austin)*, *5*(1), 58–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20962580

De Bruyne, M., Foster, K., & Carlson, J. R. (2001). Odor coding in the Drosophila antenna. *Neuron*, *30*(2), 537–552. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11395013

Faucher, C. P., Hilker, M., & de Bruyne, M. (2013). Interactions of carbon dioxide and food odours in Drosophila: olfactory hedonics and sensory neuron properties. *PLoS One*, *8*(2), e56361. doi:10.1371/journal.pone.0056361

Flügge, C. (1934). Geruchliche raumorientierung von Drosophila melanogaster. *Journal of Comparative Physiology A: Neuroethology, …*, 463–500. Retrieved from http://www.springerlink.com/index/j024411r0t1l5t82.pdf

Galizia, C. G., Munch, D., Strauch, M., Nissler, A., & Ma, S. (2010). Integrating heterogeneous odor response data into a common response model: A DoOR to the complete olfactome. *Chem Senses*, *35*(7), 551–563. doi:10.1093/chemse/bjq042

Gaudry, Q., Nagel, K. I., & Wilson, R. I. (2012). Smelling on the fly: sensory cues and strategies for olfactory navigation in Drosophila. *Curr Opin Neurobiol*, *22*(2), 216–222. doi:10.1016/j.conb.2011.12.010

Gupta, N., & Stopfer, M. (2012). Functional analysis of a higher olfactory center, the lateral horn. *J Neurosci*, *32*(24), 8138–8148. doi:10.1523/JNEUROSCI.1066-12.2012

Hallem, E. A., & Carlson, J. R. (2004). The odor coding system of Drosophila. *Trends Genet*, *20*(9), 453–459. doi:10.1016/j.tig.2004.06.015

Hay, B. A., Huh, J. R., & Guo, M. (2004). The genetics of cell death: approaches, insights and opportunities in Drosophila. *Nat Rev Genet*, *5*(12), 911–922. doi:10.1038/nrg1491

Jarman, A. P. (2002). Studies of mechanosensation using the fly. *Human Molecular Genetics*, *11*(10), 1215–1218. doi:DOI 10.1093/hmg/11.10.1215

Joiner, W. J., Crocker, A., White, B. H., & Sehgal, A. (2006). Sleep in Drosophila is regulated by adult mushroom bodies. *Nature*, *441*(7094), 757–760. doi:10.1038/nature04811

Joseph, R. M., Devineni, A. V, King, I. F., & Heberlein, U. (2009). Oviposition preference for and positional avoidance of acetic acid provide a model for competing behavioral drives in Drosophila. *Proc Natl Acad Sci U S A*, *106*(27), 11352–11357. doi:10.1073/pnas.0901419106

Kazama, H., & Wilson, R. I. (2008). Homeostatic matching and nonlinear amplification at identified central synapses. *Neuron*, *58*(3), 401–413. doi:10.1016/j.neuron.2008.02.030

Kidokoro, Y. (2003). Roles of SNARE proteins and synaptotagmin I in synaptic transmission: studies at the Drosophila neuromuscular synapse. *Neuro-Signals*, *12*(1), 13–30. doi:10.1159/000068912

Knaden, M., Strutz, A., Ahsan, J., Sachse, S., & Hansson, B. S. (2012). Spatial representation of odorant valence in an insect brain. *Cell Rep*, *1*(4), 392–399. doi:10.1016/j.celrep.2012.03.002

Kreher, S. A., Mathew, D., Kim, J., & Carlson, J. R. (2008). Translation of sensory input into behavioral output via an olfactory system. *Neuron*, *59*(1), 110–124. doi:10.1016/j.neuron.2008.06.010

Laissue, P. P., & Vosshall, L. B. (2008). The Olfactory Sensory Map in Drosophila, 102–114.

Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H., & Vosshall, L. B. (2004). Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. *Neuron*, *43*(5), 703–714. doi:10.1016/j.neuron.2004.08.019

Manning, A. (1967). The control of sexual receptivity in female Drosophila. *Anim Behav*, *15*(2), 239–250. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6030948

Martin, J.C., Keller, A., Sweeney, S. T. (2002). Targeted expression of tetanus toxin: A new tool to study the neurobiology of behavior. *Advances in Genetics*, 47:1-47, Retrieved from http://www.sciencedirect.com/science/article/pii/S0065266002470010

Matsuda, H. (1991). Effects of external and internal K+ ions on magnesium block of inwardly rectifying K+ channels in guinea-pig heart cells. *J Physiol*, *435*, 83–99. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1770455

Montell, C. (2009). A taste of the Drosophila gustatory receptors. *Curr Opin Neurobiol*, *19*(4), 345–353. doi:10.1016/j.conb.2009.07.001

Park, S. K., Shanbhag, S. R., Dubin, A. E., de Bruyne, M., Wang, Q., Yu, P., … Pikielny, C. W. (2002). Inactivation of olfactory sensilla of a single morphological type differentially affects the response of Drosophila to odors. *J Neurobiol*, *51*(3), 248–260. doi:10.1002/neu.10057

Phelan, P., & Starich, T. a. (2001). Innexins get into the gap. *BioEssays : news and reviews in molecular, cellular and developmental biology*, *23*(5), 388–96. doi:10.1002/bies.1057

Richmond, R. C., & Gerking, J. L. (1979). Oviposition site preference in Drosophila. *Behav Genet*, *9*(3), 233–241. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/115458

Rytz, R., Croset, V., & Benton, R. (2013). Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in Drosophila and beyond. *Insect Biochem Mol Biol*, *43*(9), 888–897. doi:10.1016/j.ibmb.2013.02.007

Schlief, M. L., & Wilson, R. I. (2007). Olfactory processing and behavior downstream from highly selective receptor neurons. *Nat Neurosci*, *10*(5), 623–630. doi:10.1038/nn1881

Simpson, L. L. (1986). BOTULINUM TOXIN AND TETANUS TOXIN. *Annu Rev Pharmacol Toxicol*, *26*, 427–53.

Steck, K., Veit, D., Grandy, R., Badia, S. B. I. B. I., Mathews, Z., Verschure, P., … Knaden, M. (2012). A high-throughput behavioral paradigm for Drosophila olfaction - The Flywalk. *Sci Rep*, *2*, 361. doi:10.1038/srep00361

Su, C. Y., & Carlson, J. R. (2013). Neuroscience. Circuit logic of avoidance and attraction. *Science*, *340*(6138), 1295–1297. doi:10.1126/science.1240139

Thum, A. S., Knapek, S., Rister, J., Dierichs-Schmitt, E., Heisenberg, M., & Tanimoto, H. (2006). Differential potencies of effector genes in adult Drosophila. *J Comp Neurol*, *498*(2), 194–203. doi:10.1002/cne.21022

Touhara, K., & Vosshall, L. B. (2009). Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol*, *71*, 307–332. doi:10.1146/annurev.physiol.010908.163209

Venkatesh, S. (1984). Sensilla on the third antennal segment of Drosophila Melanogaster Meigen. *Int. J. Insect Morphol. & Embryol.*, *13*(1), 51–63.

Venken, K. J., Simpson, J. H., & Bellen, H. J. (2011). Genetic manipulation of genes and cells in the nervous system of the fruit fly. *Neuron*, *72*(2), 202–230. doi:10.1016/j.neuron.2011.09.021

Vosshall, L. B., Wong, A. M., & Axel, R. (2000). An olfactory sensory map in the fly brain. *Cell*, *102*(2), 147–159. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10943836

Wicher, D., Schäfer, R., Bauernfeind, R., Stensmyr, M. C., Heller, R., Heinemann, S. H., … Schafer, R. (2008). Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature*, *452*(7190), 1007–1011. doi:10.1038/nature06861

Williamson, L. C., Halpern, J. L., Montecucco, C., Brown, J. E., & Neale, E. A. (1996). Clostridial neurotoxins and substrate proteolysis in intact neurons: botulinum neurotoxin C acts on synaptosomal-associated protein of 25 kDa. *J Biol Chem*, *271*(13), 7694–7699. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8631808

Wilson, R. I. (2013). Early olfactory processing in Drosophila: mechanisms and principles. *Annual review of neuroscience*, *36*, 217–41. doi:10.1146/annurev-neuro-062111-150533

Yamaguchi, S., Desplan, C., & Heisenberg, M. (2010). Contribution of photoreceptor subtypes to spectral wavelength preference in Drosophila. *Proc Natl Acad Sci U S A, 107*(12), 5634–5639. doi:10.1073/pnas.0809398107
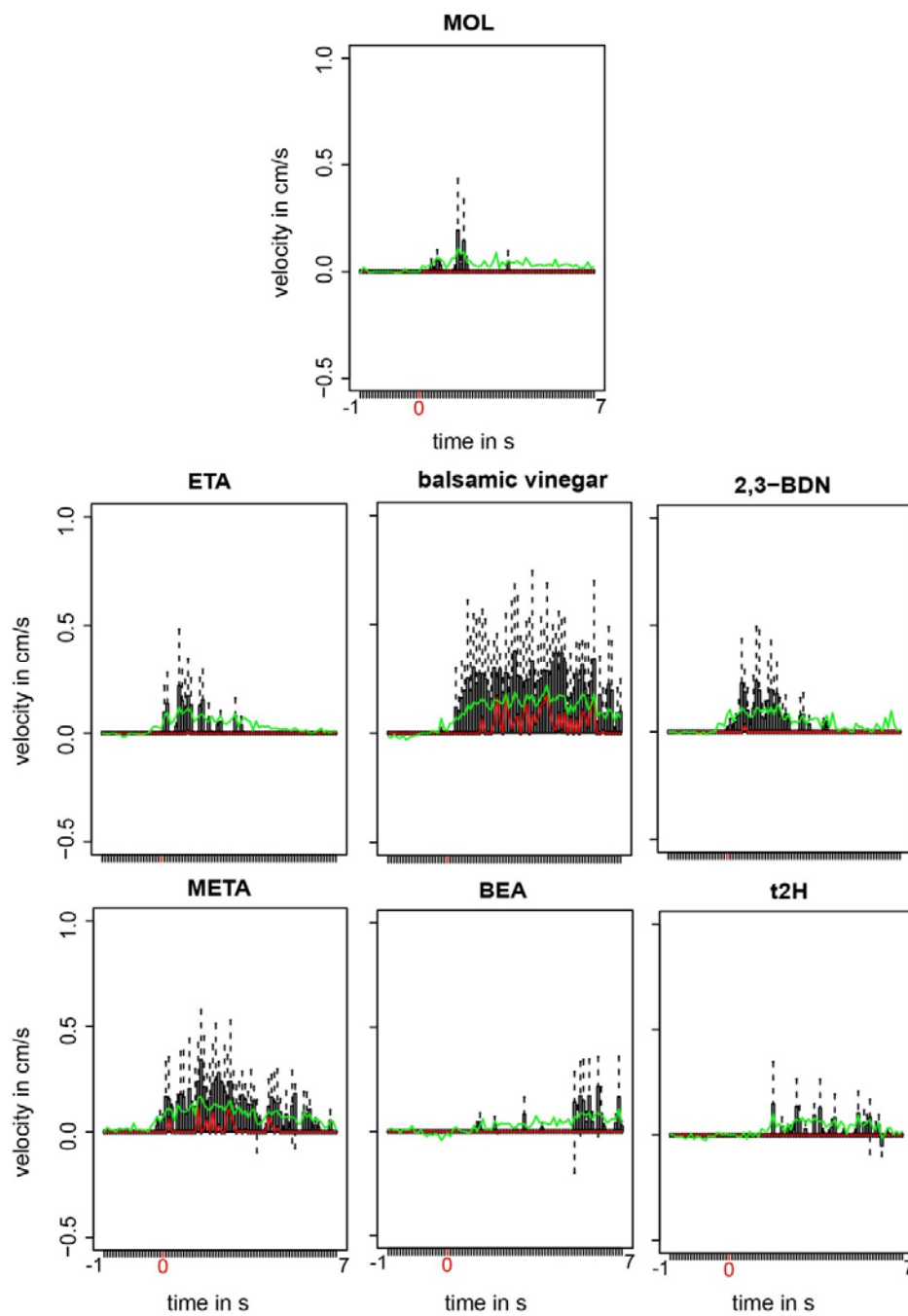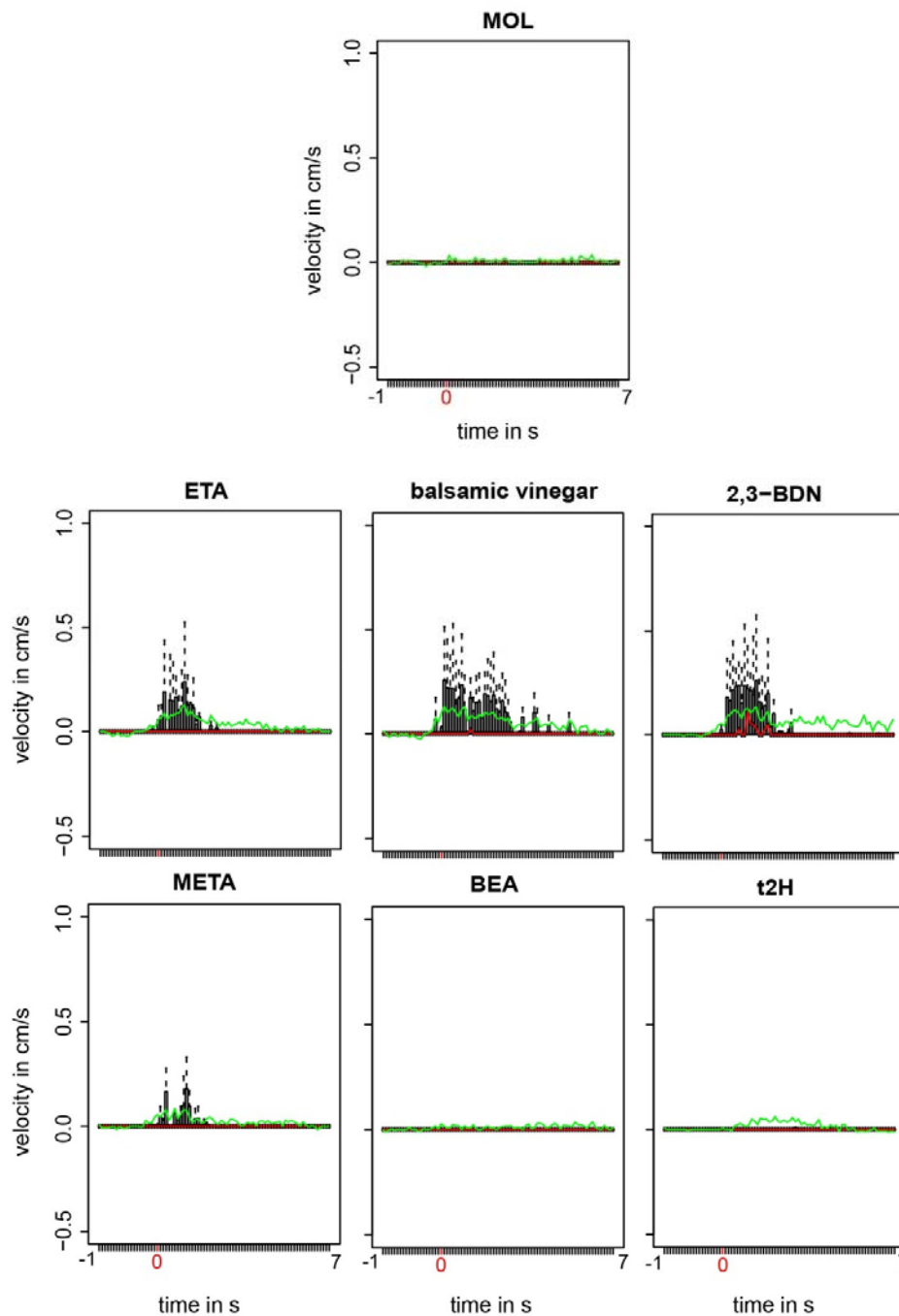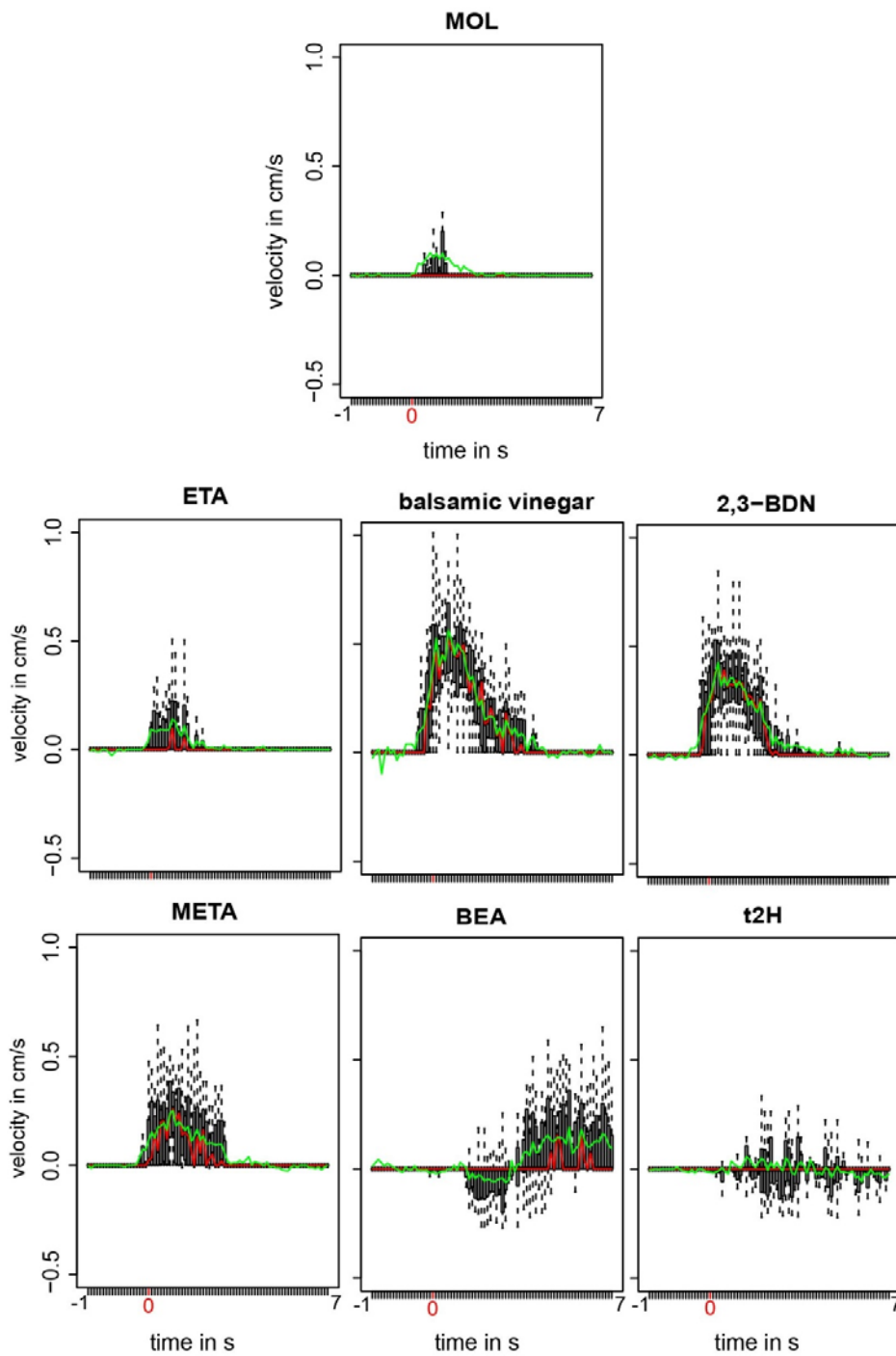
8   Supplementary
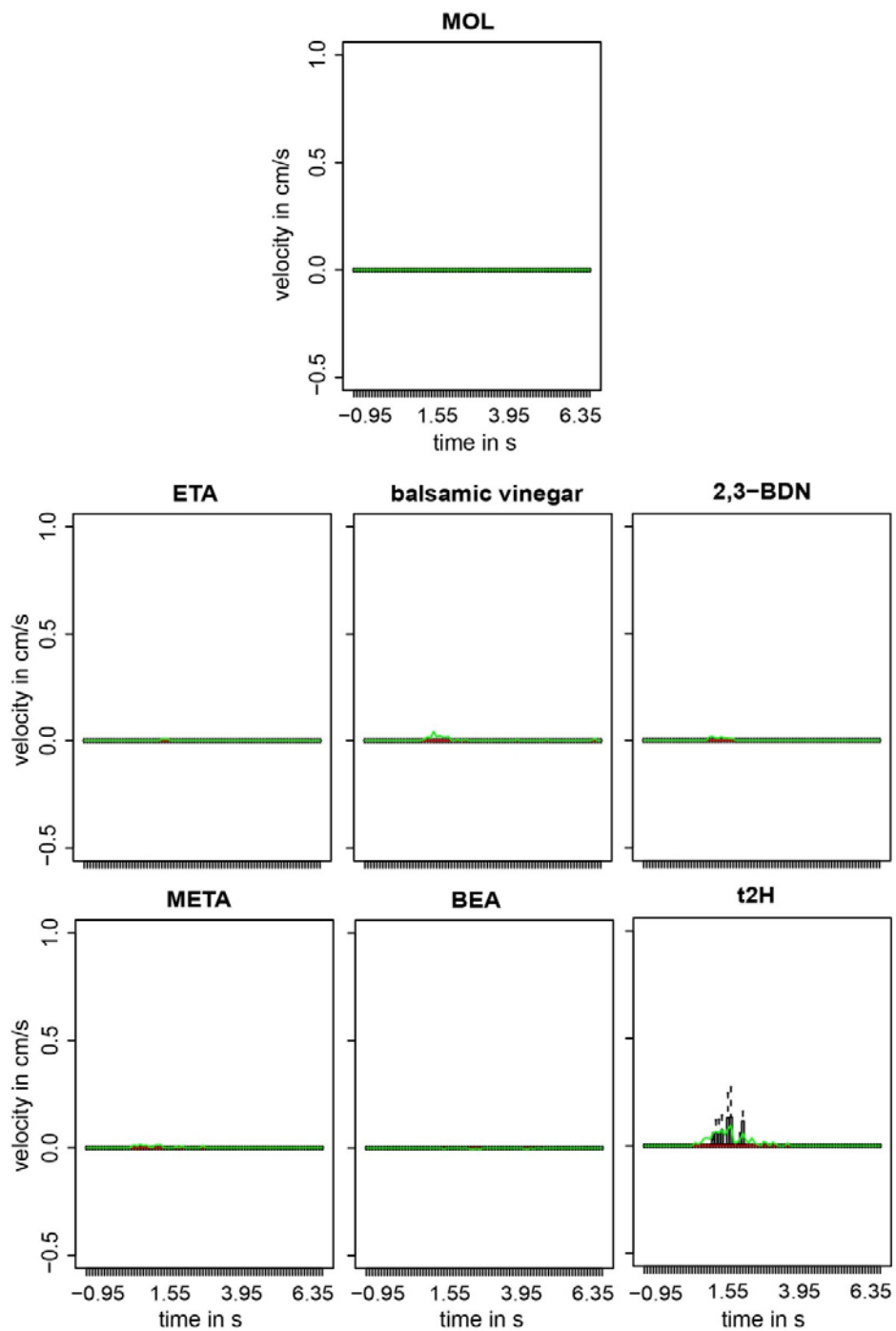
S1: A Speed overview of *OrCo*$^{-/-}$ mutant flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]
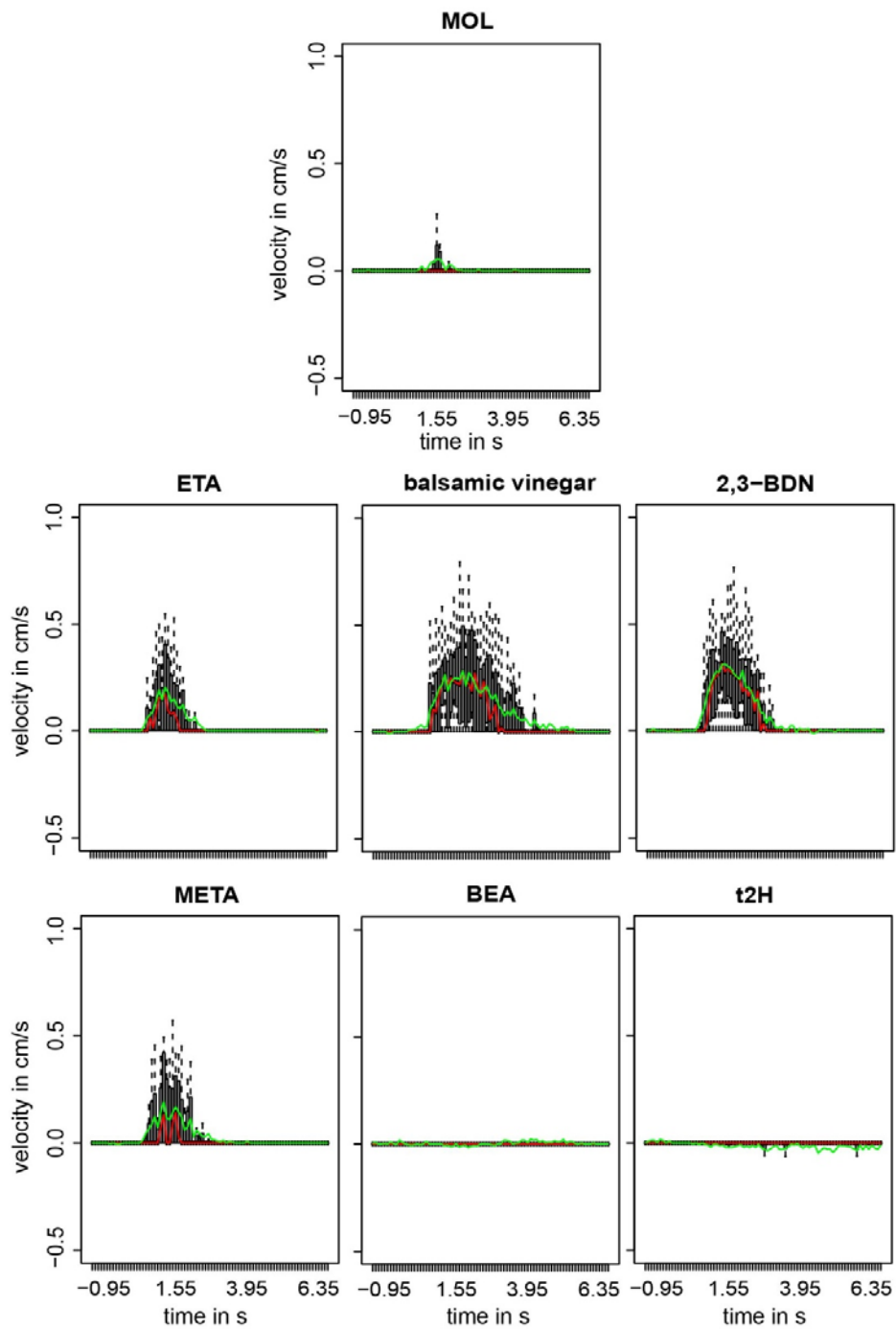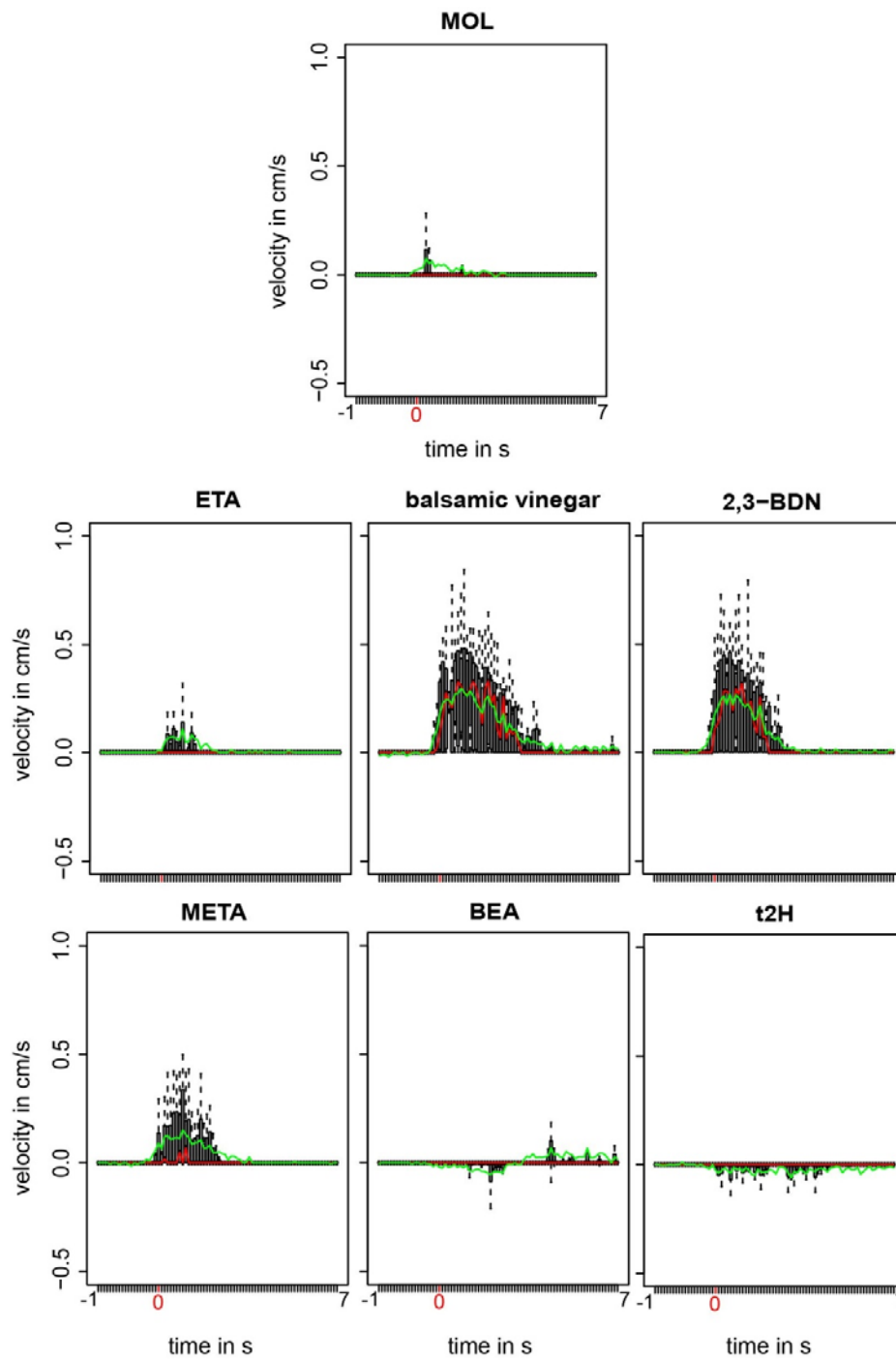
S2: A Speed overview of OrCo-Gal4 parental flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]
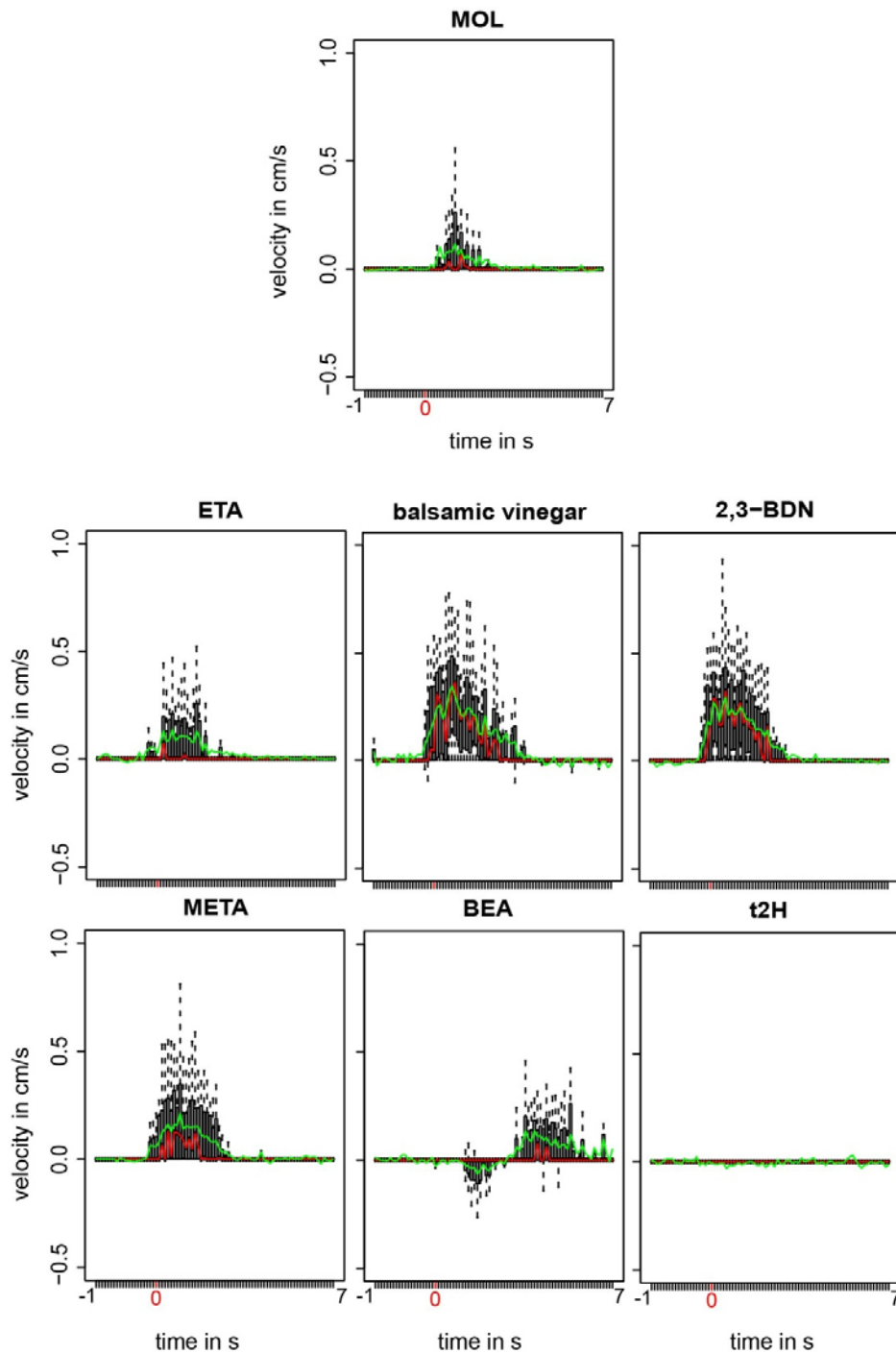
S3: A Speed overview of UAS-DTA parental flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]

S4: A Speed overview of *DTA*-expressing flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]
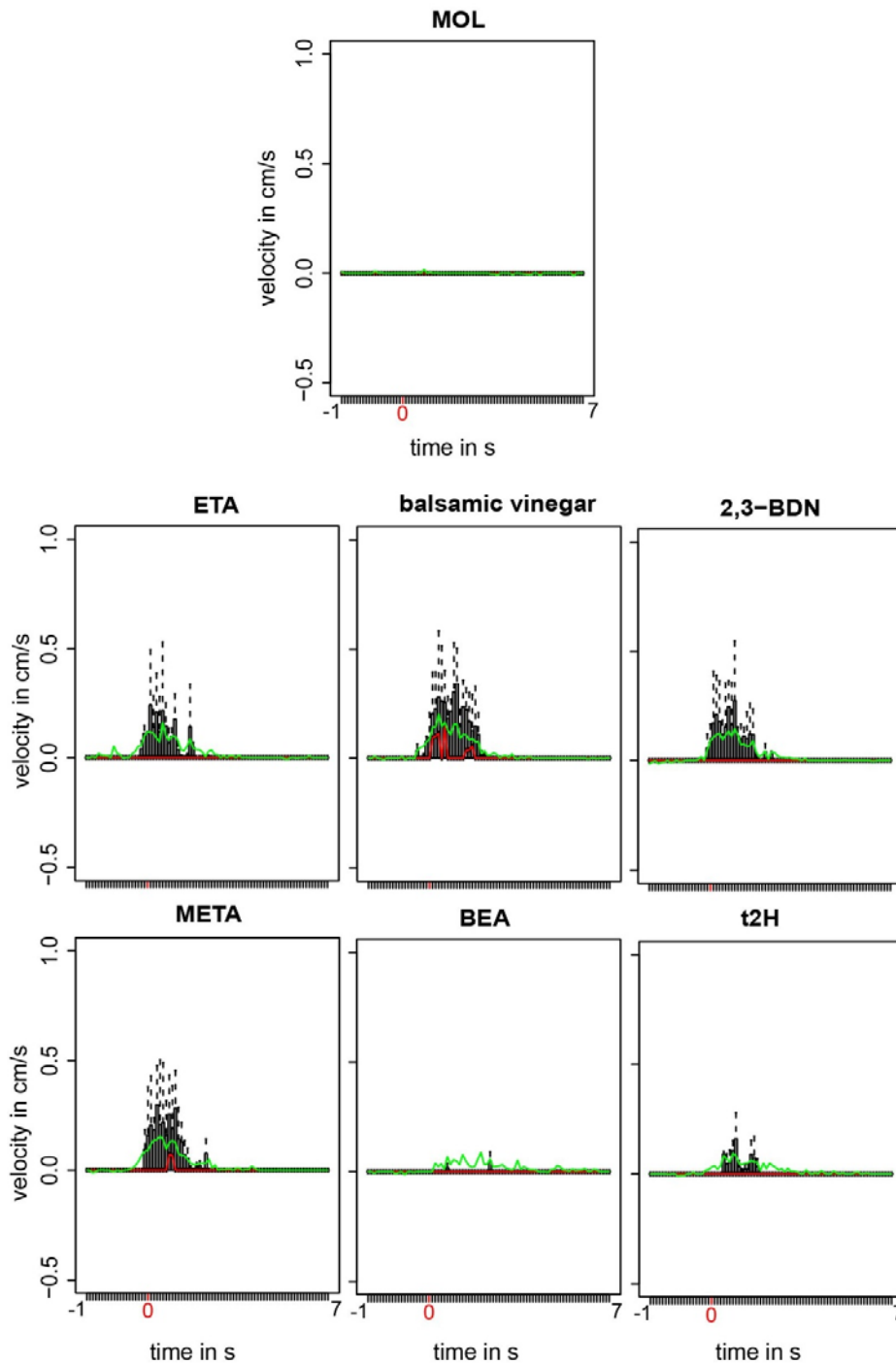
S5: A Speed overview of UAS-rpr parental flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]
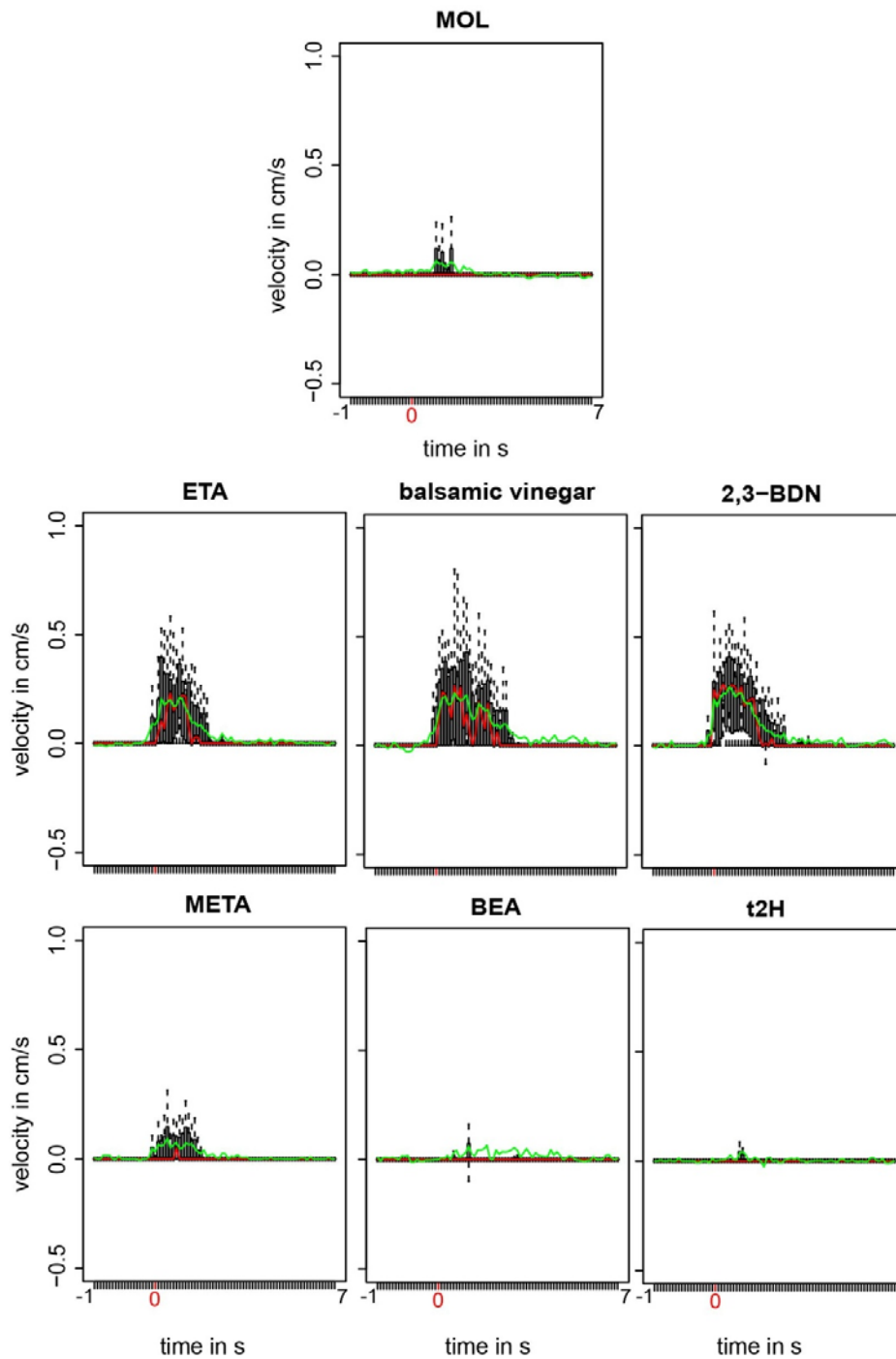
S6: A Speed overview of *rpr*-expressing flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]
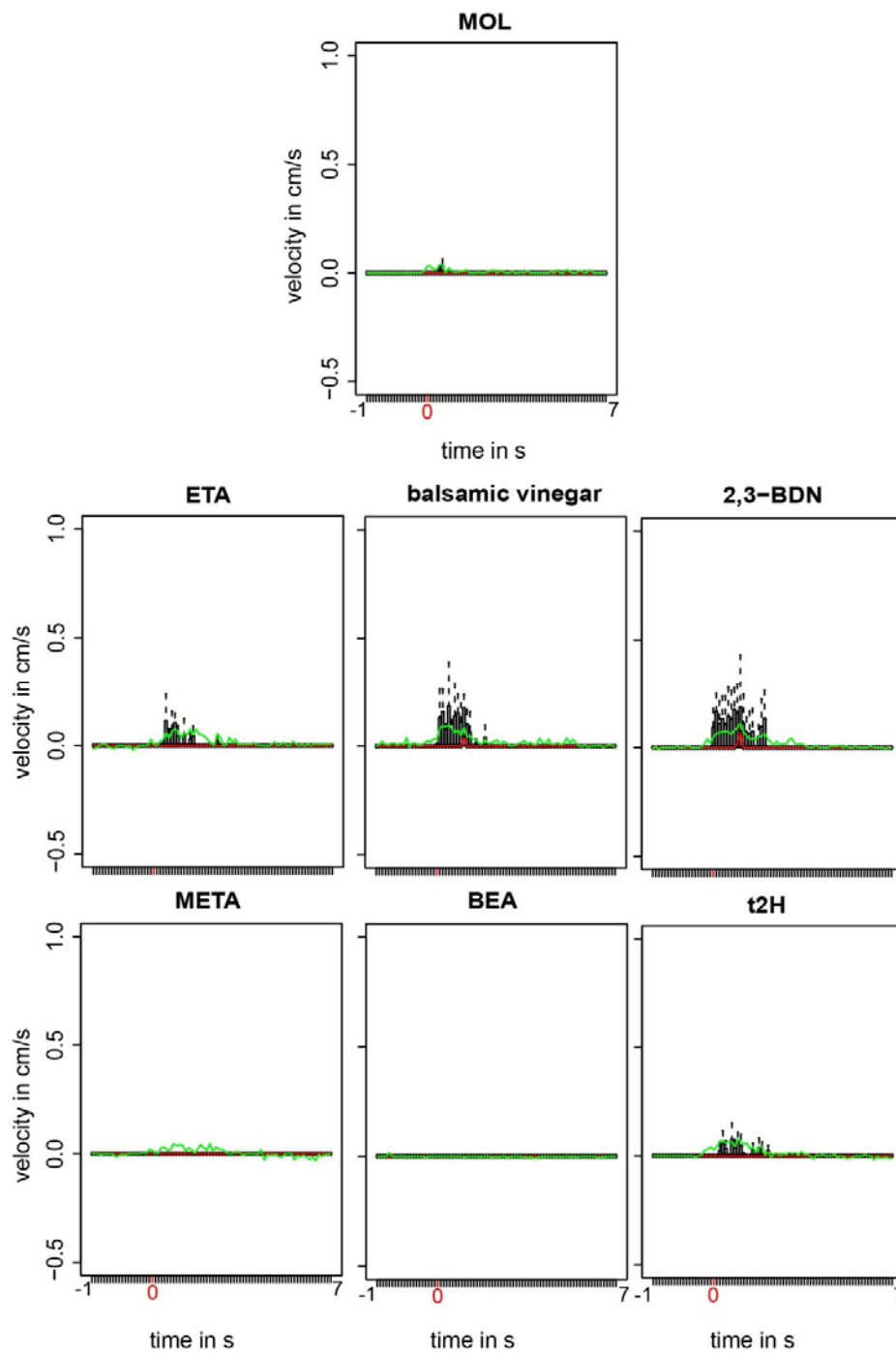
S7: A Speed overview of UAS-Kir2.1 parental flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]

S8: A Speed overview of *Kir2.1*-expressing flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]

S9: A Speed overview of UAS-TeTx parental flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]

S10: A Speed overview of *TeTx*-expressing flies. The meeting time of fly and odor is marked by the time point 0. Positive velocity values indicate an upwind direction, while negative values indicate down wind direction. green line = median, red line = mean, MOL = Mineral oil, ETA = Ethyl acetate [10^{-3}], 2,3-BDN = 2,3-Butanedione [10^{-3}], BEA = Benzaldehyde [10^{-1}], META = Methyl acetate [10^{-3}], t2H = E2-Hexanol [10^{-1}]

distillled water   balsamic vinegar
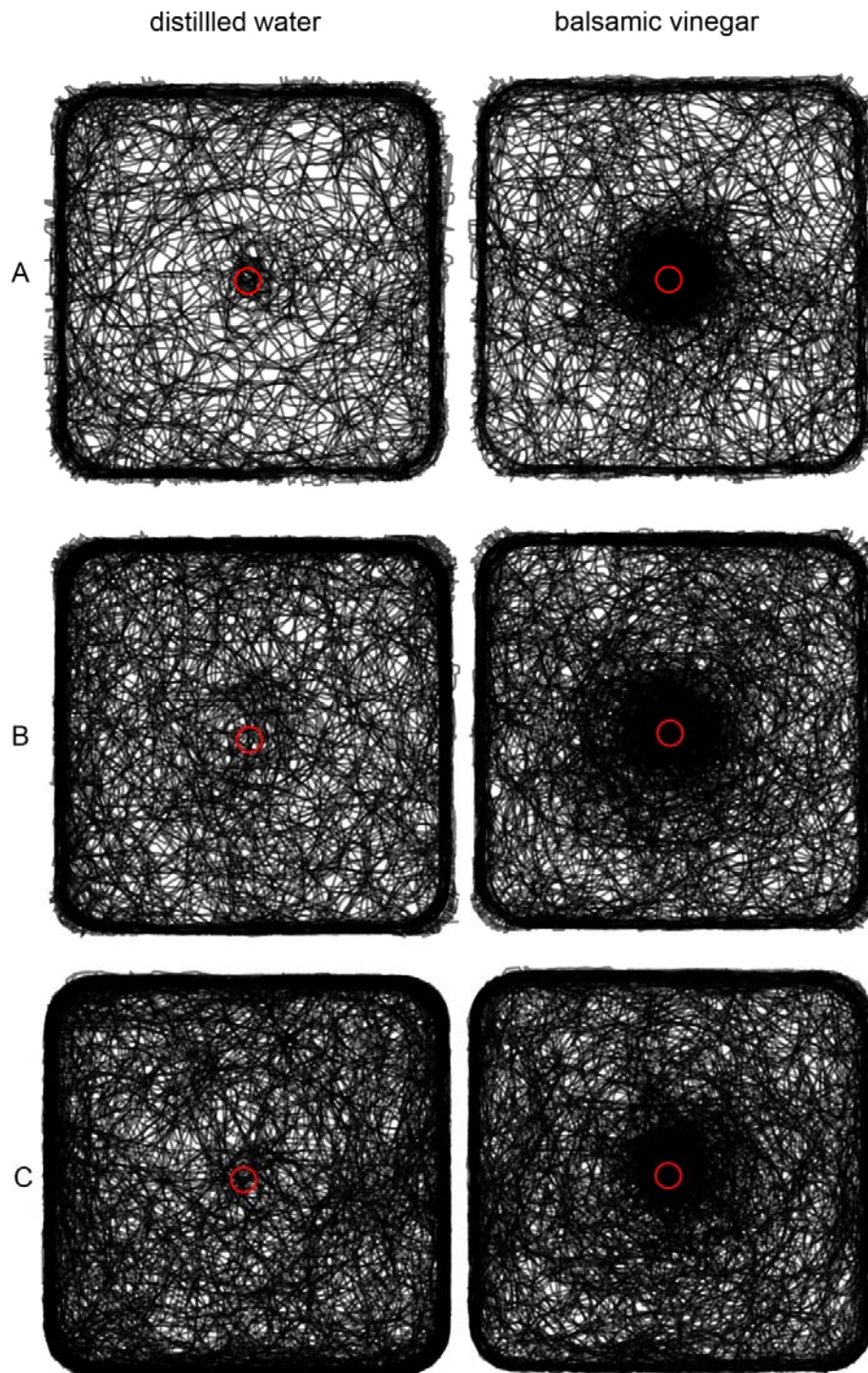
S11: Path plot of control flies. A) wild type, B) OrCo-Gal4 parental control flies, C) *OrCo*$^{-/-}$ **mutant flies** Each plot consists of overlaid searching tracks of 20 single flies. Tracking time = 10 min, red circle = odor source (to scale)
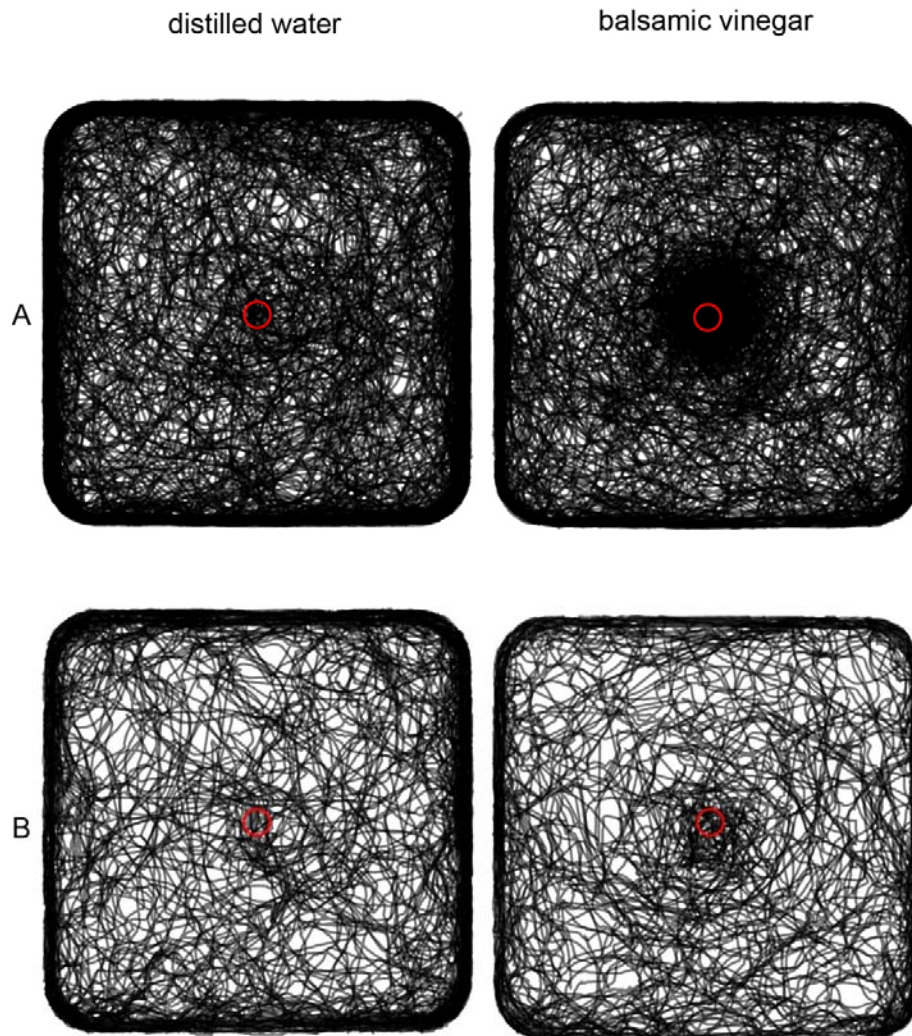
distilled water   balsamic vinegar

A

B
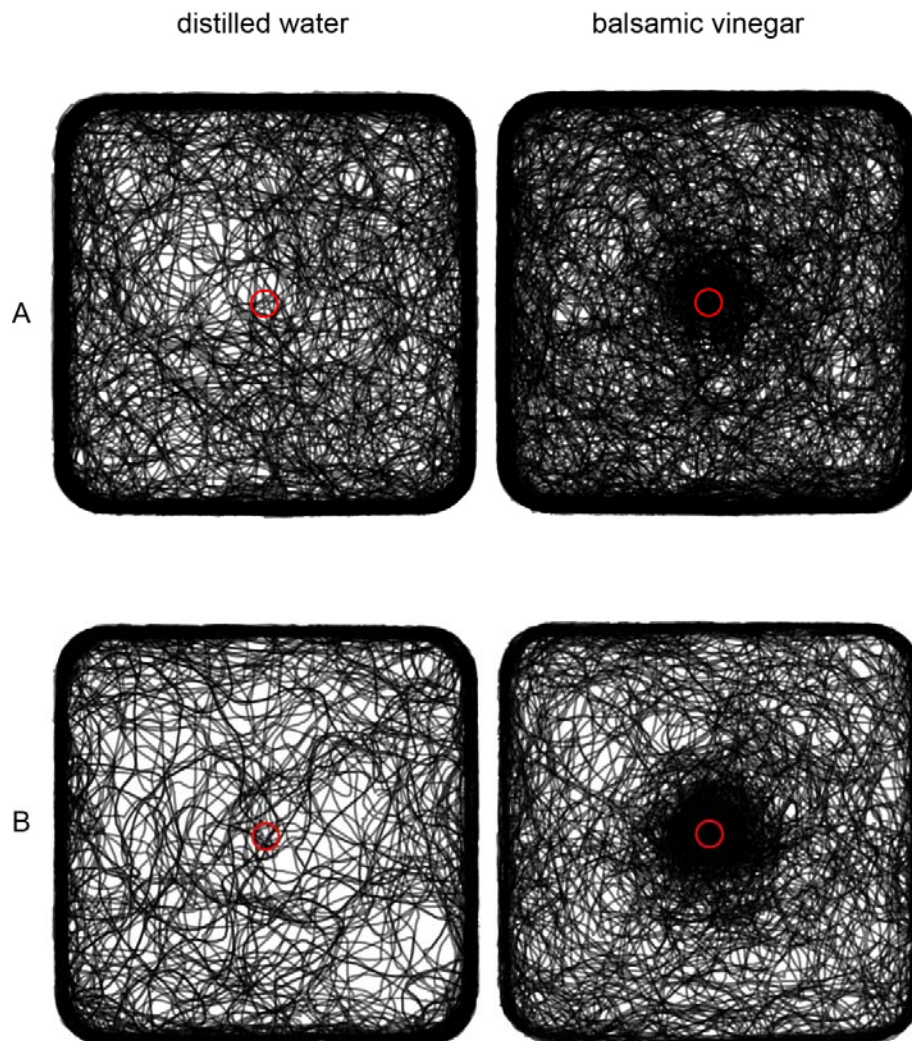
S12: Path plot of *DTA*-expressing flies. A) UAS-DTA parental control flies, B) experimental flies expressing *DTA* Each plot consists of overlaid searching tracks of 20 single flies. Tracking time = 10 min, red circle = odor source (to scale)

distilled water   balsamic vinegar
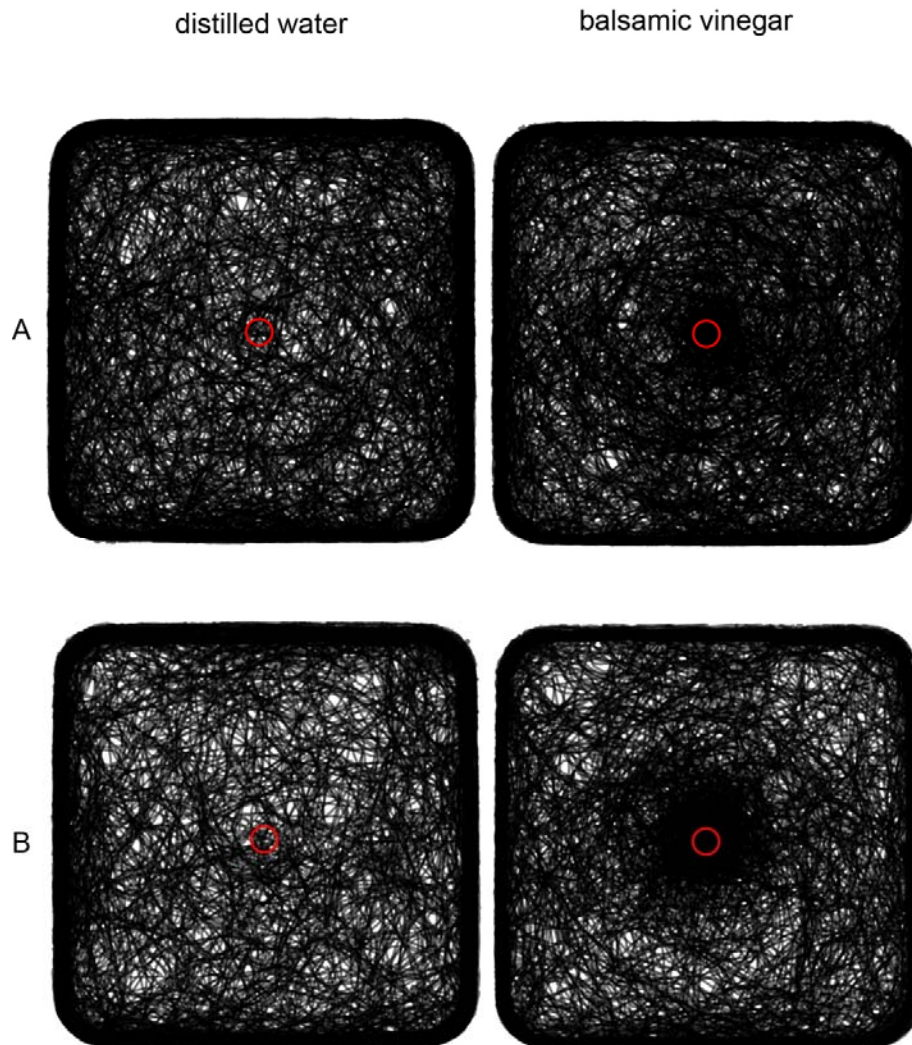
S13: Path plot of *rpr*-expressing flies. A) UAS-rpr parental control flies, B) experimental flies expressing *rpr* Each plot consists of overlaid searching tracks of 20 single flies. Tracking time = 10 min, red circle = odor source (to scale)

distilled water   balsamic vinegar
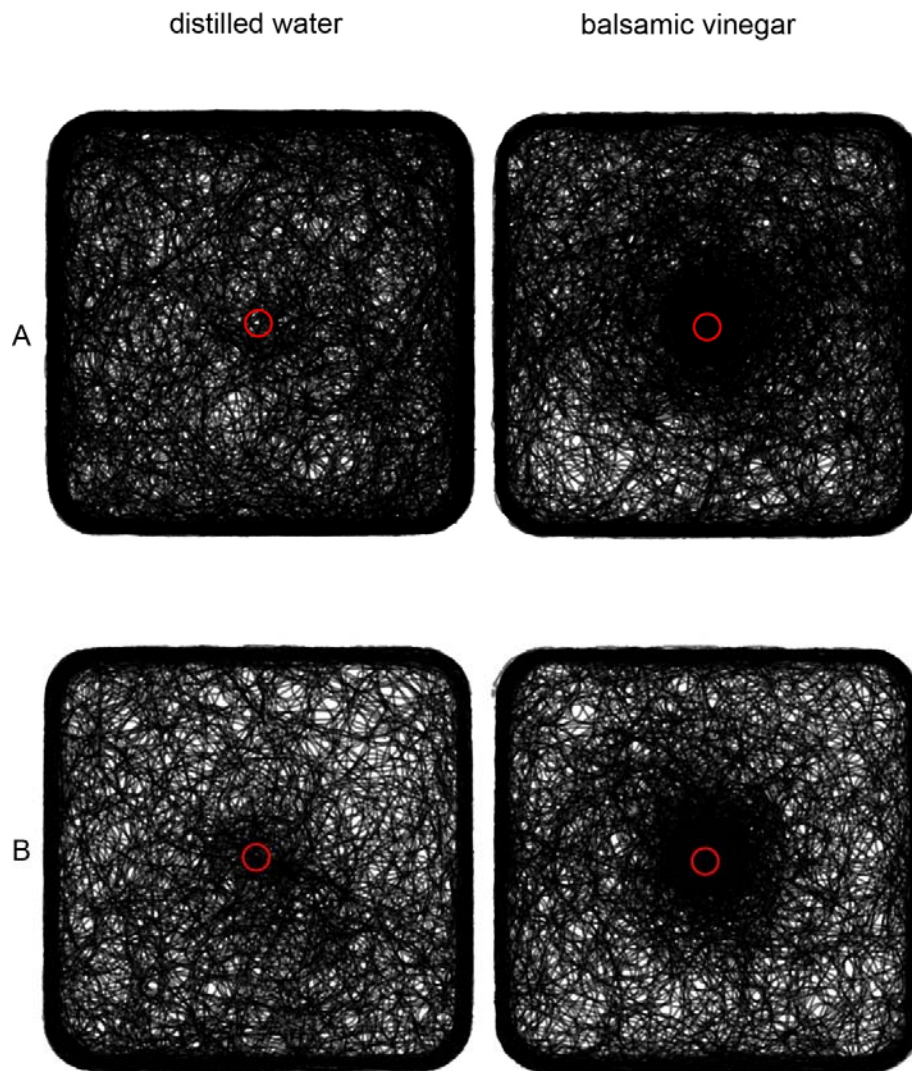
S14: Path plot of *Kir2.1*-expressing flies. A) UAS-Kir2.1 parental control flies, B) experimental flies expressing *Kir2.1* Each plot consists of overlaid searching tracks of 20 single flies. Tracking time = 10 min, red circle = odor source (to scale)

distilled water   balsamic vinegar

S15: Path plot of *TeTx*-expressing flies. A) UAS-TeTx parental control flies, B) experimental flies expressing *TeTx* Each plot consists of overlaid searching tracks of 20 single flies. Tracking time = 10 min, red circle = odor source (to scale)

9   Declaration of Original Authorship

I hereby certify that this work presented is entirely my own and the result of my investigation. The material from other sources has been fully and properly acknowledged.

Jena, 12/12/2013   ....