Friedrich-Schiller-Universität Jena

Biologisch-Pharmazeutische Fakultät

Max-Planck-Institut für Chemische Ökologie

seit 1558

Drosophila olfaction: what is the impact of selected chemical properties on the valence of an odorant?

Diplomarbeit

zur Erlangung des Grades eines

Diplom-Biochemikers

vorgelegt von

Marcus Ehlert

Jena, Dezember 2013

Declaration of authorship:

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

Jena, 19.12.2013

Acknowledgements

Abstract

Olfaction plays a crucial role in the life of insects. They are exposed to plumes containing a wide range of odorants and have to discriminate attractive and repulsive odorants. In *Drosophila melanogaster* odorant detection takes place in olfactory sensory neurons housed in three types of olfactory sensilla on the antennae and the maxillary palps. Neurons housed in basiconic and trichoid sensilla express odorant receptors (ORs) together with the coreceptor Orco, while neurons housed in the coeloconic sensilla express another receptor type, the ionotropic receptors (IRs). While ORs mainly detect food odorants and pheromones IRs are involved in the detection of acids and amines. The axons of the neurons project into the antennal lobe - the first olfactory processing center.

With a newly developed high-throughput behavioral assay, the *Flywalk*, I investigated the behavior of wild type flies to a range of odorants at different concentrations. Furthermore, I investigated if ionotropic receptors have an impact on the hedonic valence of these odorants using Orco -/- mutant flies, i.e. flies that lack functional ORs. I revealed attractive as well as repulsive behavior and also an impact of ionotropic receptors on the hedonic valence of some odorants. I next investigated the impact of physicochemical properties on the hedonic valence of an odorant. Correlation analyses revealed an impact of the vapor pressure and the boiling point with attractive compounds exhibiting significant higher vapor pressures and lower boiling points. I also found an impact of the carbon chain length, with short esters being more attractive than long esters. By performing single sensillum recordings I found that short, i.e. attractive, esters are detected by different sensilla than long, less attractive esters. This suggests that sensilla housing on specific subsets of olfactory sensory neurons are involved in the processing of attraction and aversion.

Contents

1. Introduction

1.1. Olfaction and the olfactory system of *Drosophila melanogaster*

In their habitat, most animals are surrounded with mixtures of a wide range of natural chemicals. Chemicals are able to irritate cells and it is presumed that these irritations led to the evolution of functional olfactory systems including specific chemosensory molecules and functional organs reviewed in Ache and Young (2005). It is an impressing fact that even though the olfactory systems evolved independently in vertebrates and invertebrates, they share main features regarding the detection and processing of odor information (Hildebrand and Shepherd, 1997) - which points at the crucial role of olfaction.

D. melanogaster is exposed to a multiplicity of natural odorants and to handle its life and survive, the sense of smell plays an essential role. Searching and identification of suitable food sources, mate selection affected by pheromones and locating oviposition sites are vital functions mainly enabled by olfaction (Zhu et al., 2003; Bartelt et al., 1985; Becher et al., 2012). Detecting and processing odor mixtures during free-flight requires a complex but well organized olfactory system. While odors are detected by sensilla located on the fly's antennae and palps, the further processing of olfactory information takes place in specialized brain centers.

The sensilla - fine structures for odorant detection

The surface of the antennae and palps is covered by innervated cuticular hair structures, the sensilla. Different morphological shapes led to classification into four distinct types: basiconic, trichoid, coeloconic and intermediate sensilla which are all well described by Shanbhag et al. (1999). The first three types will be further described. The basiconic sensilla are dependent on their size classified into three types (large, thin and small) and located on the antennae and maxillary palps. Every sensillum is usually innervated by two or four receptor cells and mainly activated by food-related odorants like acetates or esters (de Bruyne et al., 1999, 2001). The trichoid sensilla are located on the insect

antennae and maxillary palps. These sensilla are innervated by up to three receptor neurons and show no response to food-related odorants but to hexane extracts of fly bodies. The fly pheromone *cis*-Vaccenyl acetate (cVA) is an identified ligand of the trichoid sensilla (van der Goes van Naters and Carlson, 2007) what presumes a role as pheromone sensors. The coeloconic sensilla are located on the antennae. These sensilla are innervated by two or three receptor neurons and able to detect water vapor which indicates a role as humidity sensors, what is important for e.g. oviposition (Yao et al., 2005). In addition, coeloconic sensilla show mainly responses to acids and amines (Ai et al., 2010; Silbering et al., 2011). All sensilla are covered with cuticular pores on the surface for odorant entry and filled with aqueous lymph produced by accessory cells of the sensilla.

The sensillar lymph contains so-called odorant-binding proteins (OBP). After an odorant passed the pores and the aqueous lymph it is presumed that the OBPs solubilize the mostly hydrophobic odorants and present them to their associated receptor expressed by the neuron in this sensillum (Hekmat-Scafe et al., 2002; Park et al., 2000). Furthermore, investigation of Kim et al. (1998) has shown that *Drosophila* mutants with a lack of the OBP Lush show an absent repulsion as effect to high ethanol concentrations, which implies an involvement of OBP in ligand removing. On its way to e.g. oviposition sites or food sources *D. melanogaster* has to process numerous stimuli from a wide range of odorants. This requires a high temporal resolution of the olfactory system. Additionally, chemical compounds can cause toxic effect on the cells. Thus it becomes clear that not only the presentation of an odorant to a receptor is essential but also its degradation. Odorant-degrading enzymes such as esterases or oxidases are able to degrade stimulating odorants by chemical modification. A recent study has identified a carboxylesterase in *Drosophila*, Esterase-6, that degrade cVA to modulate sensory physiological and behavioral response dynamics (Chertemps et al., 2012).

Odorant detection on a cellular level

After an odorant passed the sensillum and its lymph it interacts with a specific odorant receptor (OR) which is located on the olfactory sensory neuron (OSN). These are the primary sensory cells for odorant detection. In *D. melanogaster* about 1200 OSNs are located in the antennae (Shanbhag et al., 1999) and about 120 in the maxillary palps (Stocker, 1994). OSNs send their dendrites into the sensillum lymph and the OR is expressed in the outer dendrite membrane. ORs are seven transmembrane domain G protein-coupled receptors (GPCR) but in contrast to other GPCRs an inverted orienta-

tion in the plasma membrane was observed (Benton et al., 2006). ORs can be activated by several odorants and in addition one odorant can activate more than one OR (de Bruyne et al., 2001). Odorant receptors can respond excitatory or inhibitory; some odorant receptors are able to respond in both modes dependent on the stimuli (de Bruyne et al., 2001). *D. melanogaster* has a repertoire of about 62 ORs (Robertson et al., 2003) with every OSN expressing one or (seldom) two odorant receptors and usually a coreceptor Or83b (Larsson et al., 2004) which is termed Orco (Vosshall and Hansson, 2011). In contrast to other ORs this chaperone protein is highly conserved and has clear homologs in other insect species. The coreceptor alone is not able to respond to odorants (Elmore et al., 2003) but after heterodimerization with the expressed OR it enhances the ligand responsiveness of the OR (Neuhaus et al., 2005). Dimers of OR and Orco are shown to be ligand-gated and cyclic-nucleotide-activated cation channels essential for signal transduction (Wicher et al., 2008). Orco null mutants show behavioral defects with *Drosophila* larvae exhibiting a loss of chemotaxis and adults exhibiting no response to most tested odorants. Additionally, defects in dendritic localization were observed (Larsson et al., 2004). Besides the described odorant receptors another distinct type of olfactory receptors has been described, the ionotropic receptors (IR). The IRs are related to ionotropic glutamate receptors but lack glutamate binding site. Benton et al. (2009) showed that 15 IR genes are expressed in a combinatorial fashion in the coeloconic sensilla of the antennae and are not coexpressed with ORs or Orco (Benton et al., 2009). Up to five coexpressed IRs are observed in one OSN implicating that these receptors are able to form multimeric complexes. In most cases one or both of the coreceptors IR8a or IR25a is also expressed in the OSN. Acids (Ai et al., 2010) and amines (Silbering et al., 2011) were shown as typical ligands of IRs.

Superior structures for odorant processing

The antennal nerve consisting of all olfactory sensory neuron (OSN) axons enters the brain and projects to a bilaterally symmetric structure, the antennal lobe (AL). Here, all axons from OSNs expressing the same receptor converge to a spherical structure called glomerulus. The AL consists of 60 glomeruli which exhibit a relation between the glomerulus size and the number of incoming axons (Vosshall et al., 2000). It is also known that OSNs from different sensillum types project to different antennal lobe regions. In the glomeruli the OSN axons have synaptic contacts to projection neurons (PN) which send their axons to higher brain centers (Hildebrand and Shepherd, 1997). Furthermore, various glomeruli are linked by local interneurons (LN). By expressing the neurotransmitter acetylcholine LNs mediate excitatory effects in *Drosophila* (Shang et al.,

2007). In contrast, inhibition was shown to be mediated by γ-aminobutyric acid (GABA) (Wilson and Laurent, 2005). There is another type of neurons, the extrinsic neurons, releasing additional neurotransmitters. The described variety of different neuron cells, their conjunction and the interplay of different neurotransmitters enables a multiplicity of processing possibilities already at the level of the AL. Recent findings suggest that e.g. the representation of hedonic valence of single odorants is already formed within the AL (Knaden et al., 2012).

Role of higher brain centers

The projection neurons in the antennal lobe send their axons to higher brain centers like the mushroom bodies and lateral protocerebrum. Mushroom bodies are shown to be involved in the processing of sex pheromones (Ferveur et al., 1995) as well as learning processes (Heisenberg et al., 1985) and are supposed to be involved in sexual behavior (Odell et al., 1995). Apart from that, neutransmission blockade in mushroom bodies of *Drosophila* resulted in a disruption of responses to attractive odorants but not to repulsive odorants. This implicates a separately processing of response modes in higher brain structures (Wang et al., 2003).
The lateral protocerebrum gets input from premotor neurons. This enables a connection from the stimulus to a fast and innate behavioral response which is thought to rely on circuits on the lateral horn (Heimbeck et al., 2001).

In conclusion, a set of different sensilla housing OSNs expressing many different ORs and IRs allows detection of a large set of odorants in *D. melanogaster*. A multitude of inhibitory and excitatory connections between different neuron types enables odorant processing within the AL. Finally, higher brain structures convert these processing patterns into an adequate behavior. For a better understanding how *D. melanogaster* manages its life-tasks, the investigation of odor-evoked behavior, which is the main topic of this thesis, is essential.

1.2. The *Flywalk*: a new technique for measuring olfaction-related behavior in *Drosophila*

The simple and fast rearing of *D. melanogaster* has made this animal to a ubiquitous study subject. Due to the finished sequencing of its genome and the easy handling for genetic experiments to determine the function of selected genes *D. melanogaster* is regarded

as a model organism. Many olfaction-related tests with *D. melanogaster* have been conducted and different techniques for tests are available. Single sensillum recordings (SSR) revealed the effect of odorants on selected OSNs of one sensillum. For example, de Bruyne et al. (2001) tested and identified responses from 47 odorants to different OSN classes in basiconic sensilla. The SSR technique enables to measure activating or inhibiting effects but gives no information on the hedonic valence of an odorant. Trap assays or T-mazes have been used extensively for behavioral tests with *Drosophila*. However, they have the main drawback that several flies become tested in one experiment and thus it is not possible to draw conclusions on the behavior of individual flies. Furthermore, free-flying *D. melanogaster* are exposed to changing odor plumes that are not comparable to the continuous odorant stimuli in classical behavior assays. Finally, the continuous stimulation in the trap assay and T-maze can cause the neuronal adaption to odorants, which again complicates the interpretation of any observed behavior. To overcome these issues Budick and Dickinson (2006) established *Drosophila* experiments in a wind tunnel in which flying *Drosophila* had to follow odorant plumes. The major backdraw of this technique, however, is that it is designed only for single individuals. For meaningful conclusions this very time consuming technique has to be repeated several times.

A newly developed high-throughput behavioral assay is the *Flywalk* (Steck et al., 2012). The technique will be described and illustrated in Chapter 2.4.3.. The system is based on the interaction between an odor-delivery system and a tracking system. The odor-delivery system produces identical odorant pulses to which up to 15 individual flies are exposed. Each fly can walk freely in a single glass tube. The movement of every fly is then recorded by a tracking system. Analyzing the data from the stimulus device and the tracking system as an open-loop paradigm allows the exact determination of the behavioral response upon stimulus arrival.

Because several flies are simultaneously tested and up to eight odor stimuli can be presented repeatedly during one experiment, one can draw meaningful conclusions from already few experiments.

1.3. Aim of this study

This study targets the following three main questions:

1. How do *D. melanogaster* wild type flies react to a range of 18 different odorants at three different concentrations?

2. What is the impact of ionotropic receptors on the hedonic valence of these odorants?

3. Which physicochemical properties of an odorant have an impact on the hedonic valence in wild type flies?

To target these questions I expose wild type flies and flies which lack functional ORs to numerous olfactory stimuli in the *Flywalk* paradigm. I furthermore conduct single sensillum recordings in order to investigate, how chain length affects the detection of odorants in different OSN types.

2. Methods

2.1. List of abbreviations

AL	Antennal lobe
ANOVA	Analysis of variance
cVA	*cis*-Vaccenyl acetate
GPCR	G protein-coupled receptor
GR	Gustatory receptor
IR	Ionotropic receptor
LED	Light-emitting diode
LN	Local interneuron
OBP	Odorant-binding protein
OR	Odorant receptor
OSN	Olfactory sensory neuron
PID	Photo-ionization detector
PN	Projection neuron
SSR	Single sensillum recording

2.2. Fly rearing

Stock cultures of *Drosophila melanogaster* wild type flies (Canton S, Bloomington) and Orco -/- mutants (BL23129, Bloomington) were separately reared in food vials containing standard agar-cornmeal medium.

Standard agar-cornmeal medium

980 ml	Water
118 g	Treacle
95 g	Cornmeal
11 g	Barm
4,1 g	Agar- Agar
3,3 ml	Methyl-4-hydroxy-benzoate (30 %)
2,4 ml	Propionic acid (99 %)

Vials were maintained at 25 ℃, 70 % relative humidity and 12 hours day-night cycle. Eclosed flies were transferred into a fresh vial containing the standard agar-cornmeal medium daily. Stock cultures were refreshed weekly by transferring eclosed flies into a big food vial and took out after one week.

2.3. Odorants

Producer	Odorant
Sigma-Aldrich, St. Louis, USA	2-Phenylethyl alcohol
	2,3-Butanedione
	3-(Methylthio)-1-propanol
	Acetophenone
	n-Butyl acetate
	γ-Butyrolactone
	β-Caryophyllene
	Ethyl acetate
	Ethyl propionate
	(R)-(-)-Fenchone
	Geranyl acetate
	n-Heptyl acetate
	n-Hexyl acetate
	Methyl acetate
	Methyl salicylate
	n-Octyl acetate
	n-Pentyl acetate
	n-Propyl acetate

Table 2.2.: Odorants used in this study

2.4. Measuring the odor-evoked behavior and activity using the *Flywalk*

2.4.1. Fly preparation

In all experiments, adult female flies (four to six days old) were used. Before the experiments, the flies were starved in a small vial containing a water-saturated plug on the bottom at 25 ℃, 70 % relative humidity and 12 hours day-night cycle.

2.4.2. Stimulus preparation

In the experiments, different dilutions (10^{-1}, 10^{-3}, 10^{-5}) of the pure odorants (see Table 2.2.) were tested. Dilutions were prepared as follows: Pure odorants were transferred into a toned 1 ml glass vial using a glass pipette. For the 10^{-1} dilution, 40 μl of the pure odorant were mixed with 360 μl mineral oil in a 1 ml glass vial by vortexing. This procedure was repeated accordingly for the lower concentrations. All odorants and dilutions were stored at 4 ℃. Dilutions lower than 10^{-1} were prepared freshly every week.

To prepare the odor stimuli for the use in the *Flywalk*, a small piece of paper tissue was laid on the bottom of a 200 μl PCR tube. The lid of the tube was cutted. 100 μl odor dilution of interest were transferred into the prepared tube. All tubes were transferred in separate odor vials and sealed with a stainless steel plug and a rubber O-Ring. Corresponding ball-stop checkvalves at both inlets and outlets prevented an uncontrolled release of the odorant from the odor vial.
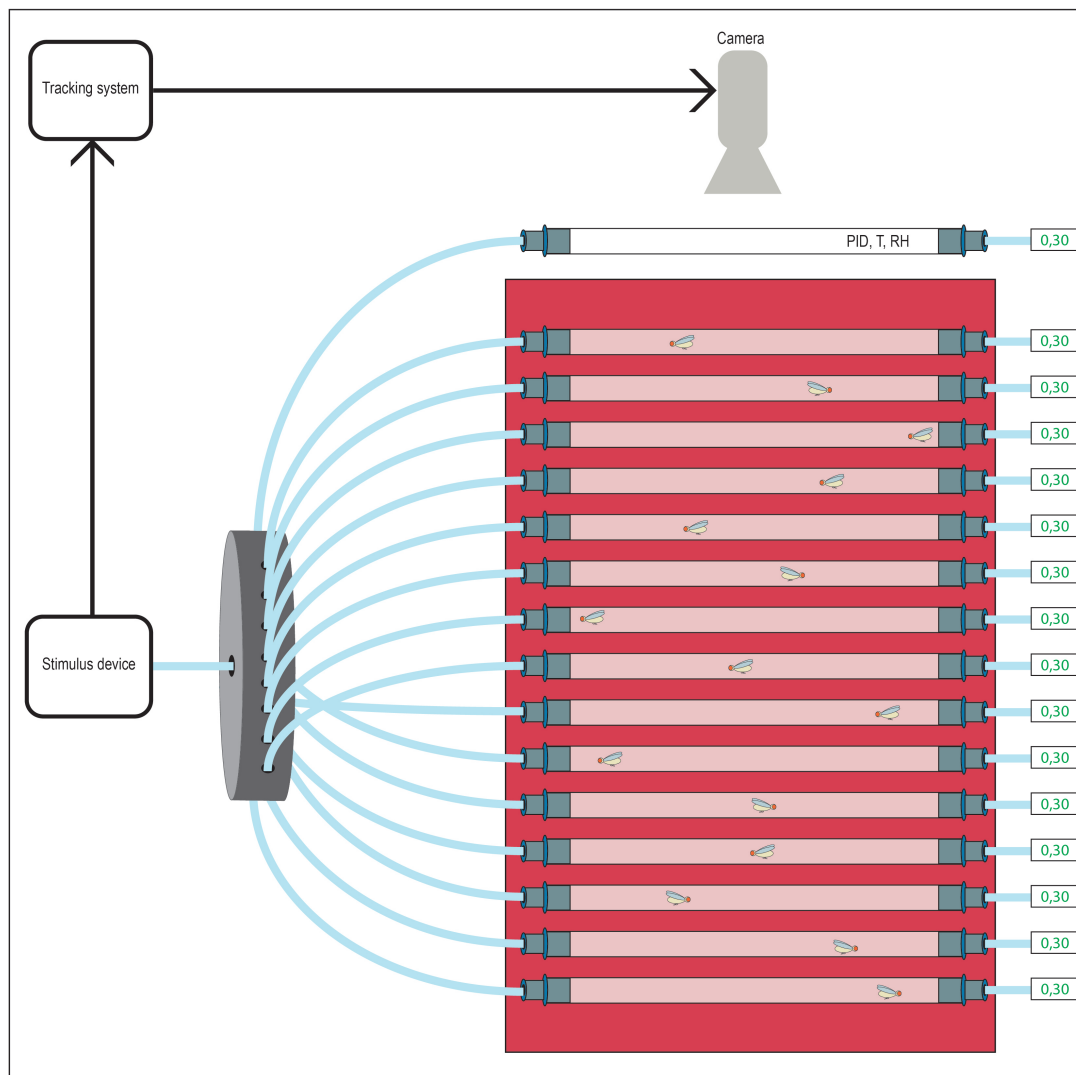
2.4.3. Experimental setup

The *Flywalk* allows the simultaneously monitoring of fifteen individual flies. Wild type flies and Orco -/- mutants starved for one day were separately placed into glass tubes with a length of 18 cm and a diameter of 0.8 cm. Flies could walk freely inside the glass tubes. 30 wild type flies of and 30 Orco -/- mutant flies were tested with every odorant. Therefore, four experiments per setup were required. Flies were placed into glass tubes in the following pattern:

Experiment	Tubes 1-5	Tubes 6-15
1	Wild type	Orco -/- mutant
2	Orco -/- mutant	Wild type
3	Wild type	Orco -/- mutant
4	Orco -/- mutant	Wild type

Table 2.3.: Distribution of flies in experimental setup

Glass tubes were then fixed on a red light LED-table, which transmitted light with a wavelength > 630 nm. Besides mineral oil as solvent control and ethyl acetate with a concentration of 10^{-3} as positive control, *Flywalk* allowed the simultaneously use of six different odorants. Prepared odor vials (as described before) were connected to olfactory stimulation device. The system served as an automated odor distributor and ensured continuous air flow of 0.3 l/m, a temperature of 25 °C, and a relative humidity of 70 %. Exact parameters were detected in an additional and empty reference tube. Wind speed in the glass tubes was 18 cm/s.

After all components were connected and fixed to the experimental system, the setup was shaded and flies were allowed to acclimatize for 30 minutes. The red LED light was the only light source in the experimental setup. Because the used light was not detectable for flies, visual-induced behavior of flies could be excluded. Before the experiment was started, valves were checked as well as all other parameters like air flow, temperature and relative humidity. Olfactory stimulation device delivered odor pulses with a stimulus length of 500 ms and an intervall of 90 seconds into the continuous air flow. Stimulus length, concentration and gradient in all tubes were proofed by a photo-ionization detector (miniPID, Model 200A, Aurora Scientific Inc. Canada). Odor-evoked behavior was recorded with a camera above the red light-table. An automatic tracking system based on the software *AnTS* and *LabVIEW 8.5* (National Instruments, Austin, Texas) recorded fly position for 10 seconds (from 3 seconds before odor stimulus up to 7 seconds after meeting time).

Figure 2.1.: Experimental setup of the *Flywalk*. Flies are separately placed in 15 glass tubes connected to the odor-delivery system. Temperature and relative humidity are detected in a reference tube. PID in reference tube detects length, concentration and gradient of stimulus in every glass tube. A red light LED-table with a wavelength > 630 nm serves as light source. Flies could walk freely inside the tubes, movement is recorded with by camera of the tracking system which is connected to the odor-delivery system. PID= Photo-ionization detector, T= Temperature, RH= Relative humidity.

2.4.4. Data collection and analysis

Timer information of the olfactory stimulus system were tracked by *LabVIEW 8.5*. To synchronize these data, a *MATLAB* routine (The Mathworks, Natick, USA) was used.

This allowed the calculation of the meeting time, when flies got in contact with the odor stimulus. Only data within a defined region of interest were considered. Synchronized data were processed with an *EXCEL* (Microsoft Corporation, Redmond, USA) routine. Analysis of odor attractiveness and fly activity, as well as generation of plots was conducted using the open source software *R*.

2.5. Single sensillum recording

Preliminary results from the *Flywalk* showed that methyl acetate and ethyl acetate are highly attractive odorants for wild type flies, in contrast, acetates with ascending chain length are not attractive. This results imply a correlation between the chain length of a molecule and odorant attractiveness. To investigate an impact of the chain length on the valence of an odorant, the technique of single sensillum recording was used. The following carboxylate esters with ascending carbon chain length were tested:

Producer	Carboxylate ester
Sigma-Aldrich, St. Louis, USA	Methyl acetate
	Ethyl acetate
	n-Propyl acetate
	n-Butyl acetate
	n-Pentyl acetate
	n-Hexyl acetate
	n-Heptyl acetate
	n-Octyl acetate

Table 2.4.: Tested carboxylate esters with ascending chain length

Seven wild type flies (Canton S, Bloomington) were tested. Odor responses of the olfactory sensory neurons in the ab1, ab2 and ab3 sensilla were tested, which are all located in the large basiconic sensilla. To distinguish different sensilla the diagnostic odorants ethyl acetate, ethyl butyrate and 2-heptanone were used. The ab1 sensillum contains four neurons A, B, C and D. The A neuron of the ab1 sensillum (ab1A) shows the strongest response to ethyl acetate. The ab2 and ab3 sensilla contain both two neurons A and B. The ab2A and ab2B neurons show only a moderate response to ethyl butyrate. The ab3A neuron show excitatory response to ethyl butyrate and the ab3B neuron to by 2-heptanone (de Bruyne et al., 2001).

2.5.1. Fly preparation

Adult female wild type flies (Canton S, Bloomington) with an age from 4 up to 6 days were starved for one day like practiced for the *Flywalk*. For SSR, a female fly was carefully placed into a 200 μl pipette tip. Only the head of the fly showed off the tip (see Figure 2.2.). The area behind the fly was then carefully cutted with a razor blade and covered with wax to avoid the escape of the fly. The prepared pipette tip was fixed on an object slide covered with wax. The ventral side of the fly showed upwards. Second and third antennal segment of the right antenna were separated by a glass capillary. Then, the prepared antenna was fixed on a cover glass.

2.5.2. Stimulus preparation

The carboxylate esters (Table 2.4.) were diluted in mineral oil like described in stimulus preparation for the *Flywalk*. Odorants were tested at concentrations of 10^{-3}. 10 μl of one odorant were pipetted on a small piece of filter paper which was placed in a glass pipette. As solvent control mineral oil was used. Furthermore, to avoid contamination and alteration in fly behavior due to the filter paper, a blank piece served as a second negative control.

2.5.3. Experimental setup

Recording and reference tungsten electrodes were sharpened by immersing in potassium nitrite solution. The prepared object slide was placed under a binocular microscope. The recording electrode was placed in the eye of the fly, the reference electrode was placed in the large basiconic sensilla. To measure alterations in spike frequency within the neurons due to an odorant, the prepared stimulus was led to a constant air flow of 0.3 l/m and then puffed to the fixed antenna. Stimulus length was 500 ms. Between different odorants, the odor-delivery system was cleaned by flushing with fresh air to avoid cross-contamination or synergetic effects of the different odorants . Spike frequencies were recorded by *Autospike32* (Syntech, Kirchzarten, Germany) from 2 seconds before until 10 seconds after stimulus. Stimulus controller IDAC-4 (Syntech, Kirchzarten, Germany) controlled odorant puff properties.
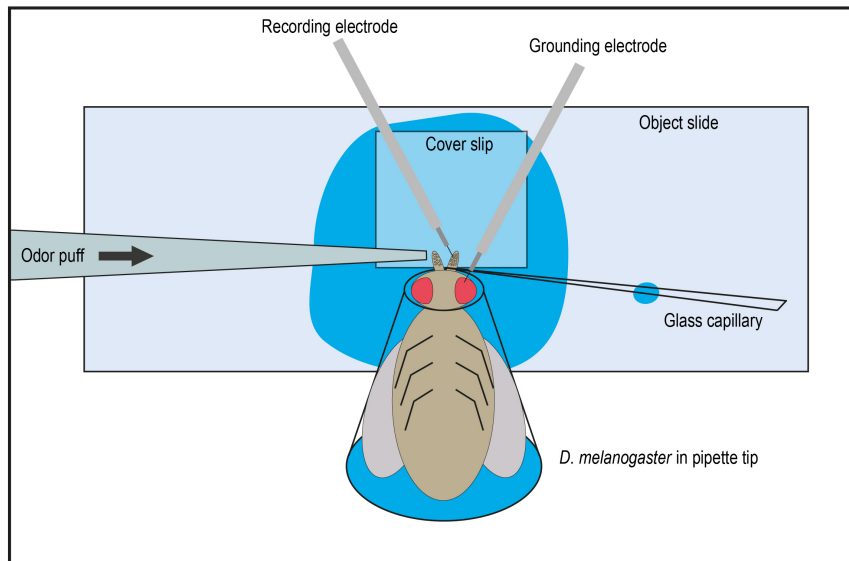
Figure 2.2.: Experimental setup of the single sensillum recording. Illustration after Badeke (2012, Friedrich-Schiller-Universitaet, Jena). The head of an adult female wild type fly shows off a pipette tip, ventral side shows upwards. A small glass capillary separates left and right antenna. Grounding electrode is placed in the right eye of the fly, reference electrode is placed in the large basiconic sensilla of the right antenna. To measure the effect of an odorant to a defined neuron, an odor puff was directed to the antenna with a stimulus length of 500 ms. Air flow was 0.3 l/min.

2.5.4. Data collection and analysis

Alteration in extracellular potentials were detected by *Autospike32* (Version 3.7). For the sensillum ab 1, the total electroantennogram was evaluated. For the ab2 and ab3 sensillum, spike amplitudes were sorted for an individual evaluation of A and B neuron. A bin width of 50 ms was used for an analysis of frequencies. For ab1, the maximum frequencies during odor stimulation of all flies were illustrated in a boxplot with *SPSS* (IBM, Armonk, USA). This procedure was also performed for ab2A, ab2B, ab3A and ab3B. Significant differences in maximum frequencies of an odor to mineral oil as control during odor stimulation were statistically evaluated by repeated measures ANOVA with *InStat3* (GraphPad software Inc., La Jolla, USA).

2.6. Characterization of the influence of selected physicochemical properties on the hedonic valence of an odorant

Correlations of the covered distance of wild type flies in the *Flywalk* and physicochemical properties (octanol-water partition coefficient, mass solubility, molecular weight, vapor pressure, and boiling point) of the odorant were tested. For this, Pearson product-moment correlation coefficient was calculated with correlation analysis performed with *SPSS* for every investigated chemical property. For the covered distance as effect of odor stimulation, the median from the covered distance of all flies was used. Chemical properties of used odorants were acquired with the aid of *SciFinder* (Chemical Abstracts Service, American Chemical Society).

3. Results

3.1. Odor-evoked behavior of *Drosophila melanogaster* wild type flies and Orco -/- mutants

The odor-evoked behavior was measured with the *Flywalk*. For every odorant, the walking speed, covered distance and activity of 30 flies were detected. A total of 18 odorants in three different concentrations were tested.

When wild type flies were tested, every tested odorant induced significant responses in the walking distance covered at least at one tested odorant concentration. For a list of all tested odorants see Table 2.2..

Positive response	Negative response	Both positive & negative responses
2,3-Butanedione	2- Phenylethyl alcohol	β-Caryophyllene
γ-Butyrolactone	3-(Methylthio)-1-propanol	Acetophenone
Ethyl acetate	(R)-(-)-Fenchone	n-Butyl acetate
Ethyl propionate	n-Heptyl acetate	n-Hexyl acetate
Geranyl acetate	Methyl salicylate	
Methyl acetate	n-Octyl acetate	
n-Propyl acetate	n-Pentyl acetate	

Table 3.1.: Odorants inducing significant responses in wild type fly behavior

In most cases, the behavior of the flies towards an odorant changed with the presented odorant concentration. For examples of odorants that were either attractive at all concentrations (2,3-butanedione), changed or lost their positive valence with changing concentrations (*n*-butyl acetate, ethyl propionate), or always induced repulsion to the flies (*n*-pentyl acetate) see Figure 3.1.-3.4.. The detailed results for every single compound and concentration are shown in appendix A.

I next tested the valence of these odorants to flies that lack functioning olfactory receptors by using *D. melanogaster* Orco -/- mutant flies. These flies exhibited significant responses to a smaller set of odorants (Table 3.2.). For examples of attraction (geranyl acetate), repellency (acetophenone), or neutral behavior (ethyl acetate) see Figure 3.5.-3.7.. The detailed results for every single compound and concentration are shown in appendix A.

Positive response	Negative response
2,3-Butanedione	Acetophenone
n-Butyl acetate	γ-Butyrolactone
Geranyl acetate	(*R*)-(-)-Fenchone
n-Octyl acetate	3-(mMthylthio)-1-propanol
	2-Phenylethyl alcohol
	n-Propyl acetate

Table 3.2.: Odorants inducing significant responses in Orco -/- mutant fly behavior
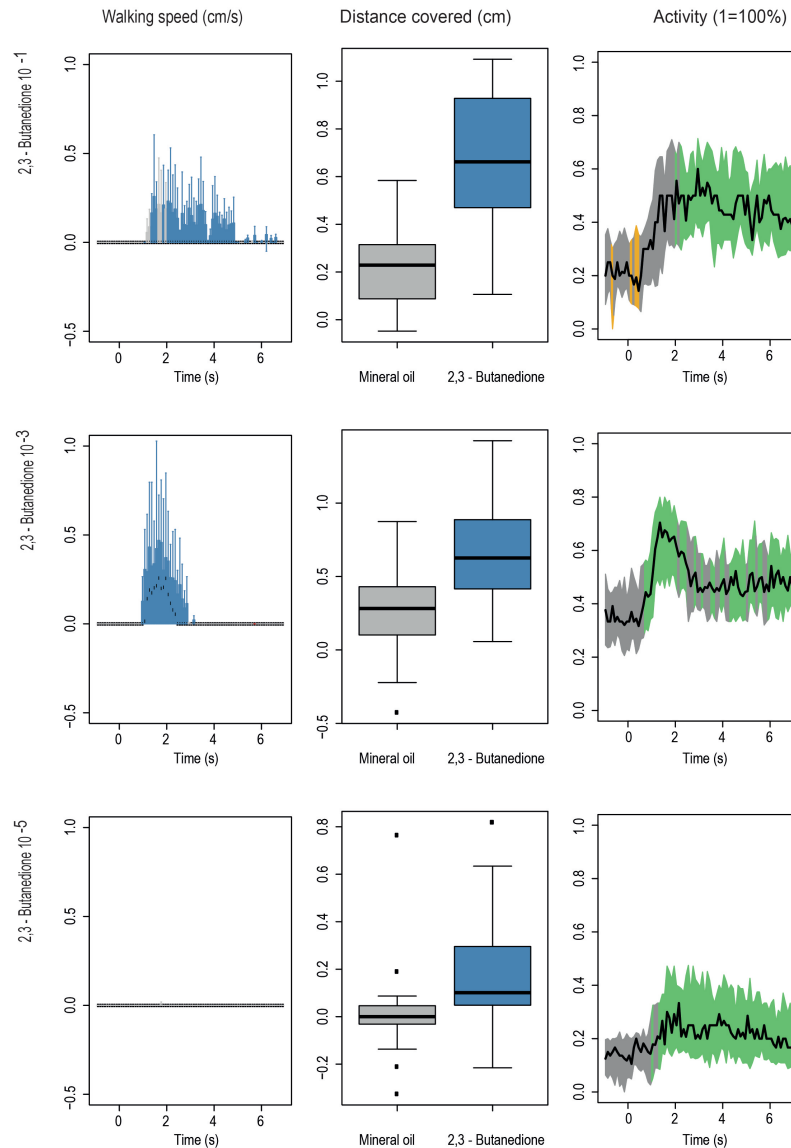
Figure 3.1.: Response of wild type flies to three different concentrations of 2,3-Butanedione. Left panels: time response from 1s before until 7s after stimulus arrival. Each boxplot depicts upwind speed during a 100 ms interval. Middle panels: upwind distance covered during 4s after stimulus arrival. Left and middle panels: black lines, median; boxes, 50% quartile; whiskers, data within 1,5-fold distance of the 50% quartile; black points, outliers. Blue box, significant upwind movement compared to solvent control (not shown); grey box, no significant movement; red box, significant downwind movement. Right panels: Total activity of flies. Line plot depicts activity measurements every 100 ms. Each fly was tested up to 40x with each odorant. Percentage of movement for each 100 ms bin and fly was calculated. Black line, median activity of all flies; shaded area, 50% quartile; green, significantly increased activity as compared to solvent control; grey, no significant difference; orange, significantly decreased activity. n=30.
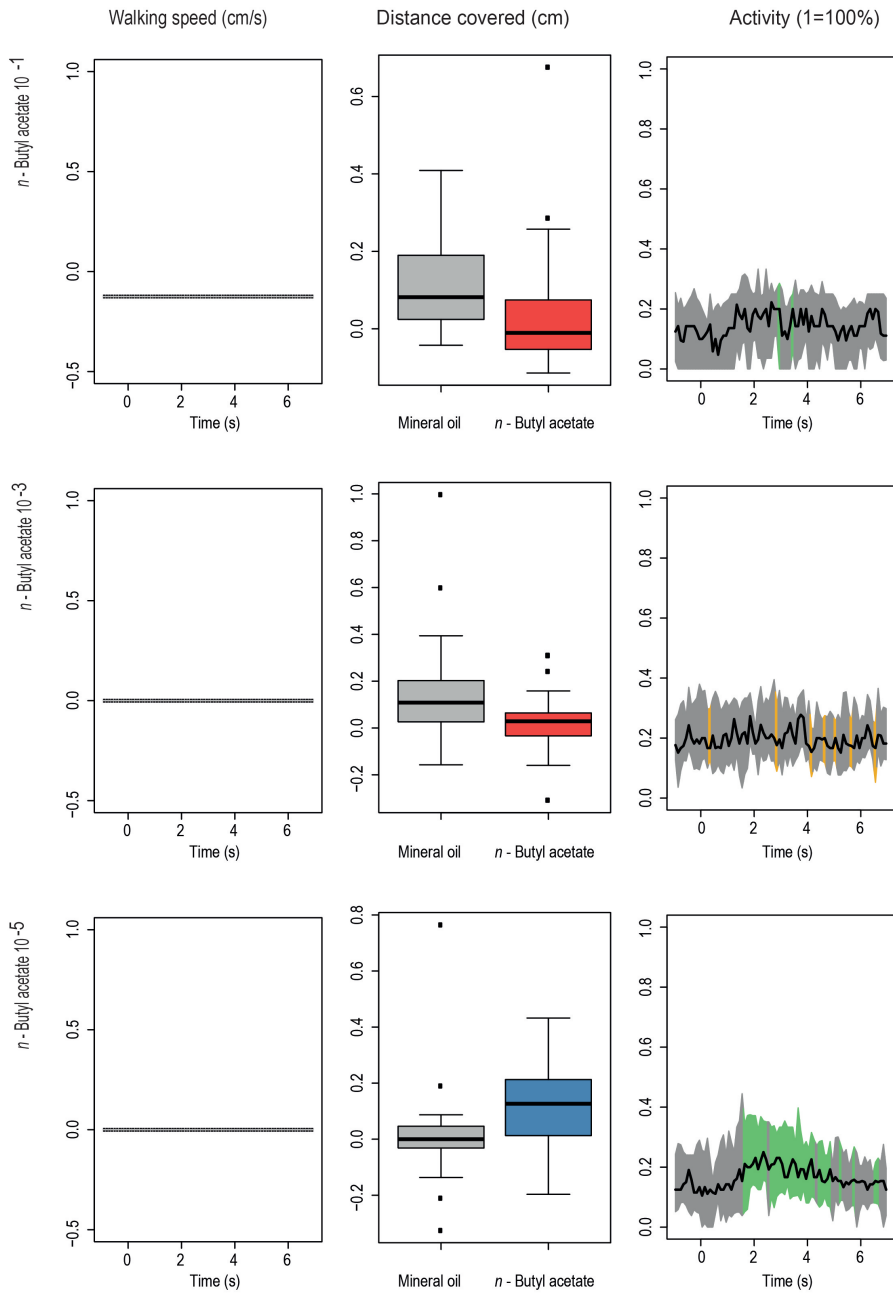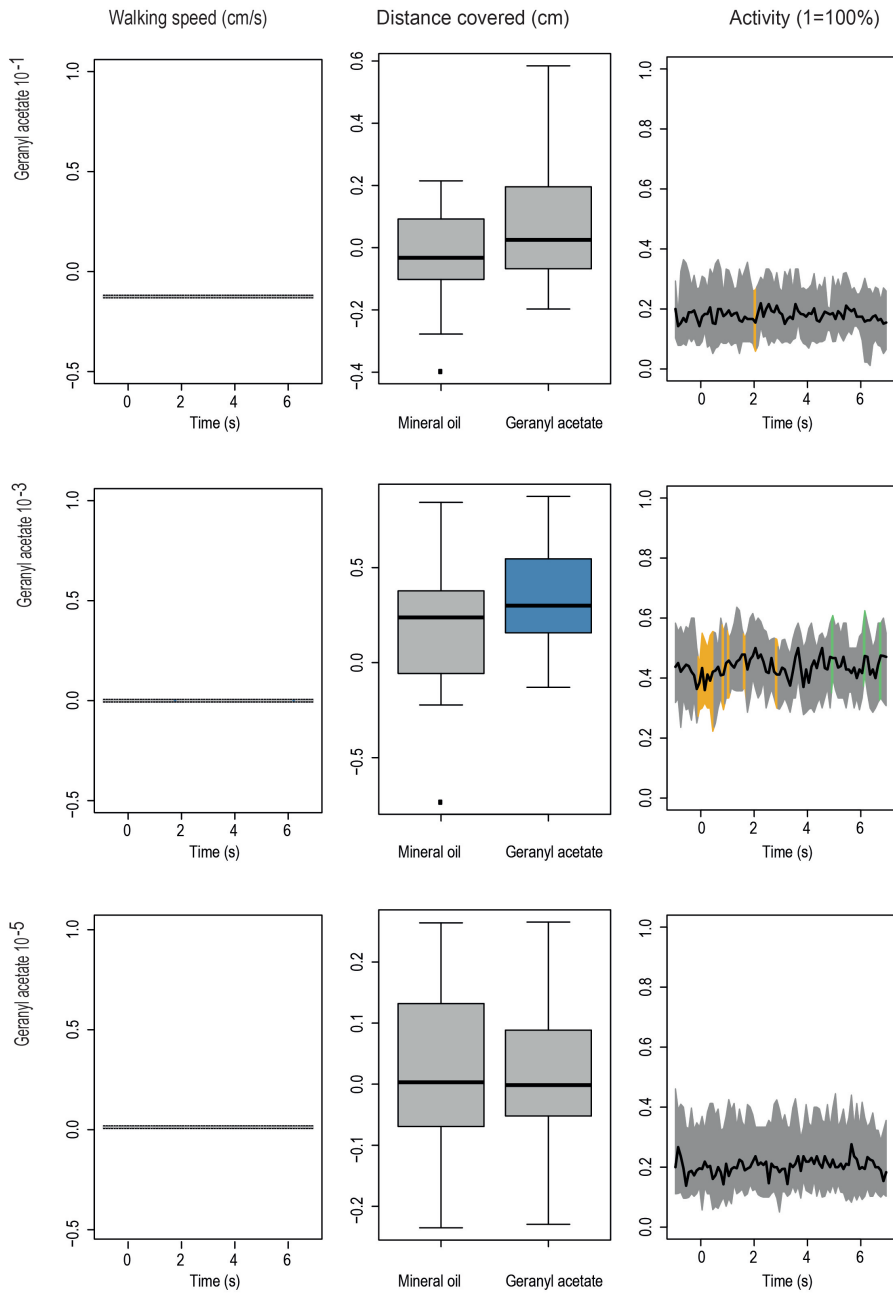
Figure 3.2.: Response of wild type flies to three different concentrations of n-Butyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
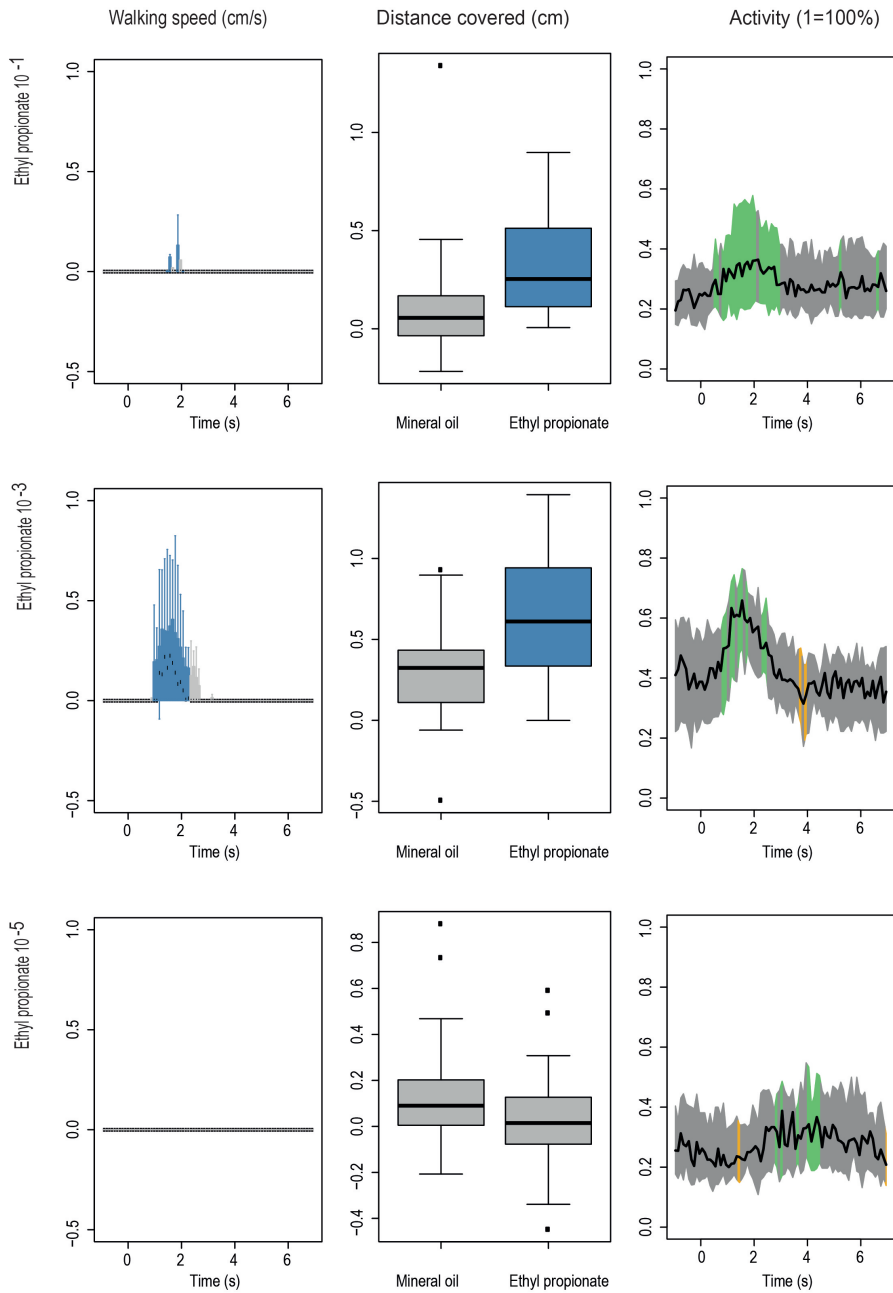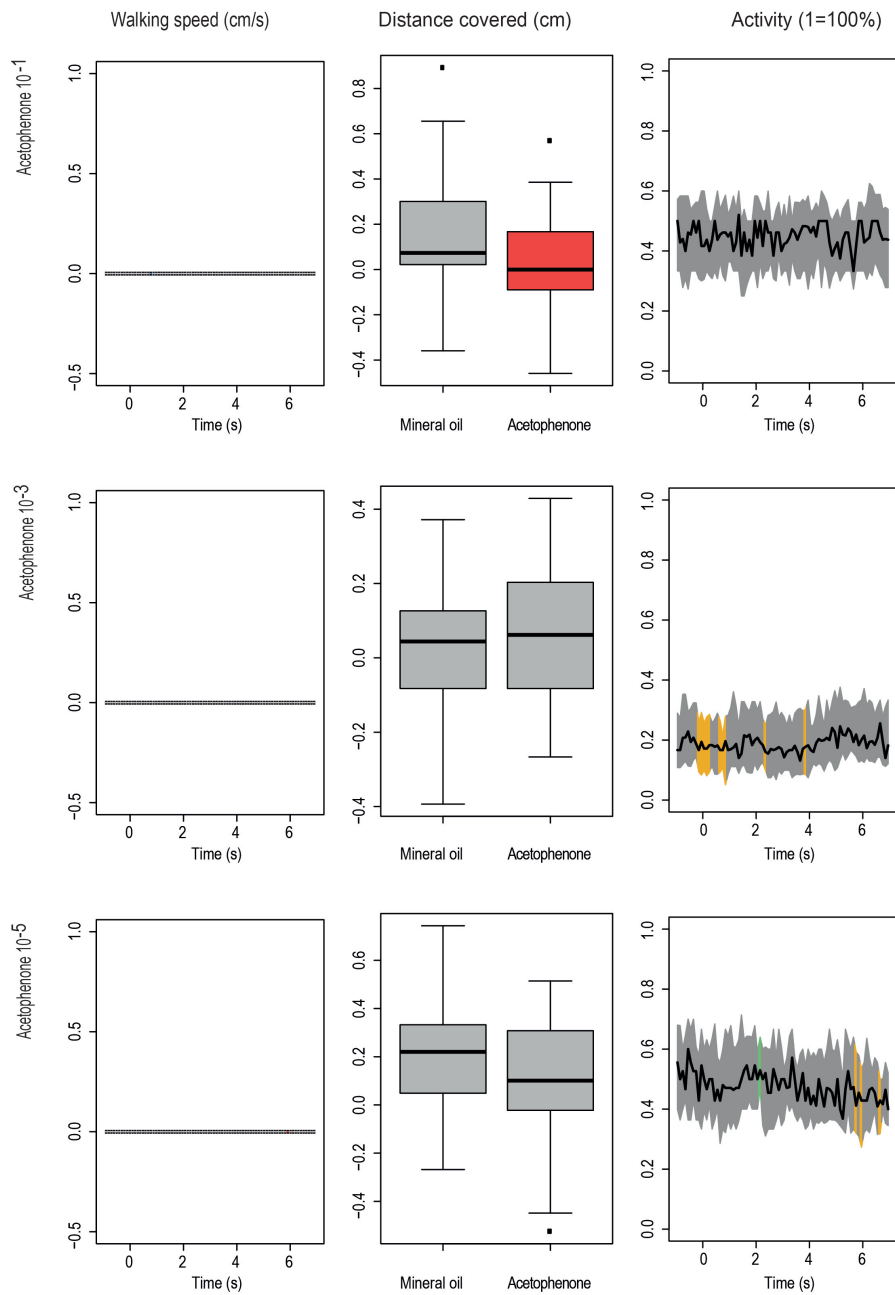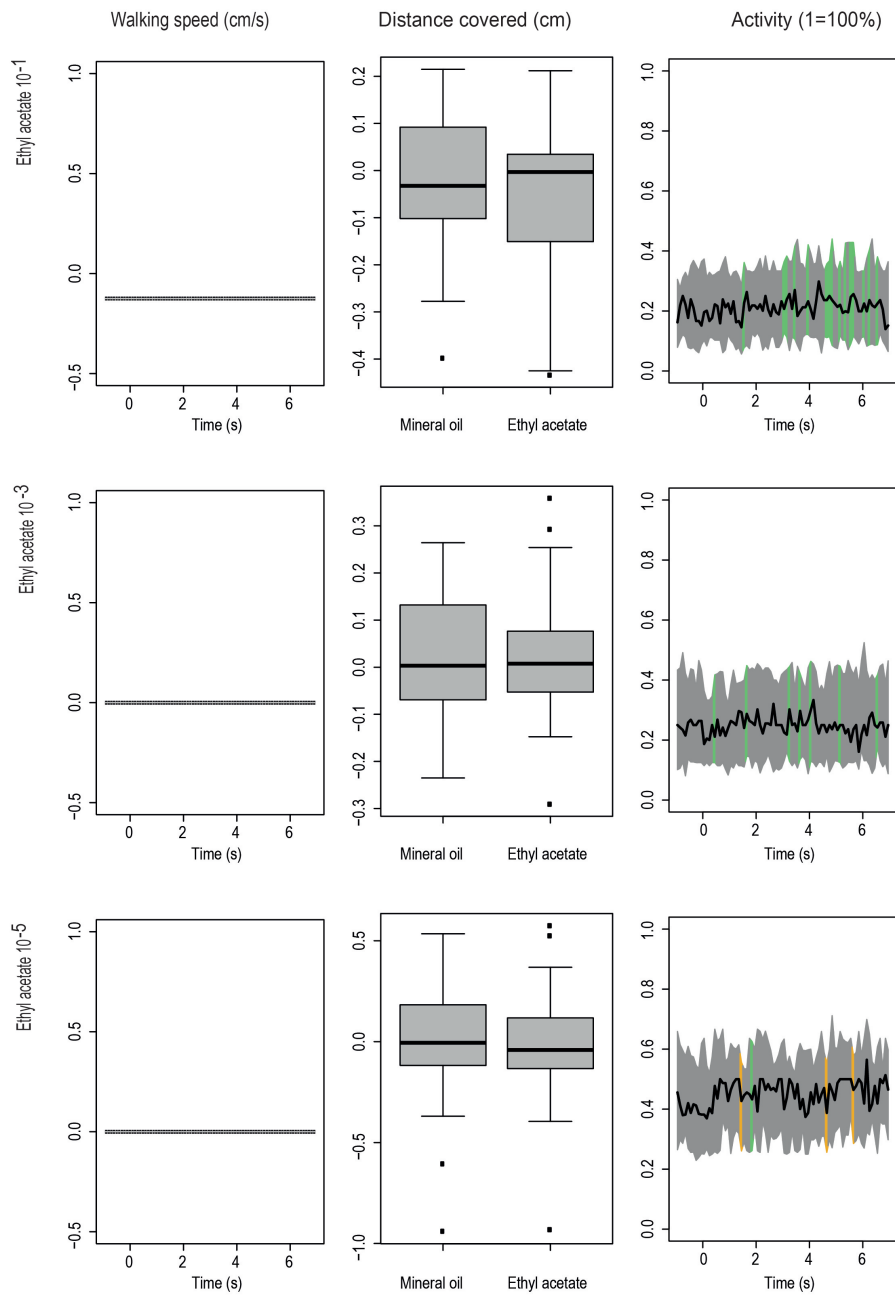
Figure 3.3.: Response of wild type flies to three different concentrations of Ethyl propionate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
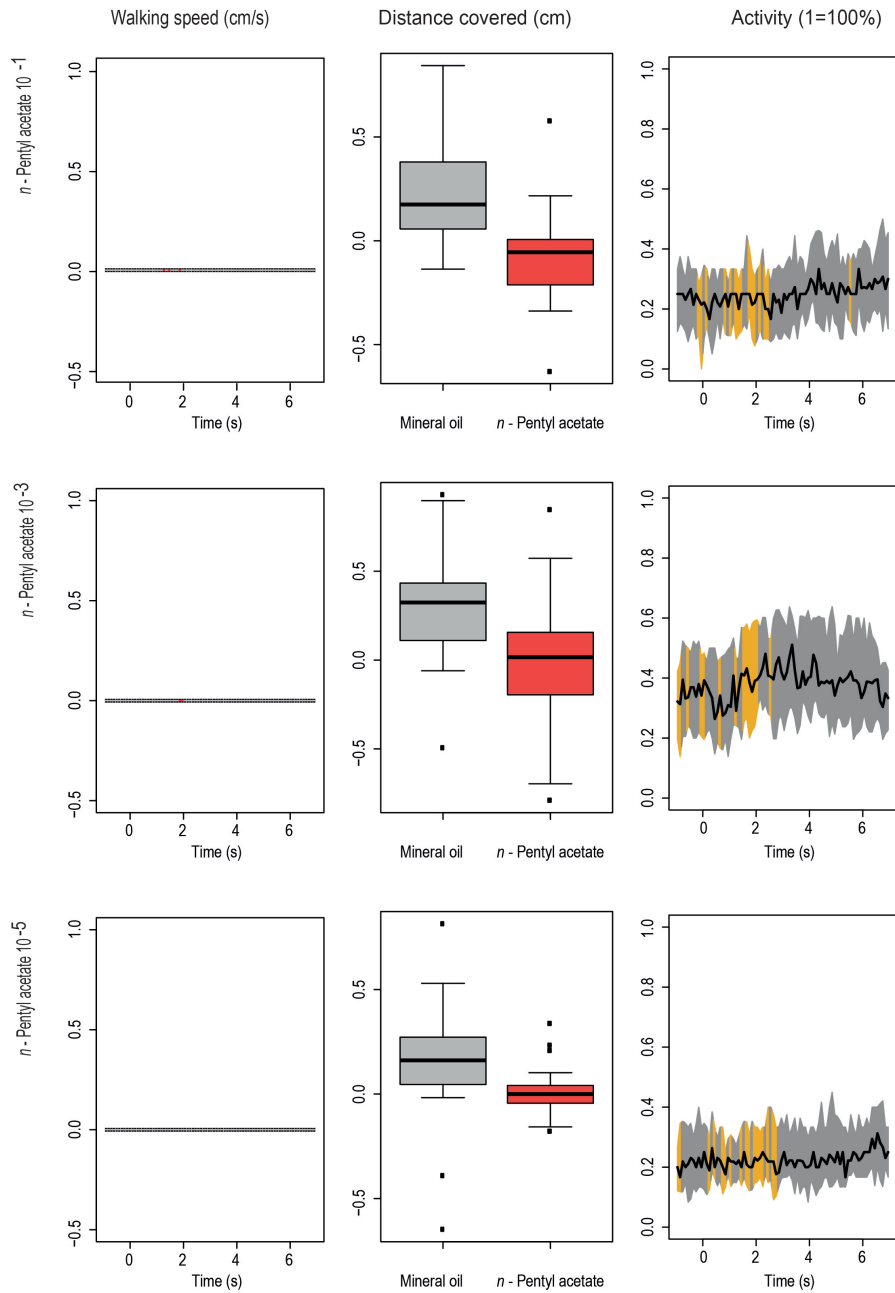
Figure 3.4.: Response of wild type flies to three different concentrations of n-Pentyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure 3.5.: Response of Orco -/- flies to three different concentrations of Geranyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure 3.6.: Response of Orco -/- flies to three different concentrations of Acetophenone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure 3.7.: Response of Orco -/- flies to three different concentrations of Ethyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

3.2. Impact of selected physicochemical properties on the odorant valence

The behavioral results gained from the *Flywalk* experiments allowed calculation of the covered distance caused by stimulation with an odorant. I concentrated on the responses to an odorant concentration of 10^{-3} that was used both for behavior and physiology. To investigate a possible link between selected physicochemical properties and the covered distance, a correlation analysis (calculation of the Pearson product-moment correlation coefficient) was conducted. In order to get an idea about the impact of e.g. hydrophyllic properties, size of the molecules etc. I tested the following chemical descriptors: the octanol-water partition coefficient, mass solubility, molecular weight, vapor pressure and the boiling point. For a list of the medians of tested odorants and their chemical properties see appendix B.

Physicochemical property	Correlation coefficient	P value (P<F)
Octanol-water partition coefficient	-0.243	0.332
Mass solubility	0.417	0.085
Molecular weight	-0.258	0.301
Vapor pressure	0.621**	0.006
Boiling point	-0.500*	0.035

Table 3.3.: Calculated correlation coefficients between medians of the covered distance and physicochemical properties and level of significance.

Significant correlations were observed for the vapor pressure and the boiling point. For the vapor pressure a positive correlation was calculated, i.e. an increase of odorant valence with increasing vapor pressure. For the boiling point a weak negative correlation was calculated, i.e. means a decrease of odorant valence with decreasing boiling point.
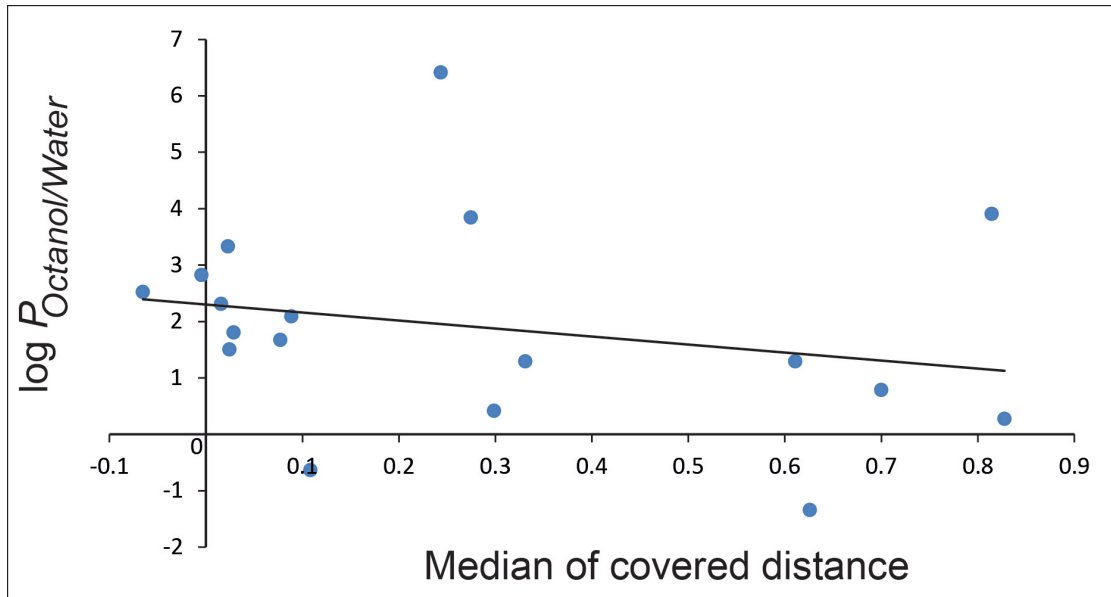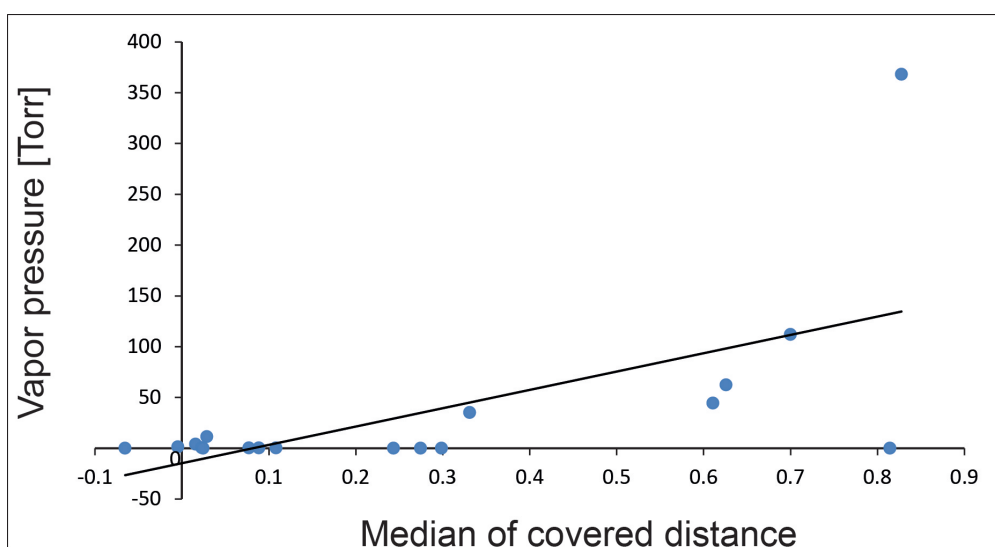
Figure 3.8.: Analysis of correlation between median of covered distance and octanol-water partition coefficient. No correlation was observed. Analysis was performed by calculating the Pearson product-moment correlation coefficient. Correlation coefficient= -0.243. For statistical data see Table 3.3..
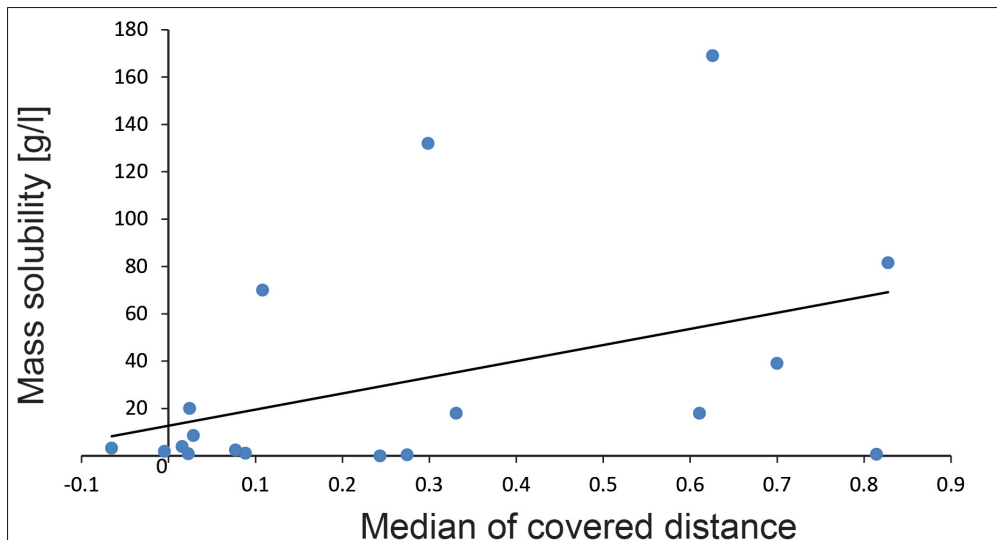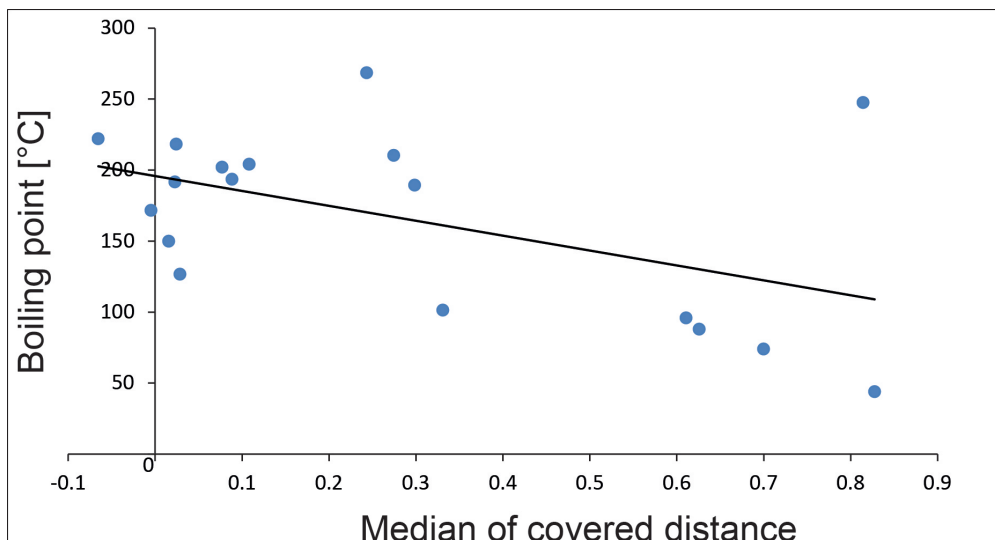

Figure 3.9.: Analysis of correlation between median of covered distance and mass solubility. No correlation was observed. Analysis was performed by calculating the Pearson product-moment correlation coefficient. Correlation coefficient= 0.417. For statistical data see Table 3.3..
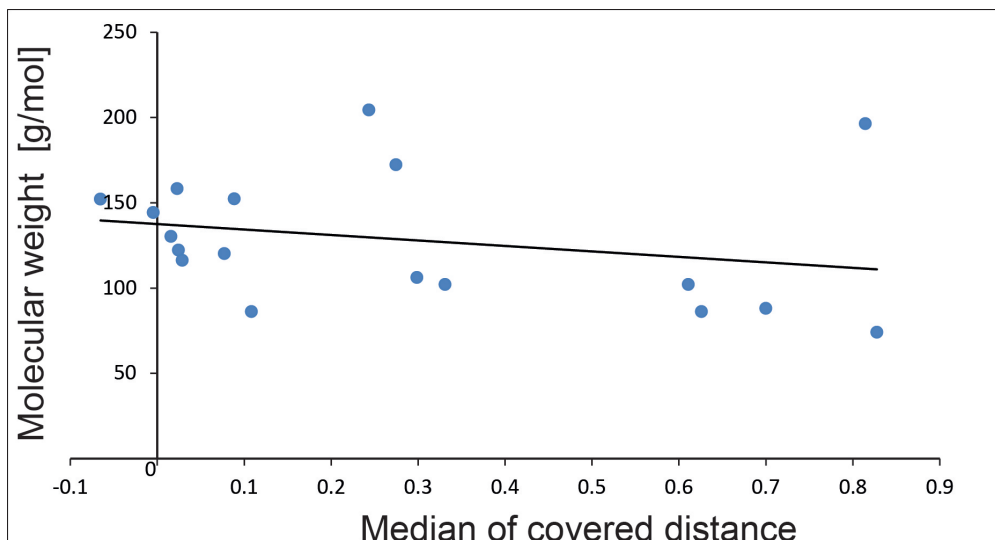
Figure 3.10.: Analysis of correlation between median of covered distance and molecular weight. No correlation was observed. Analysis was performed by calculating the Pearson product-moment correlation coefficient. Correlation coefficient= -0.258. For statistical data see Table 3.3..

Figure 3.11.: Analysis of correlation between median of covered distance and vapor pressure. A significant positive correlation was observed. Analysis was performed by calculating the Pearson product-moment correlation coefficient. Correlation coefficient= 0.621. For statistical data see Table 3.3..

Figure 3.12.: Analysis of correlation between median of covered distance and boiling point. A significant negative correlation was observed. Analysis was performed by calculating the Pearson product-moment correlation coefficient. Correlation coefficient= -0.500. For statistical data see Table 3.3..

I next asked how chain length of a molecule affects its detection and valence in *D. melanogaster*.

3.3. Impact of the chain length on the valence of an odorant

The walking distances covered of wild type flies revealed that short esters are more attractive and long esters are more repulsive (see Figure 3.13.). In single sensillum recordings (SSR) I tested how molecules with different chain lengths are detected in three basiconic sensilla ab1, ab2 and ab3. For a description of the method see Chapter 2.5.. Methyl acetate and ethyl acetate are significantly attractive to wild type flies when tested in the *Flywalk*, I decided to test the following carboxylate esters with ascending chain length:

Producer	Carboxylate ester
Sigma-Aldrich, St. Louis, USA	Methyl acetate
	Ethyl acetate
	n-Propyl acetate
	n-Butyl acetate
	n-Pentyl acetate
	n-Hexyl acetate
	n-Heptyl acetate
	n-Octyl acetate

Table 3.4.: Tested carboxylate esters with ascending chain length

Seven individual female wild type flies were tested. In every fly each one ab1, ab2 and ab3 sensilla were stimulated with the eight odorants and the solvent control. After odorant stimulation, alterations in the firing rate of tested sensilla were analyzed. Maximum frequencies for every odorant in the same sensillum were compared with mineral oil as solvent control (Figure 3.14.-3.16. and Table 3.5.-3.9.)
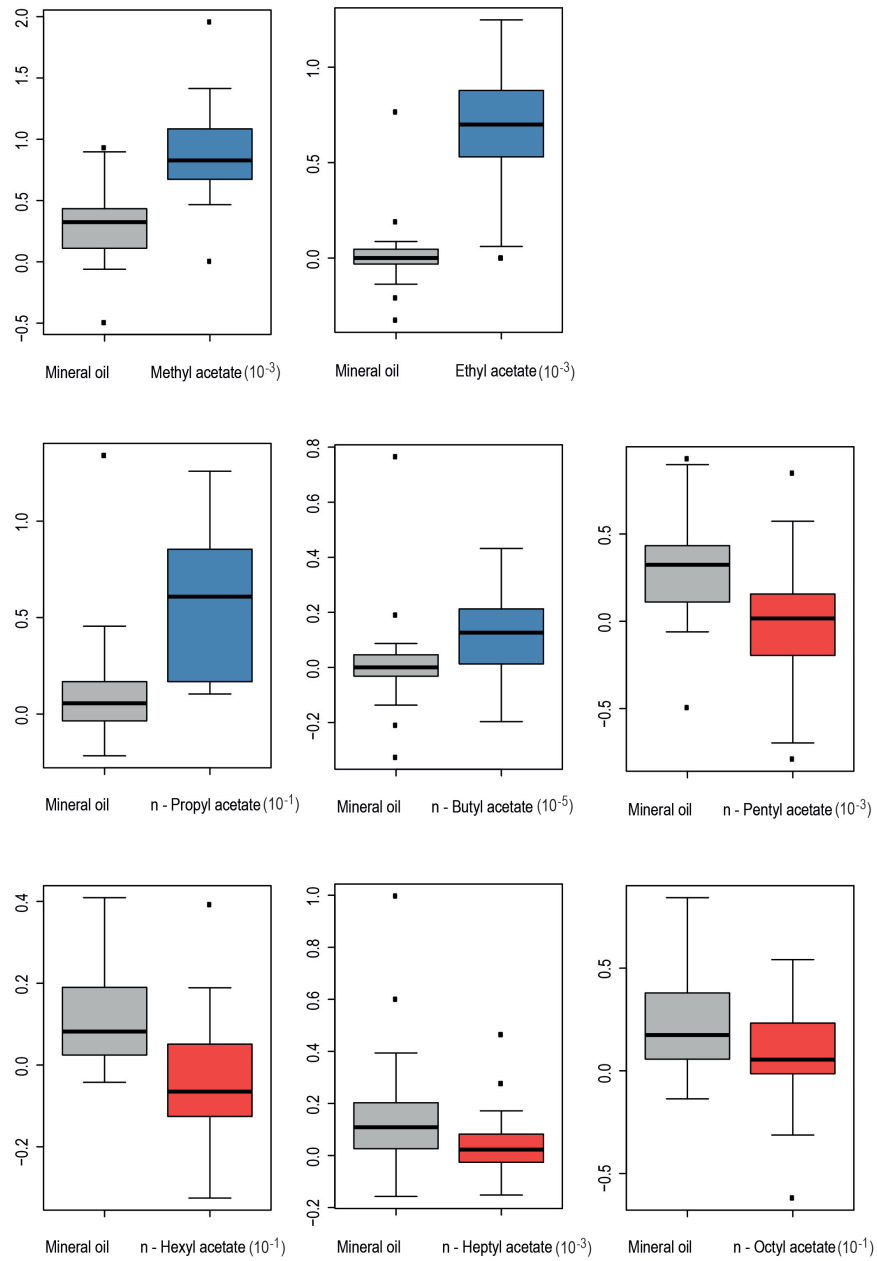
Figure 3.13.: Hedonic valence of tested carboxylate esters. The illustration shows the walking distances covered of wild type flies. The results of the odorant concentrations with the strongest impact are shown.
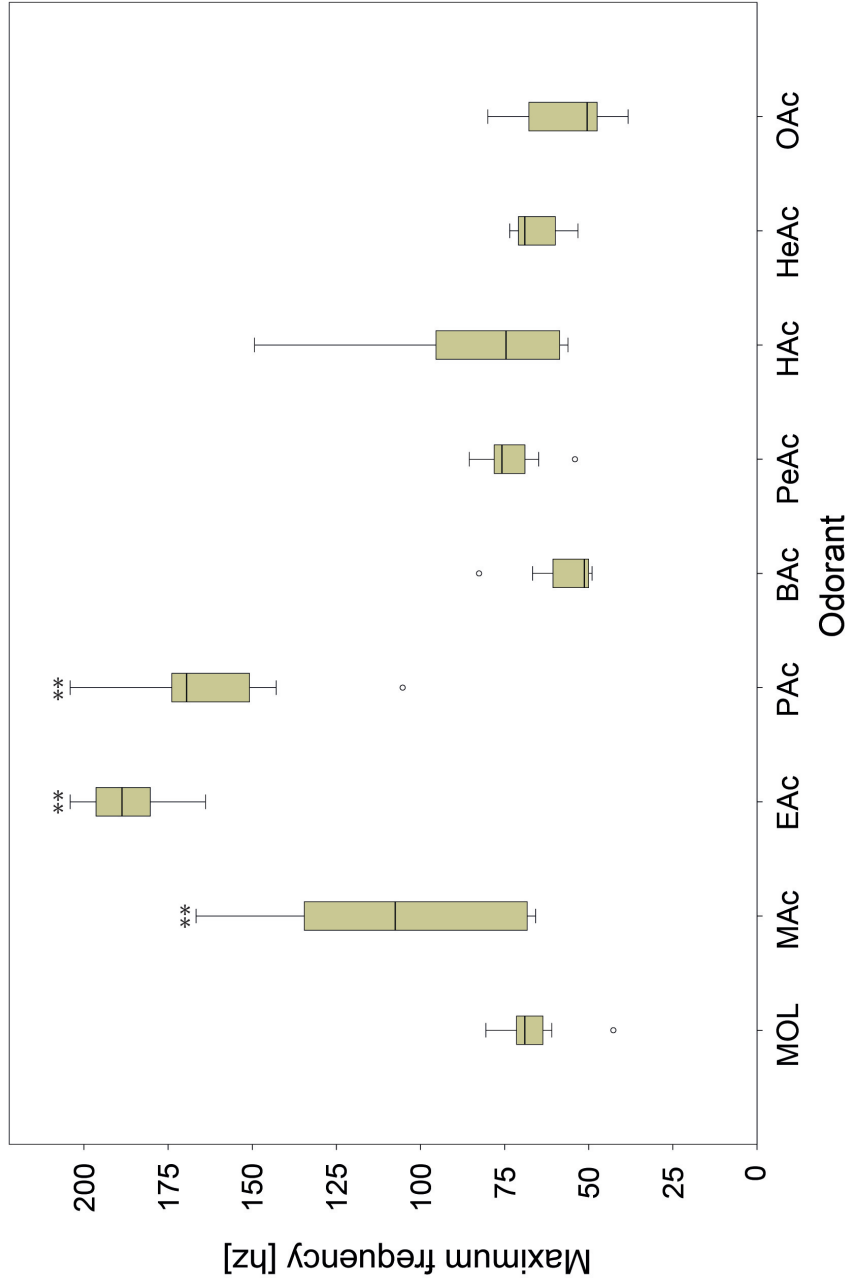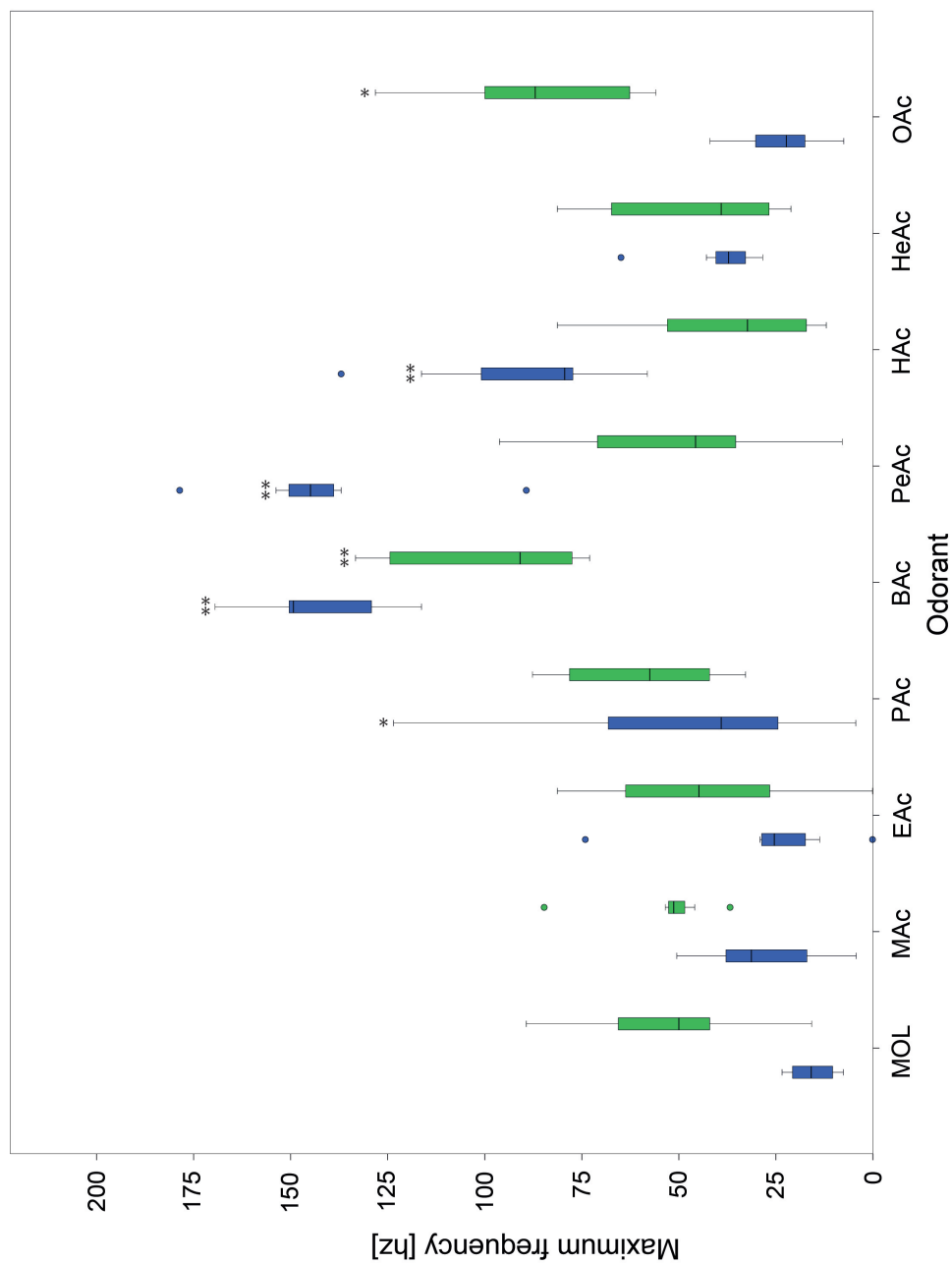
Figure 3.14.: Single sensillum recording of ab1 sensilla. Boxplots of maximum firing frequencies of all tested flies for the corresponding odorant. Outlier are depicted as circles. Significant differences to mineral oil are depicted as asterisks, which indicate the corresponding level of significance. n=7, statistical analysis by repeated measured ANOVA. For detailed data see Table 3.5.. MOL= Mineral oil, MAc= Methyl acetate, EAc= Ethyl acetate, PAc= n-Propyl acetate, BAc= n-Butyl acetate, PeAc= n-Pentyl acetate, HAc= n-Hexyl acetate, HeAc= n-Heptyl acetate, OAc= n-Octyl acetate.
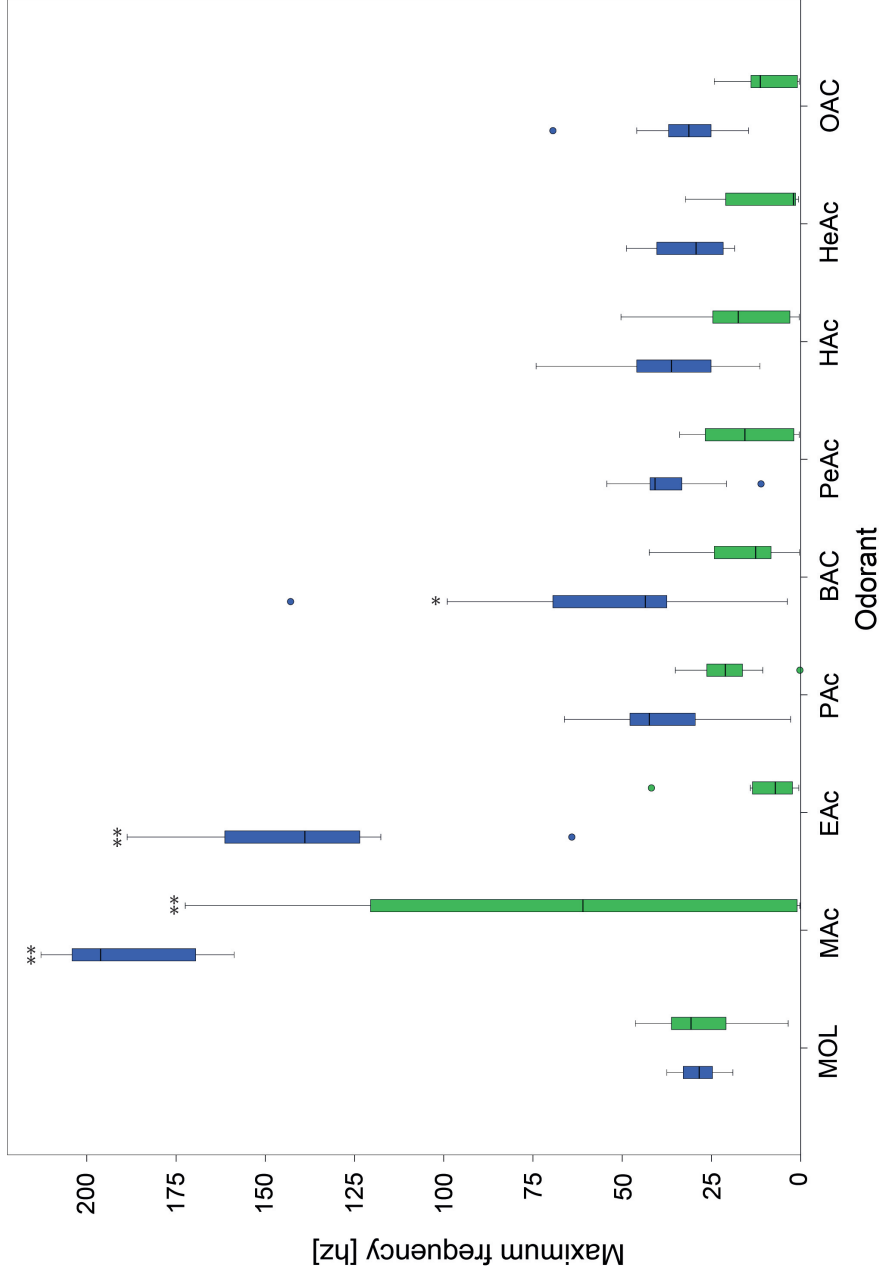
Figure 3.15.: Single sensillum recording of ab2 sensilla. Boxplots of maximum firing frequencies of all tested flies for the corresponding odorant. Outlier are depicted as circles. Results of A neuron are colored in blue, results of B neuron in green. Significant differences to mineral oil are depicted as asterisks, which indicate the corresponding level of significance. n=7, statistical analysis by repeated measured ANOVA. For detailed data see Table 3.6.-3.7.. MOL= Mineral oil, MAc= Methyl acetate, EAc= Ethyl acetate, PAc= n-Propyl acetate, BAc= n-Butyl acetate, PeAc= n-Pentyl acetate, HAc= n-Hexyl acetate, HeAc= n-Heptyl acetate, OAc= n-Octyl acetate.

Figure 3.16.: Single sensillum recording of ab3 sensilla. Boxplots of maximum firing frequencies of all tested flies for the corresponding odorant. Outlier are depicted as circles. Results of A neuron are colored in blue, results of B neuron in green. Significant differences to mineral oil are depicted as asterisks, which indicate the corresponding level of significance. n=7, statistical analysis by repeated measured ANOVA. For detailed data see Table 3.8.-3.9.. MOL= Mineral oil, MAc= Methyl acetate, EAc= Ethyl acetate, PAc= n-Propyl acetate, BAc= n-Butyl acetate, PeAc= n-Pentyl acetate, HAc= n-Hexyl acetate, HeAc= n-Heptyl acetate, OAc= n-Octyl acetate.

While ab1 was mainly activated by esters with short chain lengths (Figure 3.14.), the A neuron in ab2 responded to molecules of short and medium lengths, while the B neuron was activated only by methyl acetate (Figure 3.15.). In ab3 the A and B neuron became activated by molecules with medium and long chain lengths, however, the B neuron was activated by a smaller set of odorants (Figure 3.16.).

Compared odorants	P value (P<F)
Mineral oil : Ethyl acetate	<0.01
Mineral oil : Methyl acetate	<0.01
Mineral oil : n-Propyl acetate	<0.01
Ethyl acetate : Methyl acetate	0.0015
n-Propyl acetate : Methyl acetate	0.0020
Ethyl acetate : n-Propyl acetate	*not significant*

Table 3.5.: Significant differences between significant odorants in the ab1 sensilla. For graphical analysis see Figure 3.14..

Compared odorants	P value (P<F)
Mineral oil : Methyl acetate	<0.01
Mineral oil : Ethyl acetate	<0.01
Mineral oil : n-Butyl acetate	<0.05
Methyl acetate : Ethyl acetate	0.0024
Methyl acetate : n-Butyl acetate	<0.0001
Ethyl acetate : n-Butyl acetate	<0.0001

Table 3.6.: Significant differences between significant odorants in the A neuron of the ab2 sensilla. For graphical analysis see Figure 3.15..

Compared odorants	P value (P<F)
Mineral oil : Methyl acetate	<0.01

Table 3.7.: Significant differences between significant odorants in the B neuron of the ab2 sensilla. For graphical analysis see Figure 3.15..

Compared odorants	P value ($P<$F)
Mineral oil : n-Butyl acetate	<0.01
Mineral oil : n-Pentyl acetate	<0.01
Mineral oil : n-Hexyl acetate	<0.01
Mineral oil : n-Propyl acetate	<0.05
n-Butyl acetate : n-Pentyl acetate	*not significant*
n-Butyl acetate : n-Hexyl acetate	0.0089
n-Butyl acetate : n-Propyl acetate	0.0020
n-Pentyl acetate : n-Hexyl acetate	0.0110
n-Pentyl acetate : n-Propyl acetate	0.0038
n-Hexyl acetate : n-Propyl acetate	*not significant*

Table 3.8.: Significant differences between significant odorants in the A neuron of the ab3 sensilla. For graphical analysis see Figure 3.16..

Compared odorants	P value ($P<$F)
Mineral oil : n-Butyl acetate	<0.01
Mineral oil : n-Octyl acetate	<0.05
n-Butyl acetate : n-Octyl acetate	*not significant*

Table 3.9.: Significant differences between significant odorants in the B neuron of the ab3 sensilla. For graphical analysis see Figure 3.16..

4. Discussion

4.1. The odor-evoked behavior of *Drosophila melanogaster* wild type flies to selected natural chemicals

I investigated the odor-evoked behavior of *D. melanogaster* to 18 odorants with the *Fly-walk* paradigm. Every odorant was tested at three different concentrations 10^{-1}, 10^{-3} and 10^{-5} and I focused on three response parameters, i.e. walking speed, distance covered and activity of all tested flies as described in 3.1.. In summary, different patterns of odor-evoked behavior were observed. My results agree with Steck et al. (2012) where increased fly movement due to attractive odorants also results in increased fly activity and repulsive odorants often show a decrease in activity called freezing. Although mineral oil was used as solvent control positive results for this compound in the walking distance covered were observed. Besides the described olfactory receptors the sensory system of *D. melanogaster* contains also mechanoreceptive compartments on the maxillary palps (Singh and Nayak, 1985) and antennae (Foelix et al., 1989); it is possible that the movement upon stimulation with mineral oil is caused by a mechanical stimulus due to the opening of the valves within the stimulus device. This mechanoreceptive effect complicated the identification of repulsive odorants. While strongly attractive odorants can cause an obviously higher upwind movement, a repulsive could already be interpreted when an odorant provoked an upwind movement that was smaller than that of the mineral oil control. For a valid identification of repulsive odorants distinct alterations in walking speed and fly activity as observed for benzaldehyde (Steck et al., 2012) are useful. Most odorants had previously been tested in single sensillum recordings to investigate the effect on specific sensilla (de Bruyne et al., 2001) or odorant receptors (Hallem and Carlson, 2006). Therefore my results expand existing physiological data to the odor-evoked behavior. Detailed results for every single odorant and concentration are shown in appendix A.

Attractive odorants

Seven of 18 odorants elicit significant upwind movements (see Table 3.1.) suggesting that these odorants are attractive to *D. melanogaster*. Exclusively methyl acetate and 2,3-butanedione evoked attractive behavior at every tested concentration. However, even for those two odorants the level of attractiveness changed with changing concentrations. Although an effect of the odorant concentration on the hedonic valence had been shown before for an individual odor (Semmelhack and Wang, 2009), my results suggest that an impact of an odorant concentration on hedonic valence is a widespread phenomenon in *Drosophila* olfaction. Previous analyses with single sensillum recordings (SSR) revealed the effects of 110 odorants on selected odorant receptors in *D. melanogaster* (Hallem and Carlson, 2006). Methyl acetate was shown to induce the strongest excitatory response with over 200 spikes/s to the olfactory receptor Or59b; one could therefore speculate, that Or59b is expressed in neurons that govern positive hedonic valence. 2,3-Butanedione was shown to elicit weak responses of up to 100 spikes/s in neurons expressing different ORs. Nevertheless this odorant provoked strong upwind movements and increased the activity of the flies. However, as Hallem and Carlson (2006) tested only a subset of ORs other ORs not induced in that study could be responsible for the strong effect of 2,3-butanedione. Alternatively the weak activation of some of the neurons is sufficient for governing a strong attractiveness. Remarkable was that 71% of the attractive odorants were carboxylate esters. With the exception of geranyl acetate, all short carboxylate esters attracted the flies. The carboxylate ester ethyl acetate was previously identified in breeding sites of *D. melanogaster* (Jaenike, 1982) and its attractive effect on fly behavior was shown in Steck et al. (2012). Furthermore, it was shown that this odorant targets neurons housed in large basiconic sensilla (de Bruyne et al., 2001). The strong behavioral responses to carboxylate esters of my study correspond well with Hallem and Carlson (2006) who found many esters among the physiologically most active compounds.

Repulsive odorants

Seven of 18 odorants elicit exclusively downwind movements in the walking distance covered compared with the solvent control (see Table 3.1.) which indicates a repulsive impact on *D. melanogaster*. As for attractive odorants the impact of a specific odorant was dependent on its concentration with only *n*-pentyl acetate inducing a nearly similar downwind movement at every tested concentration. Interestingly, 57% of the repulsive odorants were carboxylate esters. However, in contrast to the attractive ones the repulsive esters had a longer chain length. Furthermore, both alcoholic compounds of the

18 odorants, 3-(methylthio)-1-propanol and 2-phenylethyl alcohol, revealed a repulsive impact on fly behavior mainly at high concentrations. However, as both compounds in addition either contained sulfur or an aromatic ring, no general conclusion regarding the hedonic valence of odorants can be drawn.

Odorants that evoked both attractive and repulsive fly behavior

As mentioned before, I observed that some odorants were more attractive at lower concentrations. This effect became remarkable in four odorants that were significantly attractive at low, but repulsive at high concentrations: n-butyl acetate, n-hexyl acetate, acetophenone and β-caryophyllene. Both carboxylate esters affected repulsive fly behavior at high concentrations and attractive behavior at the lowest concentration. These findings are in accordance with results from Stensmyr et al. (2003) who tested beside other odorants both acetates and observed repulsion at higher and attraction at lower concentrations. Acetophenone and β-caryophyllene - like the strictly repellent compound 2-phenylethyl alcohol - contain an aromatic ring which again might hint at a role of aromatics in repulsion.

My next target was to reveal if ionotropic receptors are involved in the detection of the tested odorants.

4.2. The impact of ionotropic receptors on the hedonic valence of tested odorants

To reveal the impact of ionotropic receptors on the hedonic valence of tested odorants I used *D. melanogaster* Orco -/- mutant flies in the *Flywalk* paradigm. These flies are known to lack functional ORs to numerous olfactory stimuli (Larsson et al., 2004). Four odorants attracted Orco -/- mutant flies: 2,3-butanedione, n-butyl acetate, geranyl acetate and n-octyl acetate. In contrast, six odorants had a repulsive impact: acetophenone, γ-butyrolactone, (R)-(-)-fenchone, 3-(methylthio)-1-propanol, 2-phenylethyl alcohol and n-propyl acetate. The responses were observed at different odorant concentrations. If a significant response in the walking distance covered was detected, up- or downwind movement was mostly close to that of the mineral oil control. Only 2,3-butanedione elicited significant responses at more than one odorant concentration (10^{-1}, 10^{-3}) at which the strongest upwind movement was observed at 10^{-3}. Although an attractive impact was observed alterations in fly activity were not as strong as in wild type flies. The repulsion to 2-phenylethyl alcohol, 3-(methylthio)-1-propanol and acetophe-

none agrees with the impact to wild type fly behavior.

The odor-evoked behavior in Orco -/- mutant flies implies an involvement of ionotropic receptors (IR) in the detection and classification of these odorants. Olfactory sensory neurons (OSN) on the coeloconic sensilla were shown to express neither odorant receptors including Orco nor gustatory receptors (Yao et al., 2005; Scott et al., 2001; Couto et al., 2005), with the exception of Or35a/Or83b-expressing neurons. However, IRs are expressed on four types of coeloconic sensilla termed ac1-ac4 (Benton et al., 2009). Identified ligands of ionotropic receptors were acids and amines (Ai et al., 2010; Yao et al., 2005), but also hygroreception was observed. A previous electrophysiological screen on IR expressing neurons (Silbering et al., 2011) revealed further ligands. 2,3-Butanedione was identified as a ligand with a strong excitatory effect to the IR75a expressing neuron housed in the ac2 sensilla. As Orco -/- mutant flies were attracted to this odorant, the neuron expressing IR75a could be involved in a neuronal circuit governing attraction. Other odorants that induced significant behavior in Orco -/- mutant flies have not been identified as IR ligands yet. Whether they are detected by IRs or whether in this case the attraction is governed by gustatory receptors (GR) which were first described in Clyne et al. (2000) remains unclear. While most ligands of gustatory receptors seem to be nonvolatile but solvable in water (like sugars, bitter compounds and nonvolatile pheromones (Montell, 2009)) at least one neuron expressing a gustatory receptor has been shown to respond to the volatile CO_2 (Jones et al., 2007; Kwon et al., 2007). As the odorants I used in the *Flywalk* paradigm were not previously tested or identified as GR ligands, it remains open, whether the response observed in Orco -/- mutant flies are due to IR-expressing neurons, GR-expressing neurons or a combination of both - further tests with the same odorants but *D. melanogaster* flies that lack the function of selected gustatory receptors (maybe by gene ablation) might solve this question.

4.3. The impact of selected physicochemical properties on the hedonic valence of odorants

Having investigated the odor-evoked behavior of wild type flies in the *Flywalk* I asked if physicochemical properties have an impact on the hedonic valence of an odorant. Previous investigation of Haddad et al. (2008) suggested that the perception of an odorant depends not only on the chemical class but also on a range of other physicochemical descriptors. Performing a principal component analysis, Knaden et al. (2012) investigated whether these physicochemical descriptors are indicators for odorant valence. Neither

coherent clustering of attractive or aversive odorants nor a correlation between the Euclidean distances of physicochemical properties and hedonic valences of odorant pairs was observed.

In order to investigate the impact of physicochemical properties I focused on the hedonic valence indicated by the median of the walking distance covered of all tested flies at an odorant concentration of 10^{-3}. I performed correlation analyses between the hedonic valence of an odorant and five different physicochemical properties: the octanol-water partition coefficient, mass solubility, molecular weight, vapor pressure and the boiling point (Table 3.3.). The octanol-water partition coefficient indicates a lipophilic or hydrophilic character of the odorant. I wondered if this property and furthermore the solubility have an impact on odorant valence. Furthermore, I wondered if the molecular weight has an impact on the hedonic valence. For these three physicochemical properties no correlation was found. Significant correlations were only found for vapor pressure and (much weaker) for the boiling point. Comparing the vapor pressure of an odorant and its hedonic valence revealed a significant positive correlation. Increasing vapor pressure means increasing odorant volatility so that more volatile odorants have an increased hedonic valence. This might also account for the effect that short carboxylate esters evoked attractive behavior in wild type flies while esters with long carbon chains were repulsive.

I next investigated, whether the attractive and repulsive esters were detected by the same set of sensilla. Therefore, I performed single sensillum recordings on the large basiconic sensilla ab1-3 and analyzed the maximum spike frequency in response to a set of carboxylate esters ranging from methyl acetate to n-octyl acetate. The ab1 sensilla showed strong excitatory responses to methyl acetate, ethyl acetate and n-propyl acetate. A similar result was observed in the ab2 sensilla in which especially the ab2A neurons showed excitatory responses to methyl acetate, ethyl acetate and n-butyl acetate. Significant responses to short carboxylate esters were not observed in the ab3 sensilla but ab3A neurons showed excitatory responses to long carboxylate esters ranging from n-propyl acetate to n-heptyl acetate. Furthermore, the ab3B neurons exhibited excitatory responses to n-butyl acetate and n-octyl acetate. It therefore would be interesting to activate these sensilla to a larger set of odorants and to test, whether attractive compounds usually activate neurons housed by ab1 and ab2 while repellent activate those in ab3.

Investigation of the human's ability to discriminate series of aliphatic alcohols and aldehydes revealed a negative correlation between the discrimination performance and

structural similarity in terms of the carbon chain length (Laska and Teubner, 1999). This suggests that the chain length affects the quality of odorants. The olfactory systems of vertebrates and invertebrates show similarities in terms of signal detection and processing (Hildebrand and Shepherd, 1997), suggesting that the carbon chain length has also an impact on the odorant quality in insects. By conditioning the proboscis extension reflex with odorants varying in chain length and functional group, Guerrieri et al. (2005) revealed that both properties determine the generalization responses of honeybees. For molecules that differ only in chain length a decreasing gradient in generalization depending on the difference in the number of carbon atoms was observed. Relating to my question if the chain length has an impact on the hedonic valence of odorants no published data exist.

Due to my findings carboxylate esters with long and short carbon chain lengths were detected by olfactory sensory neurons (OSN) in different sensillum types. OSNs from different sensillum types project to different regions in the antennal lobe. Hence, it is possible that short and large carboxylate esters are separately processed in different AL regions. Calcium imaging of the honeybee AL showed that the carbon chain length is the main common variable influencing the glomerular response profile to hydrocarbons with different functional groups (Sachse et al., 1999). It is possible that the stimulus of a short carboxylate ester is detected by ab1 and ab2 sensilla and projected to glomeruli which are involved in attraction behavior, while the activation by long chained esters activates repellent specific regions in the antennal lobe. Knaden et al. (2012) have shown, that the medial part of the antennal lobe mainly responds to attractive odorants, while the lateral part seems to govern repulsion. DM1 was shown as a target glomerulus of the ab1 sensilla (Couto et al., 2005) and is identified to mediate attraction to vinegar (Semmelhack and Wang, 2009). The ab2 sensilla targets among others to the glomerulus DM4 which was shown to be strongly activated by attractive odorants (Knaden et al., 2012). DM1 and DM5 are located in the medial part of the antennal lobe and the excitatory effect of short esters on the ab1 and ab2 sensilla agrees with the result of Knaden et al. (2012) that the medial part mainly responds to attractive odorants. One identified target glomerulus of the ab3 sensilla is DM2. This glomerulus is on the medial part of the antennal lobe and is activated by attractive compounds. However, my results showed that the repulsive effect of long chained esters on fly behavior is mediated by ab3 sensilla. This is not in agreement with Knaden et al. (2012) who postulated that repulsive effects are governed by the lateral part of the antennal lobe. One reason for this contradiction may be that there are so far unidentified target glomeruli of the ab3 sensilla besides DM2.
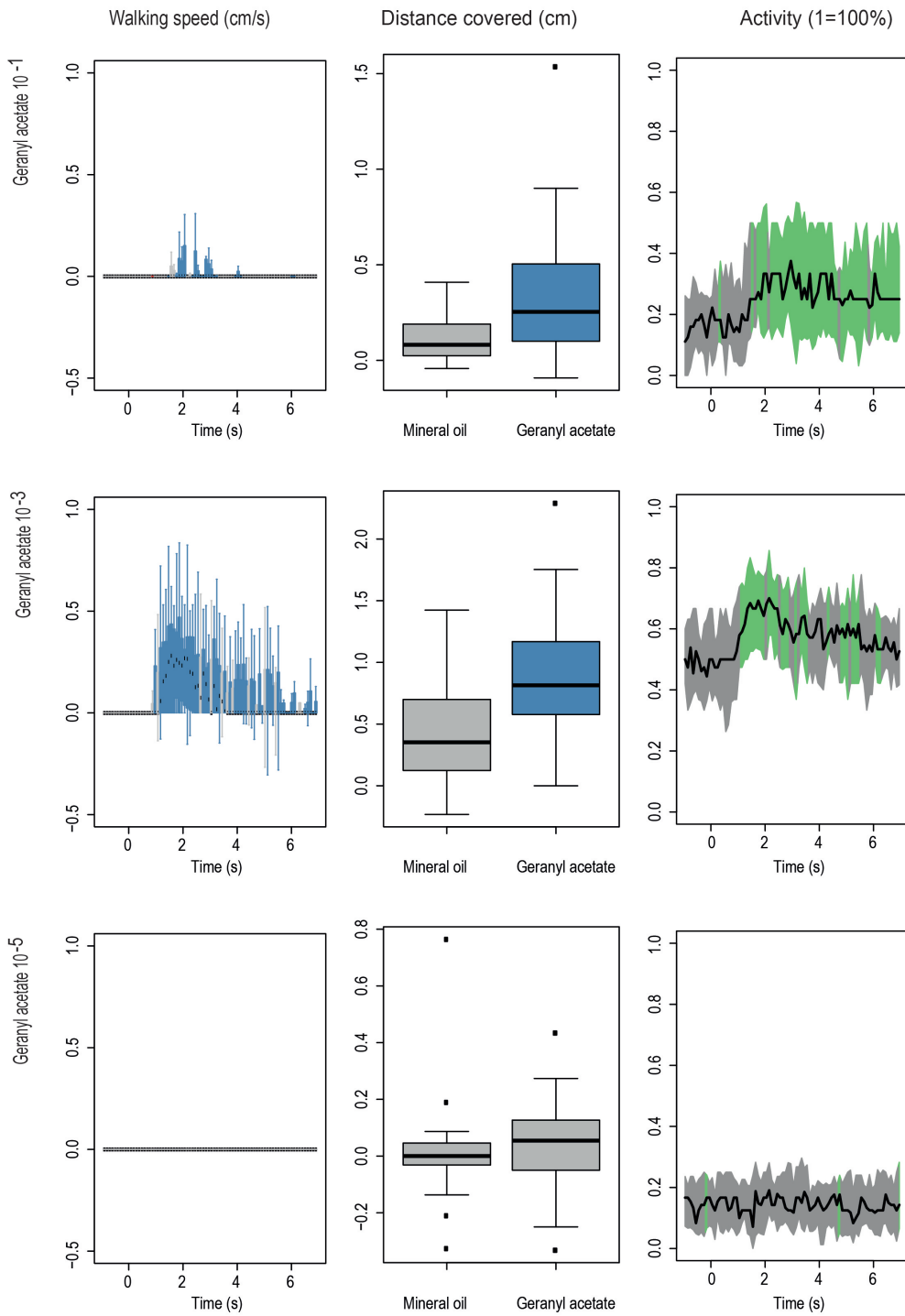
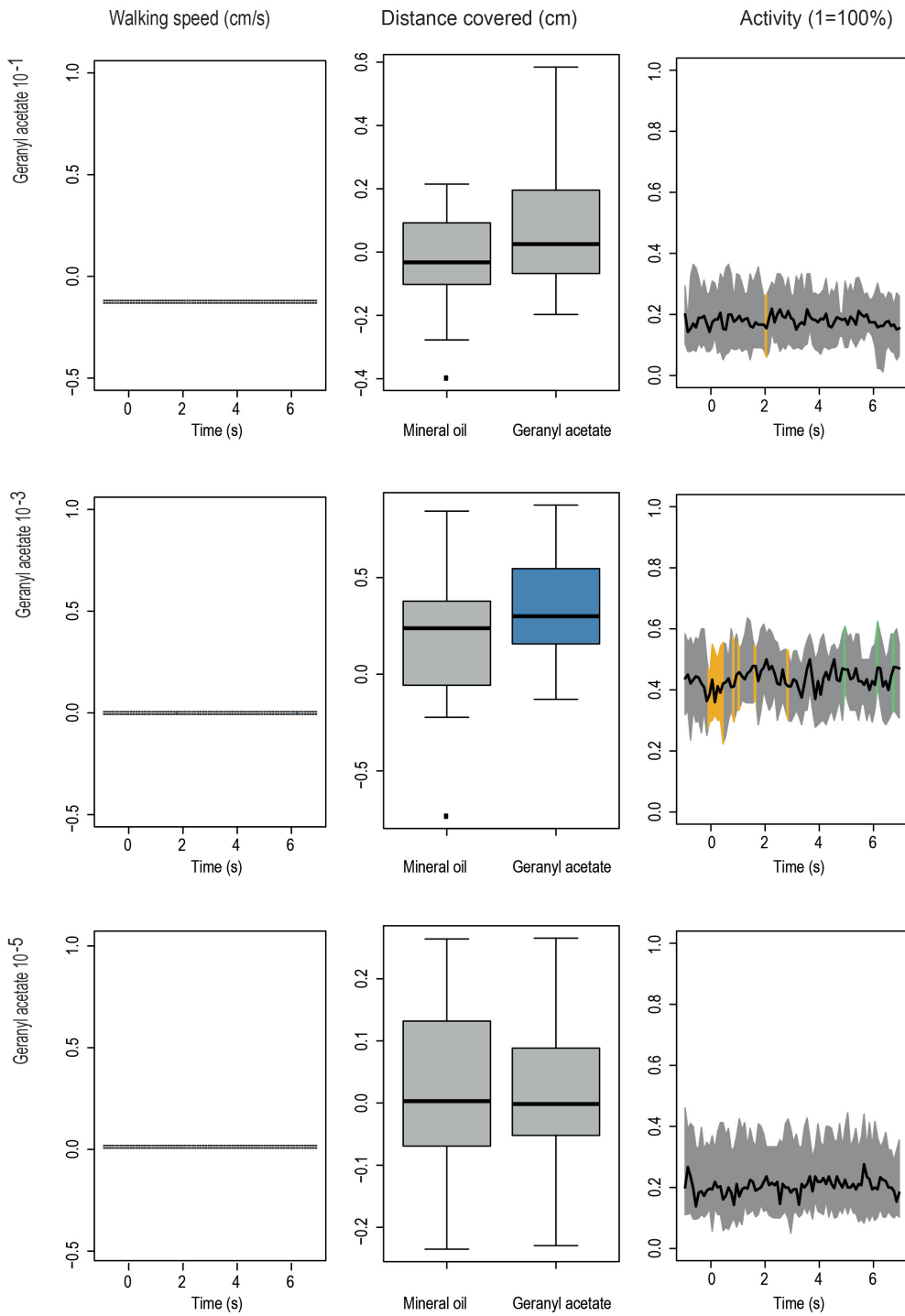# A. Results of odorant valence from the *Flywalk*
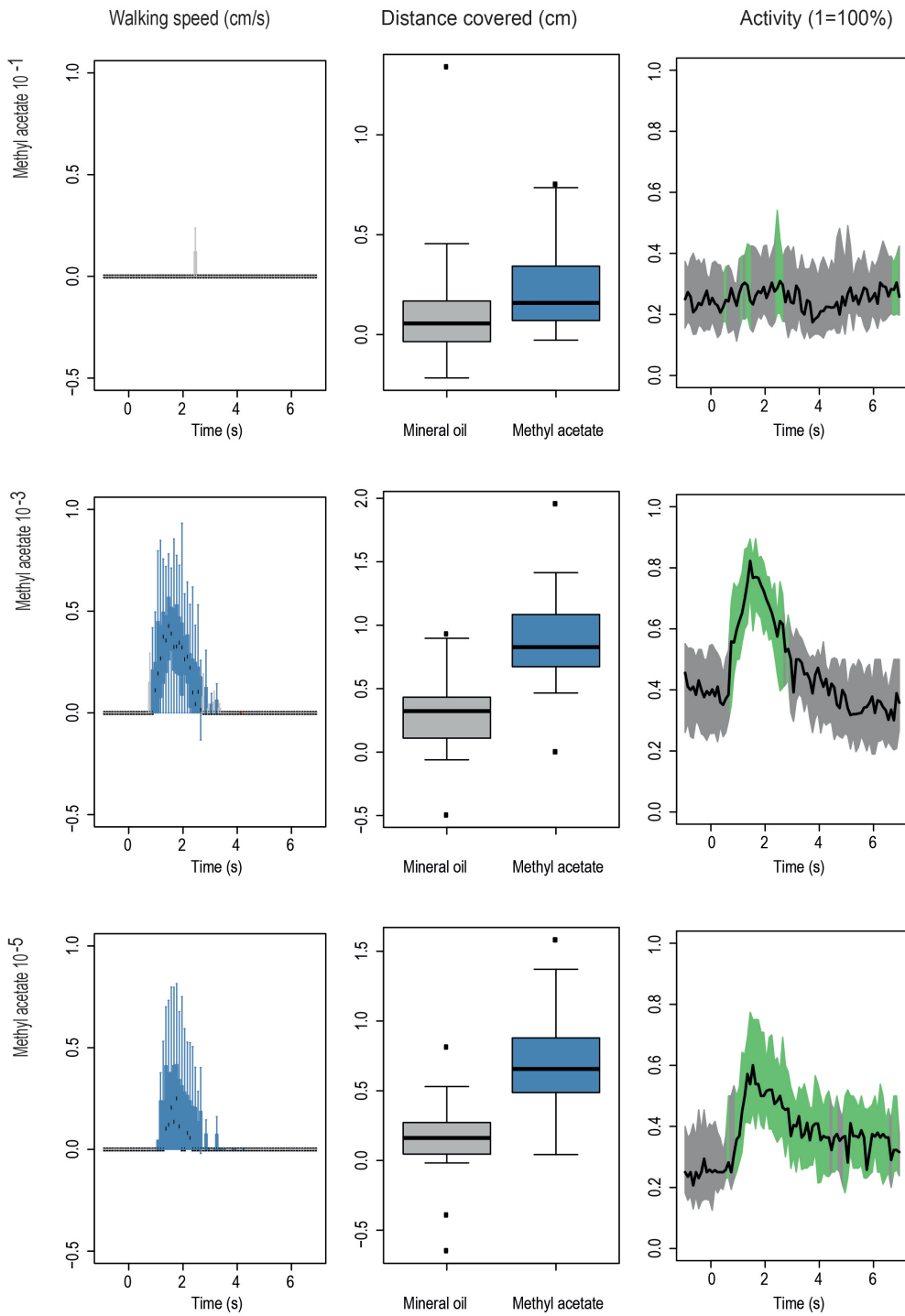
Figure A.1.: Response of wild type flies to three different concentrations of Methyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
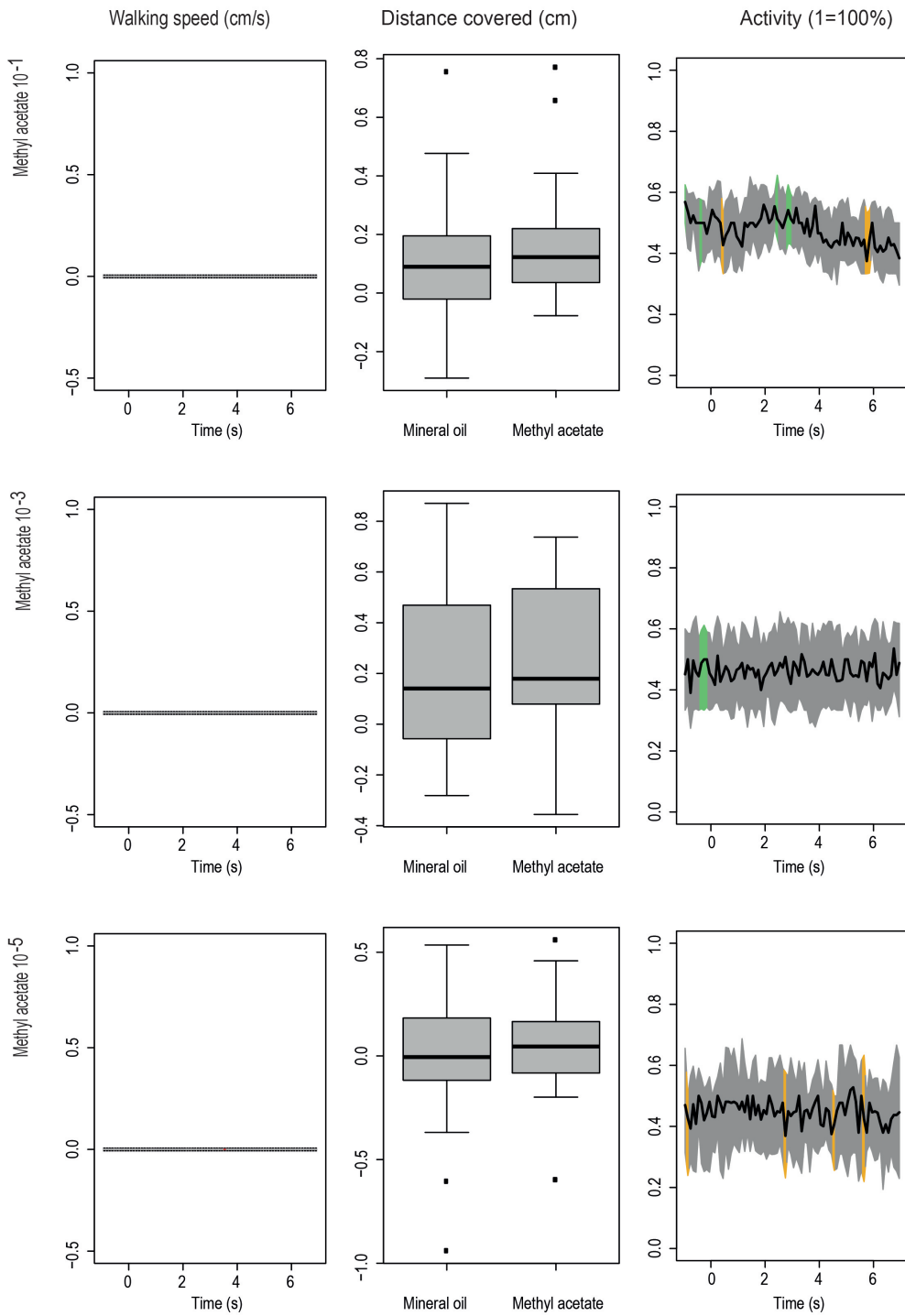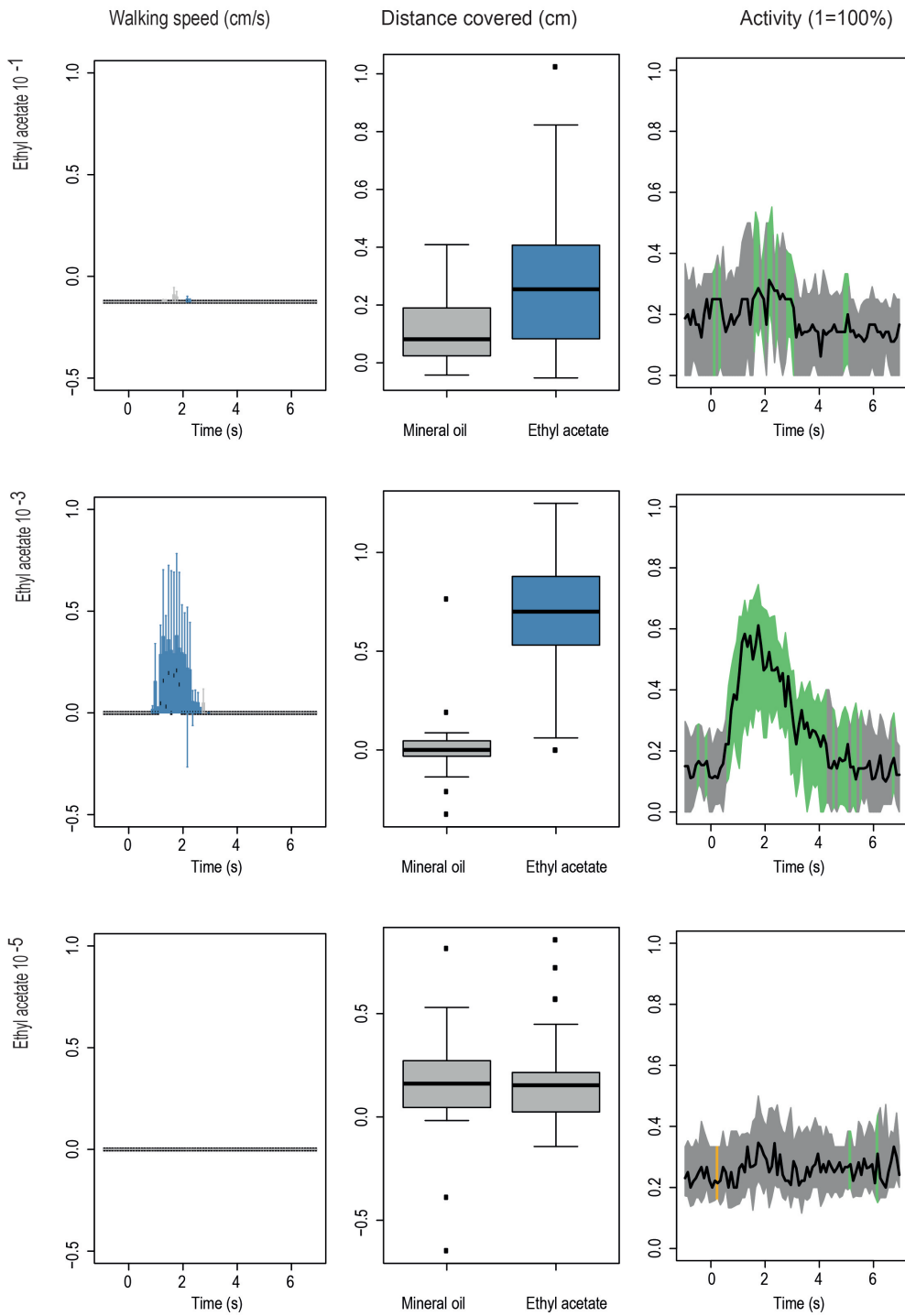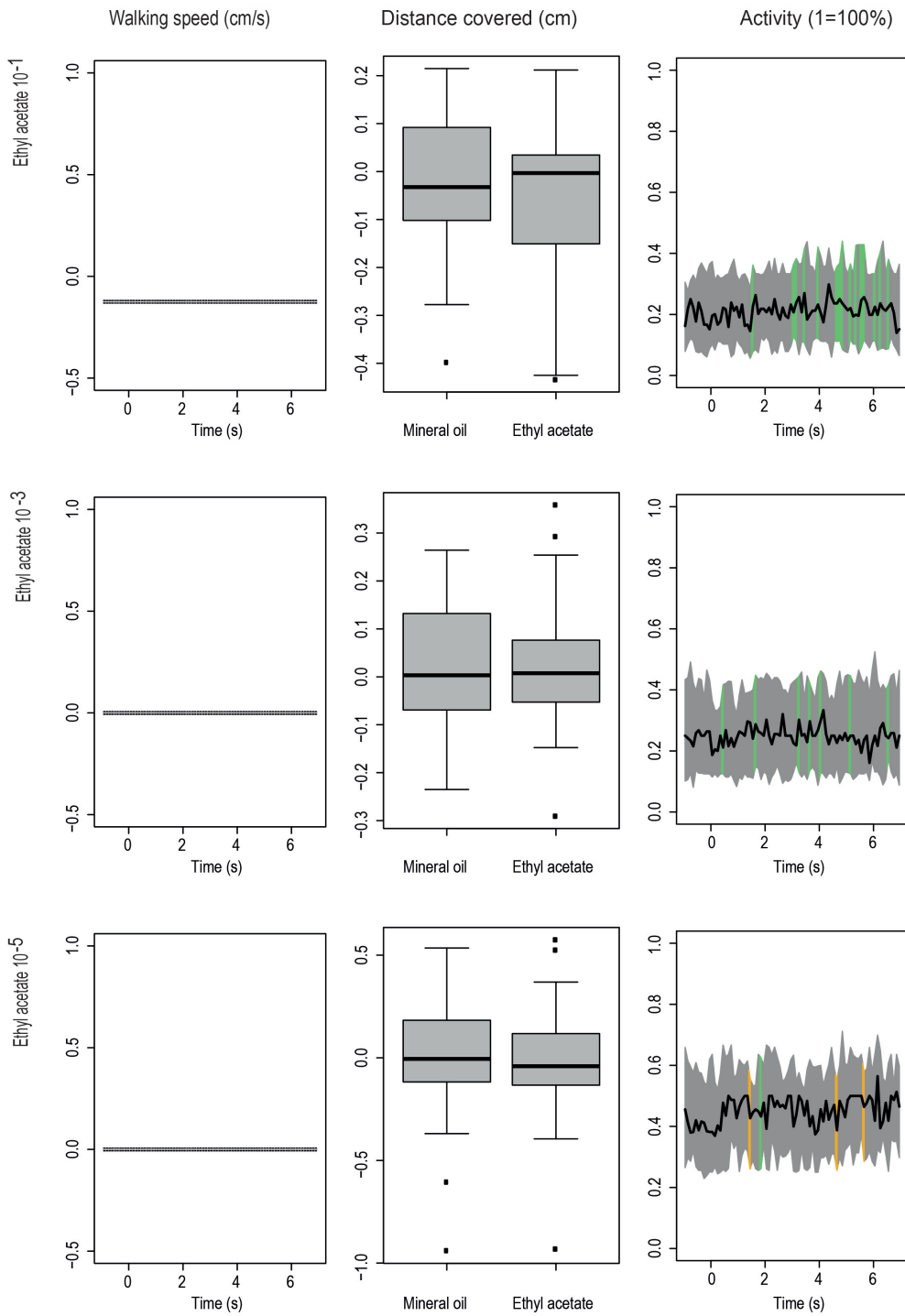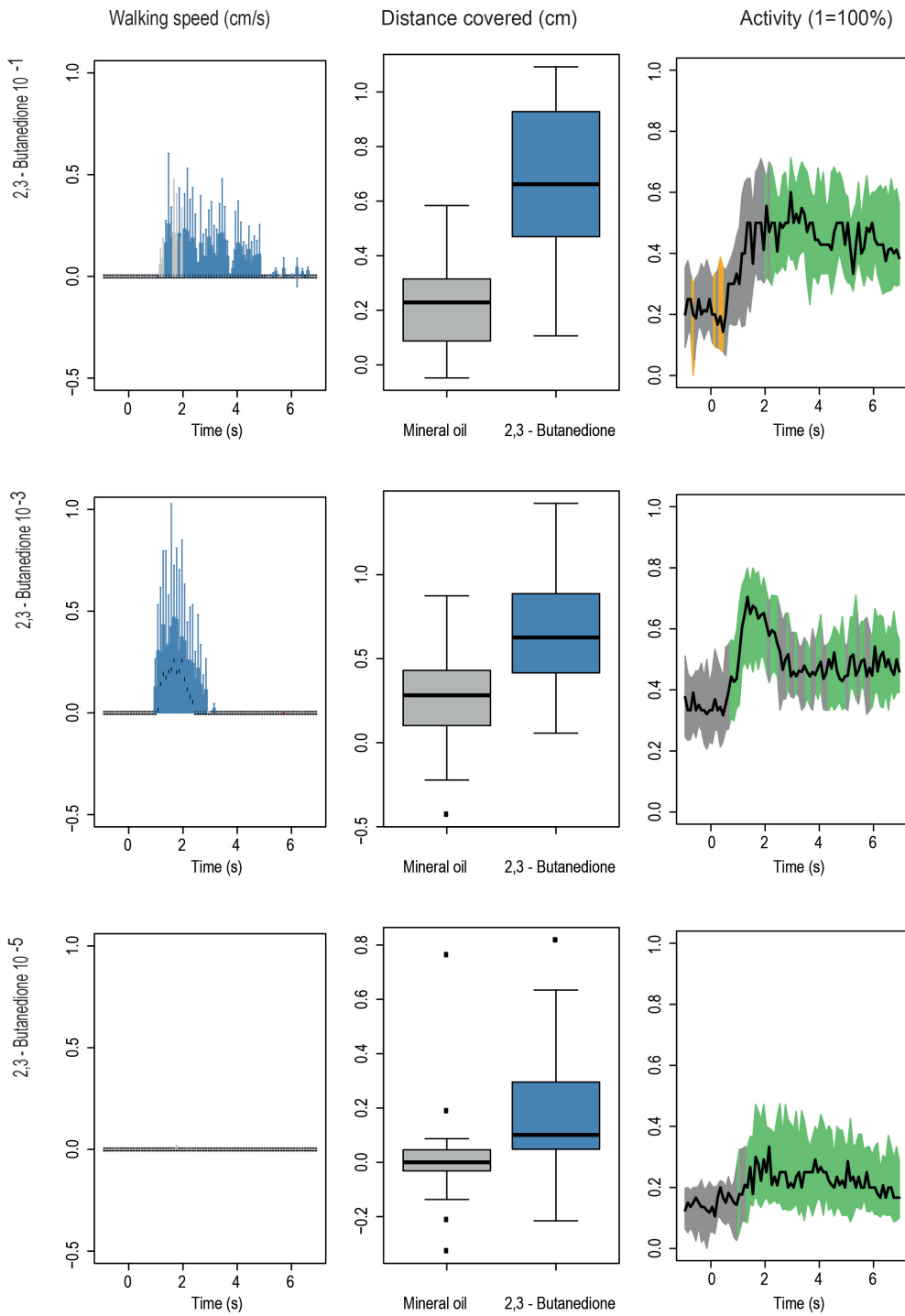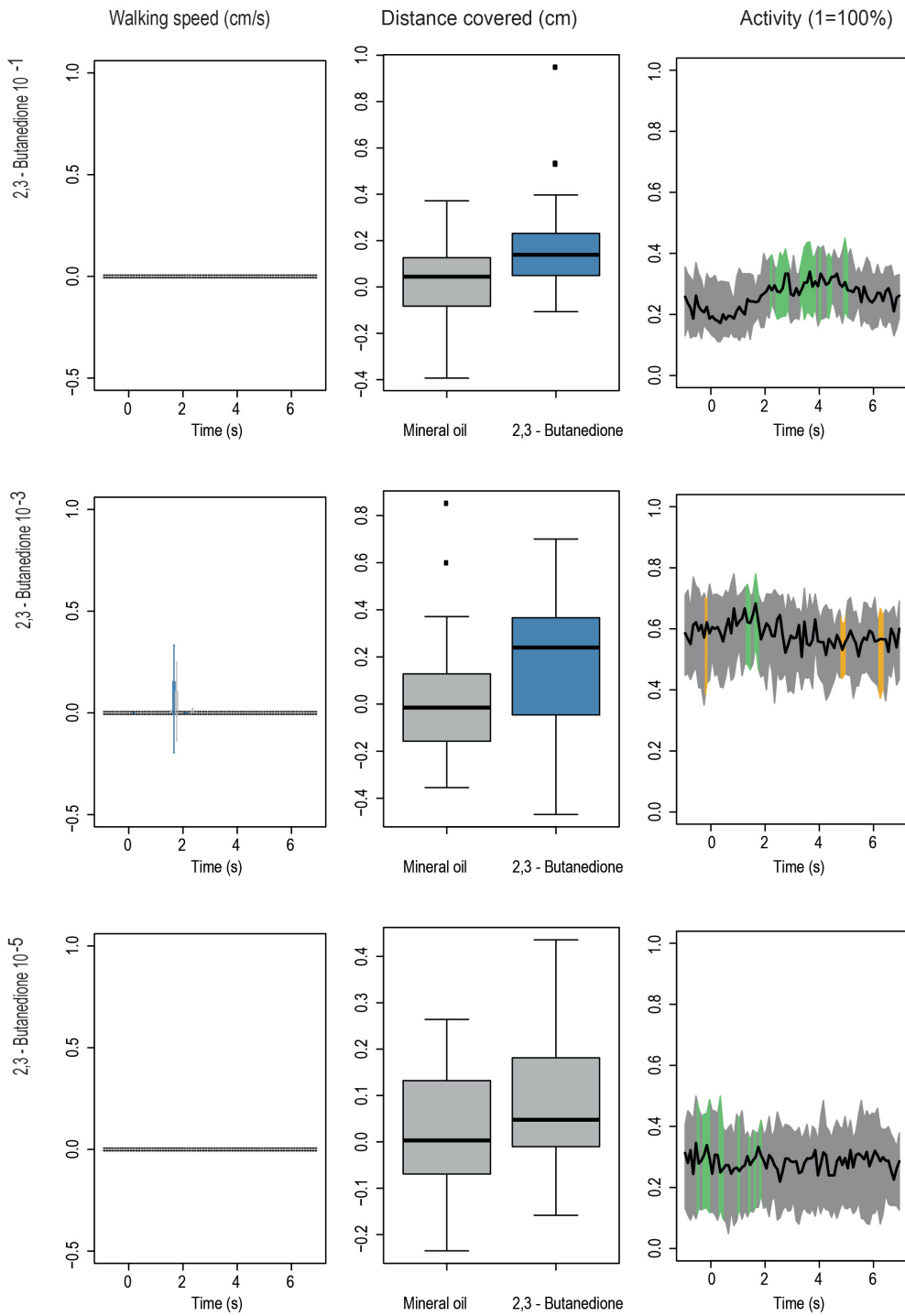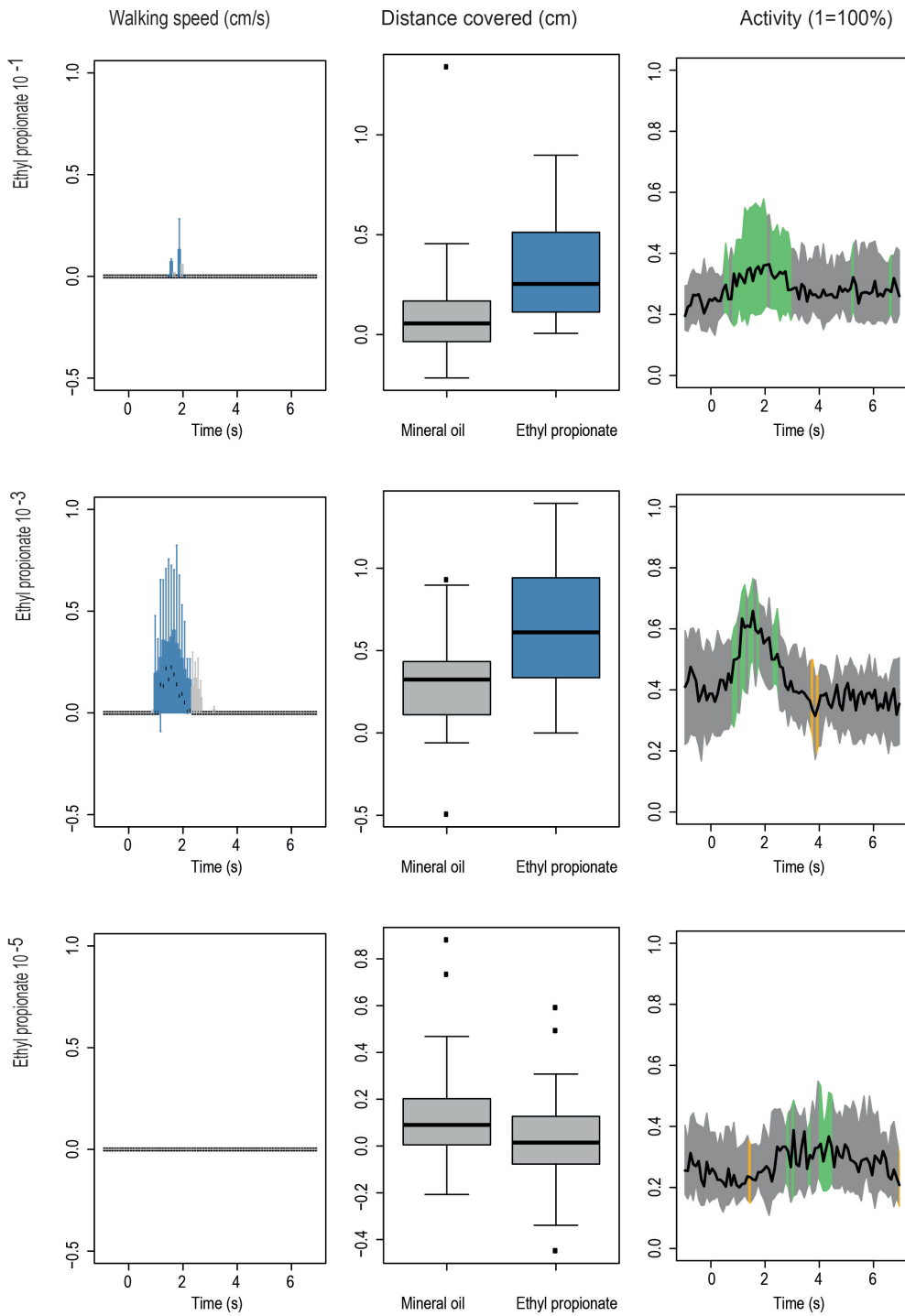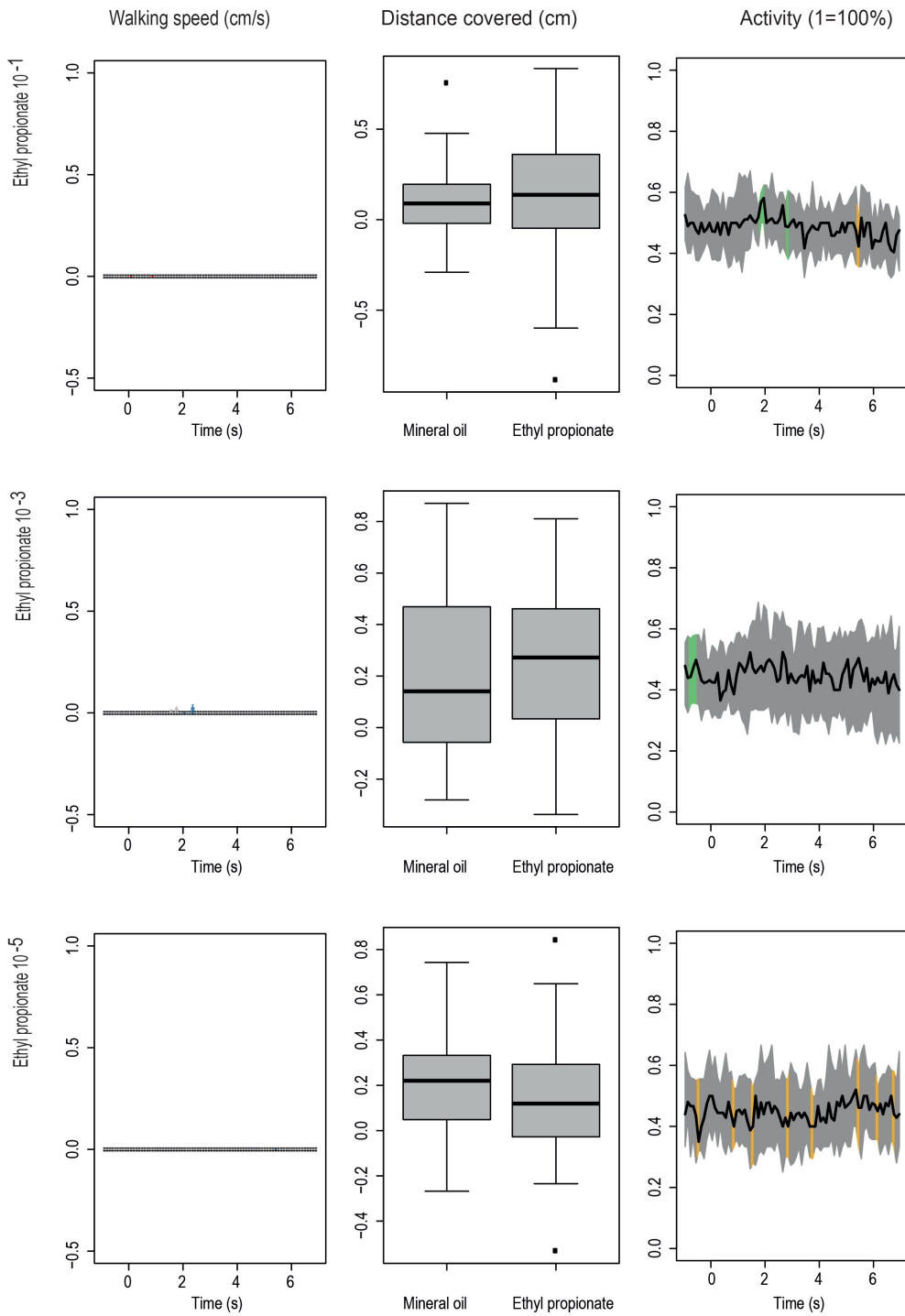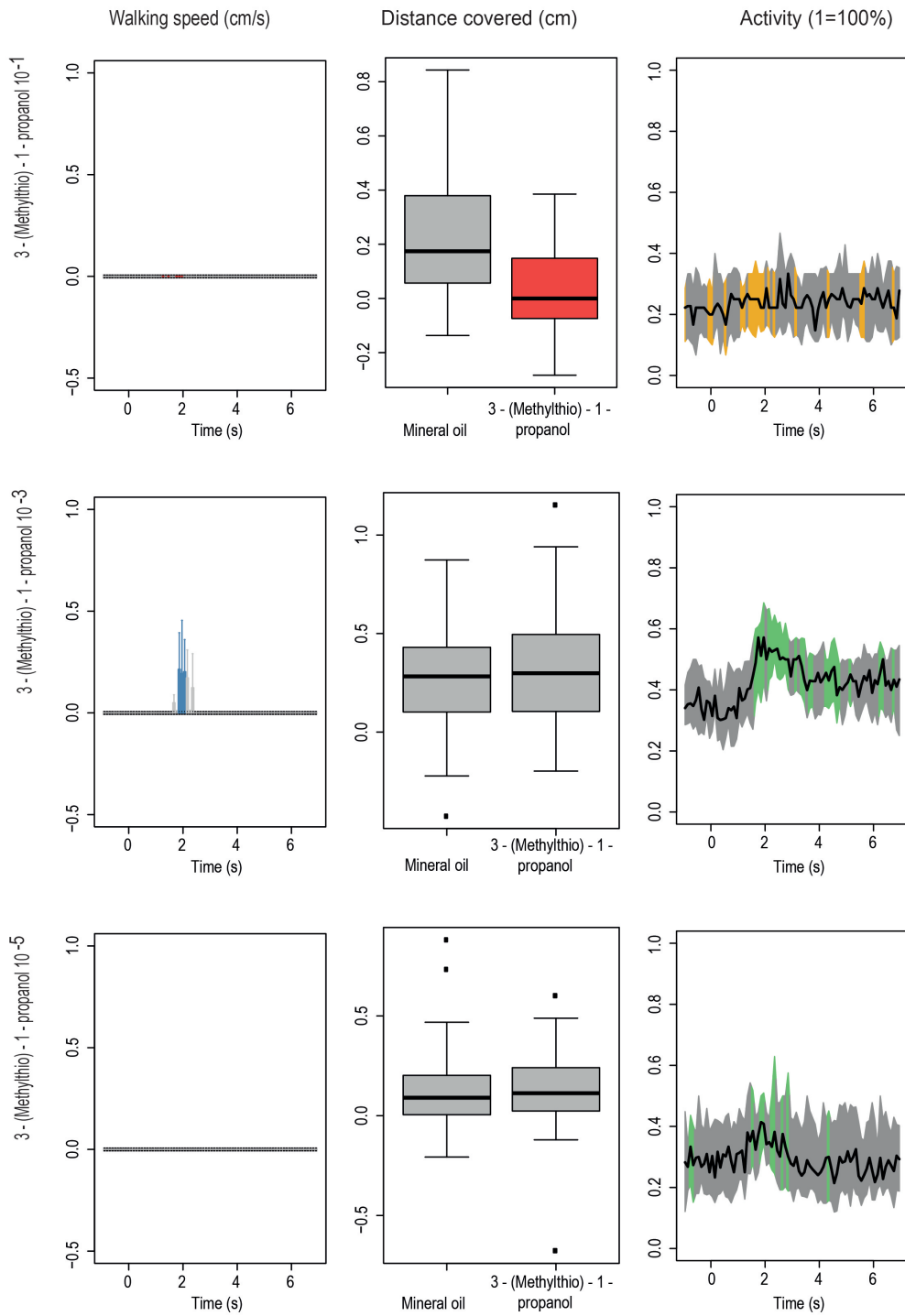
Figure A.2.: Response of Orco -/- flies to three different concentrations of Methyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
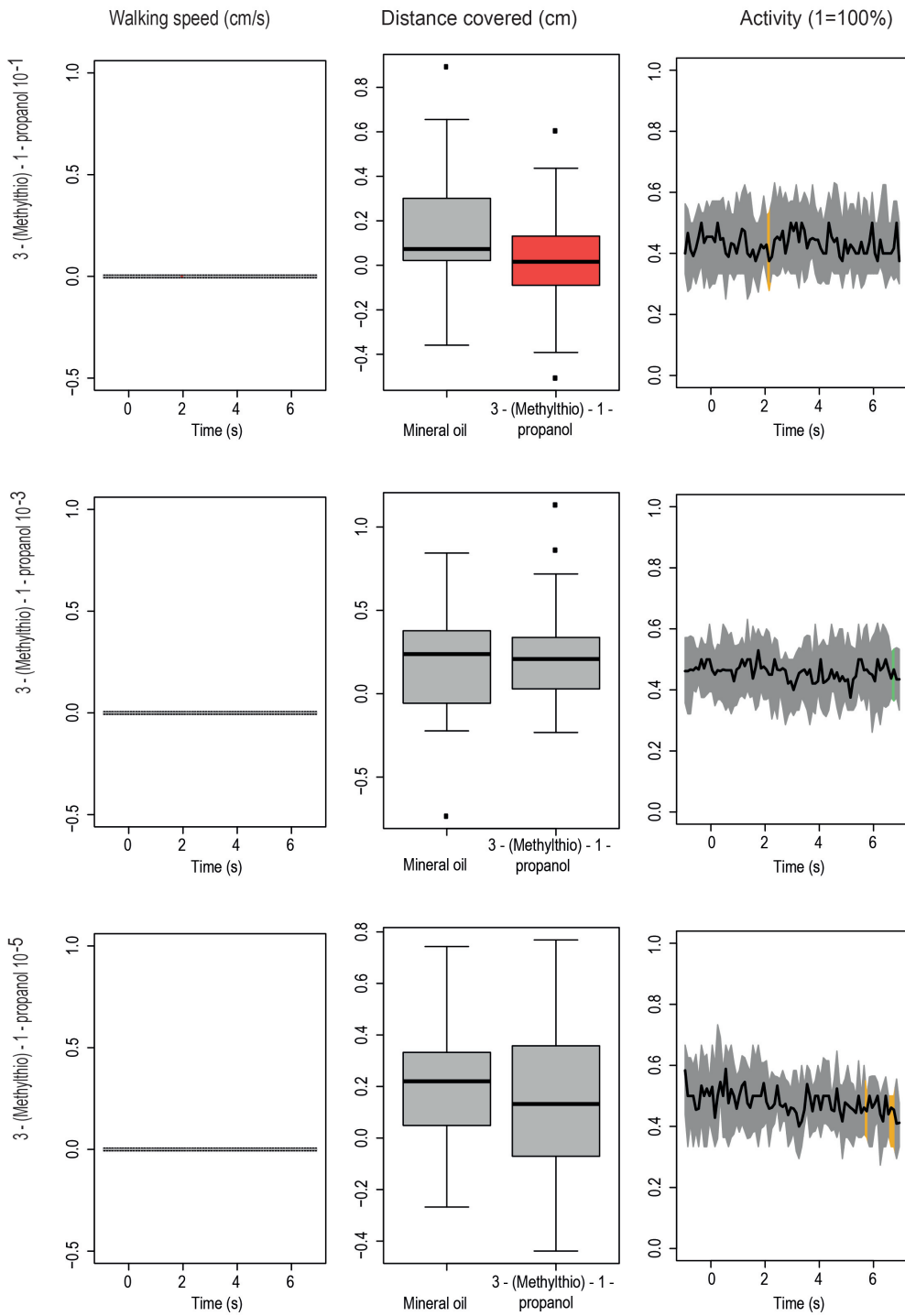
Figure A.3.: Response of wild type flies to three different concentrations of Geranyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.4.: Response of Orco -/- flies to three different concentrations of Geranyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
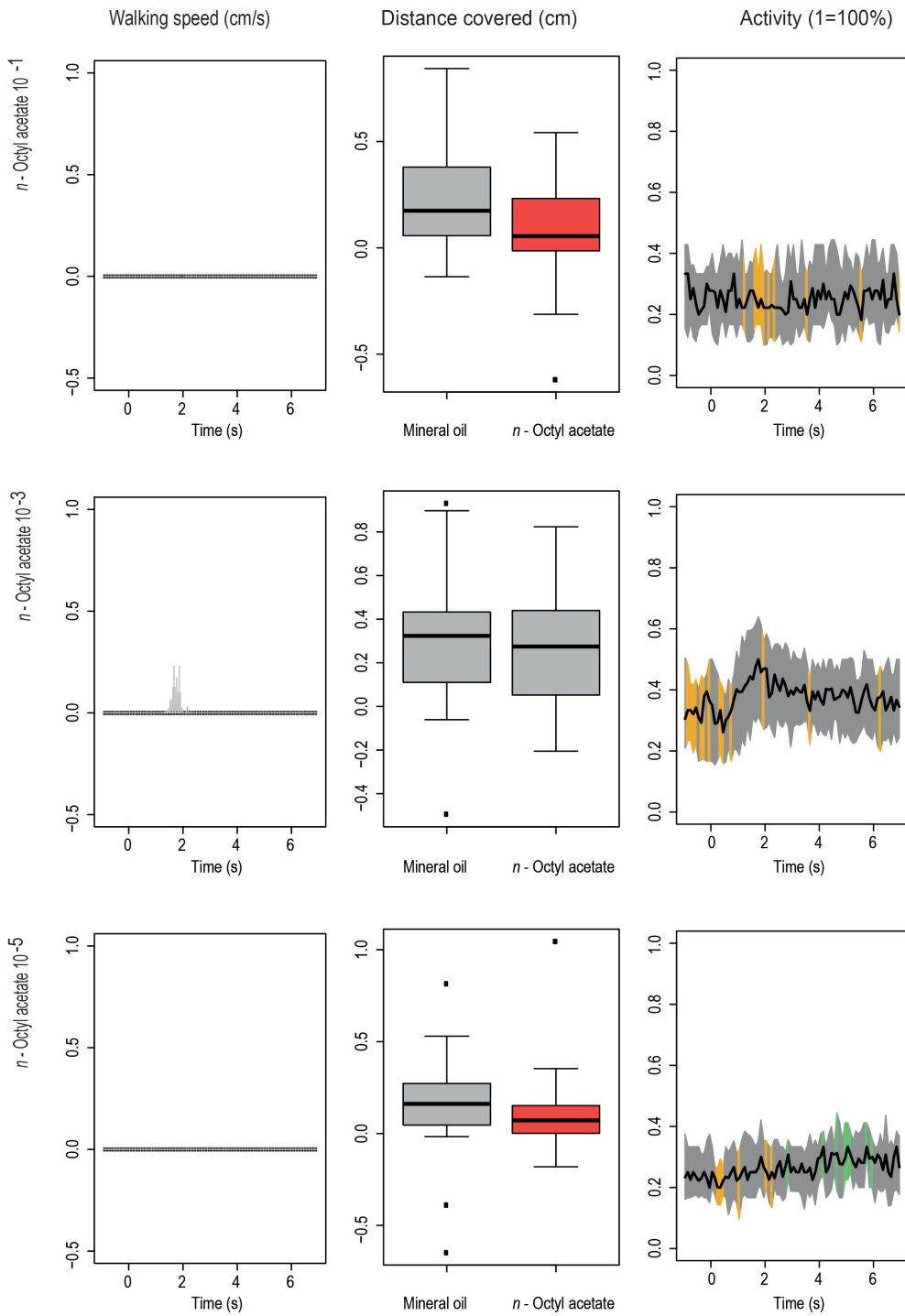
Figure A.5.: Response of wild type flies to three different concentrations of Ethyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.6.: Response of Orco -/- flies to three different concentrations of Ethyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

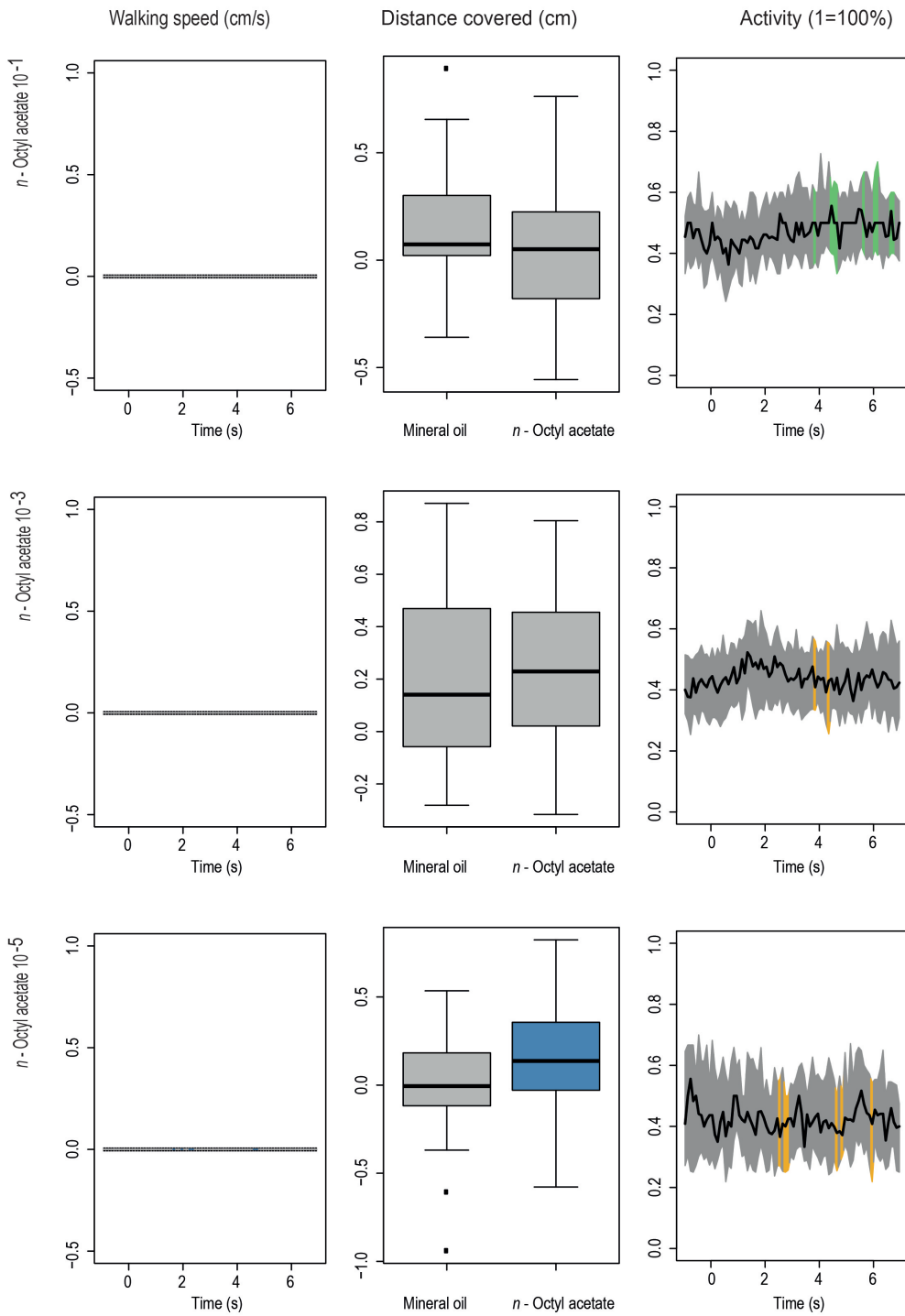**Figure A.7.: Response of wild type flies to three different concentrations of 2,3-Butanedione.** For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.8.: Response of Orco -/- flies to three different concentrations of 2,3-Butanedione. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

51

Figure A.9.: Response of wild type flies to three different concentrations of Ethyl propionate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.10.: Response of Orco -/- flies to three different concentrations of Ethyl propionate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
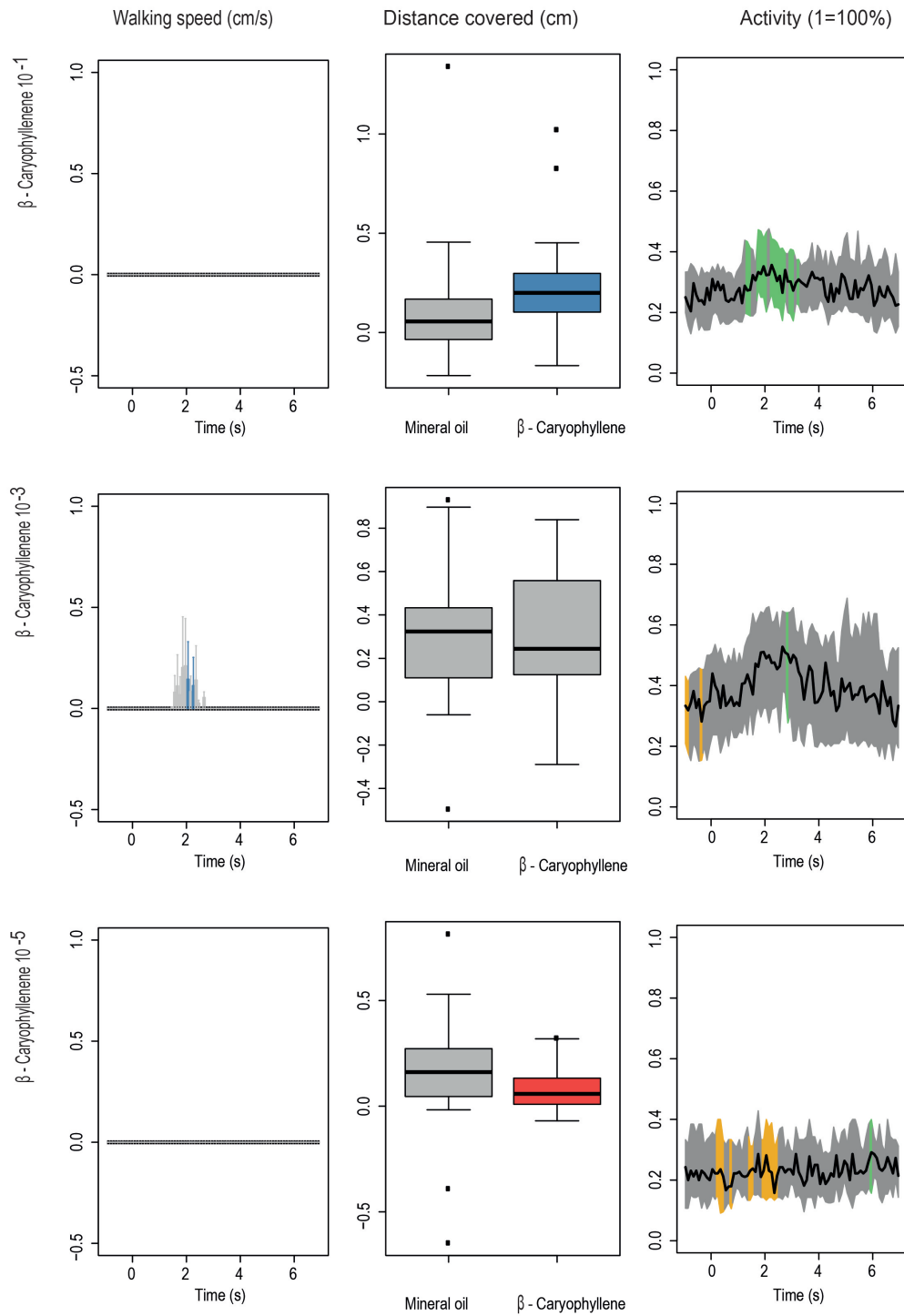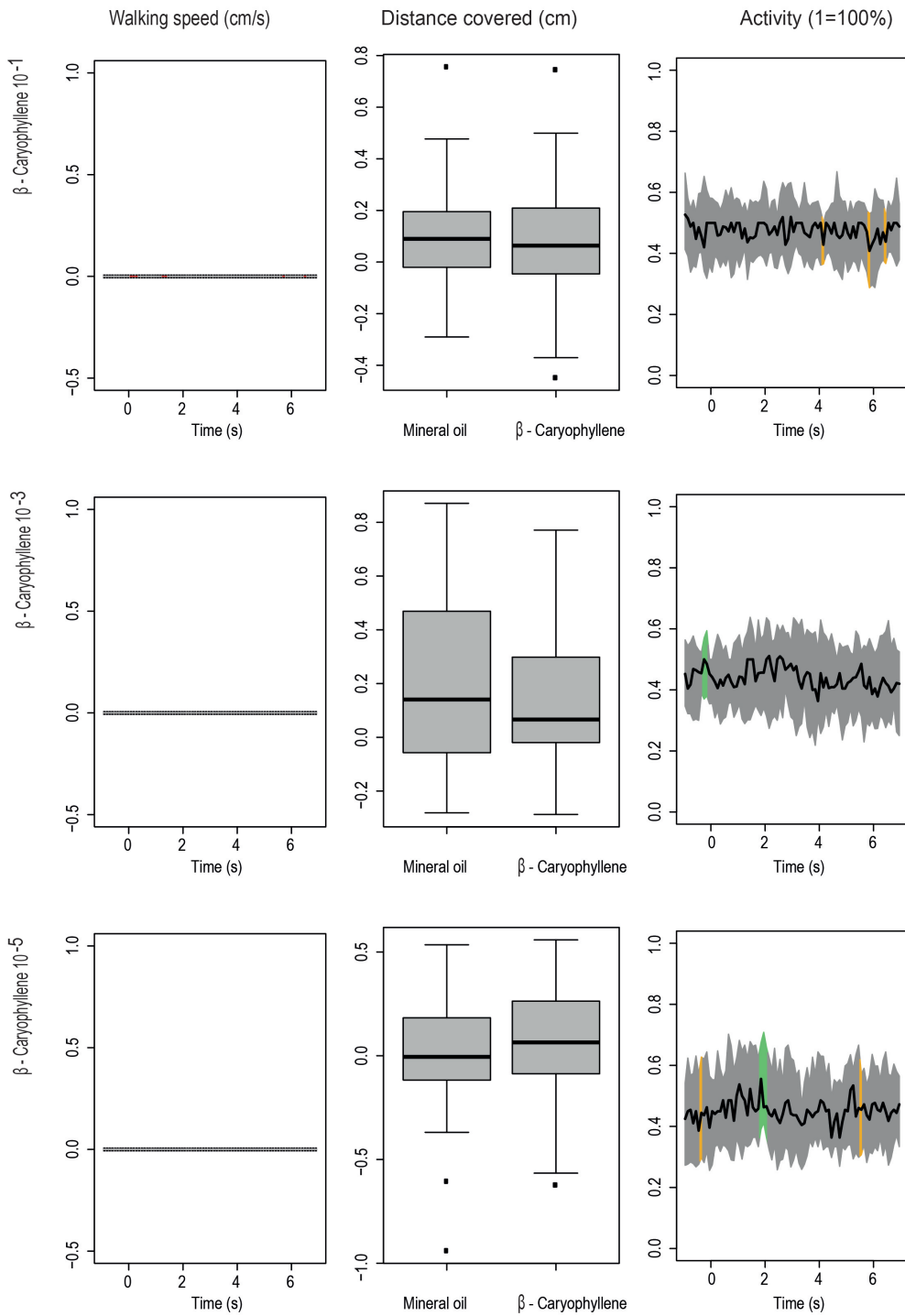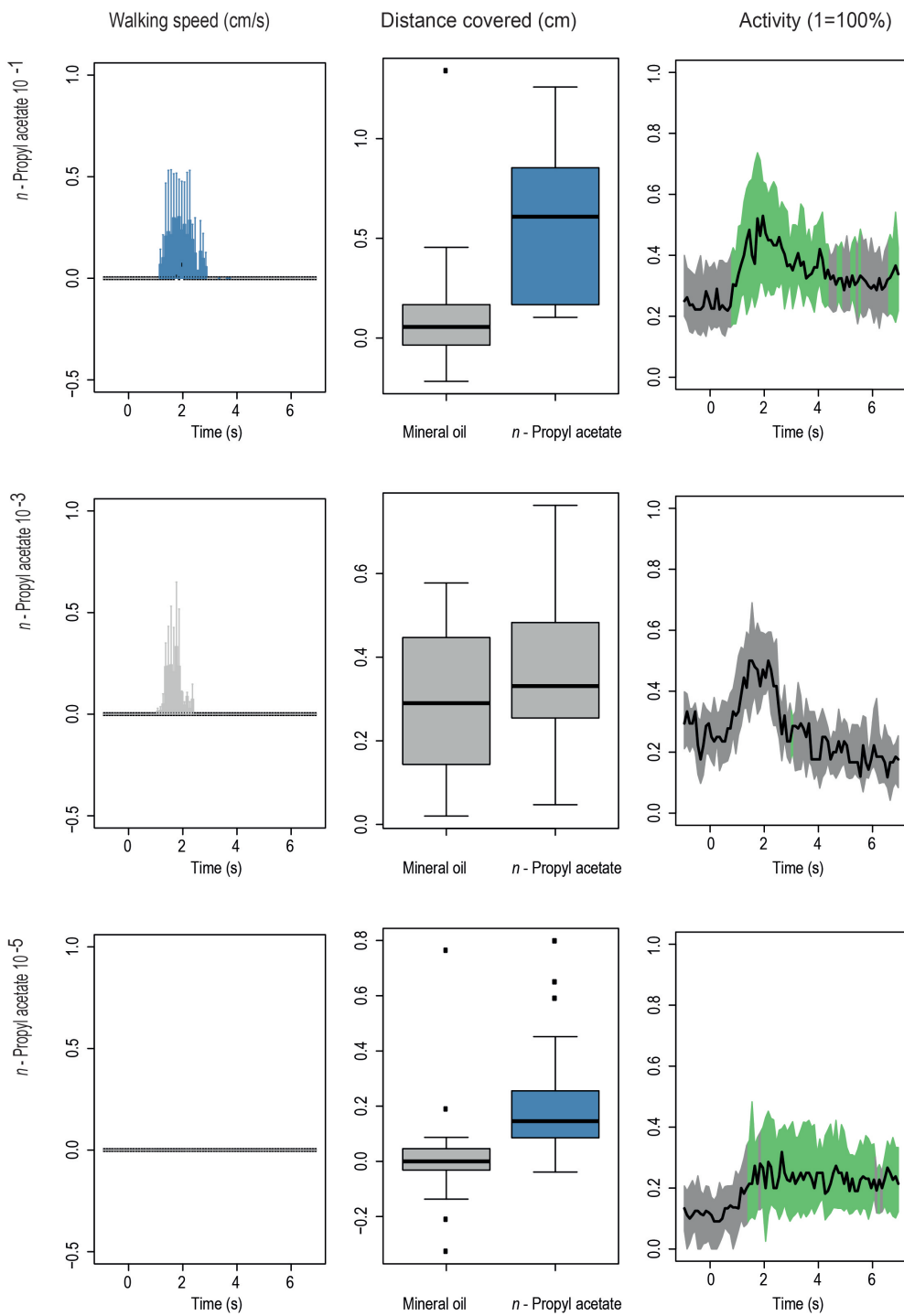
Figure A.11.: Response of wild type flies to three different concentrations of to 3-(methylthio)-1-propanol. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.12.: Response of Orco -/- flies to three different concentrations of 3-(methylthio)-1-propanol. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.13.: Response of wild type flies to three different concentrations of n-Octyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
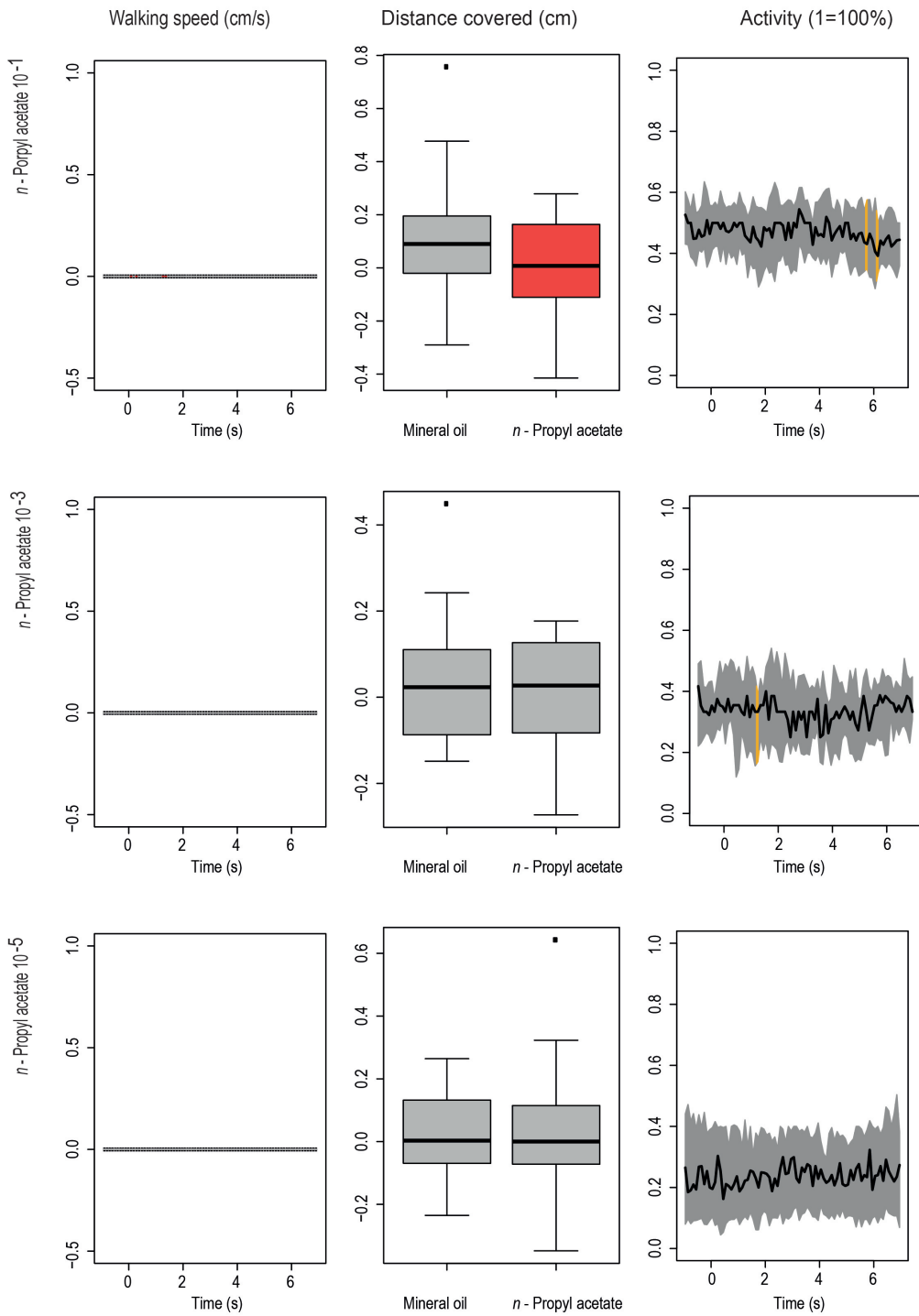
Figure A.14.: Response of Orco -/- flies to three different concentrations of *n*-Octyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

57

Figure A.15.: Response of wild type flies to three different concentrations of β-Caryophyllene. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
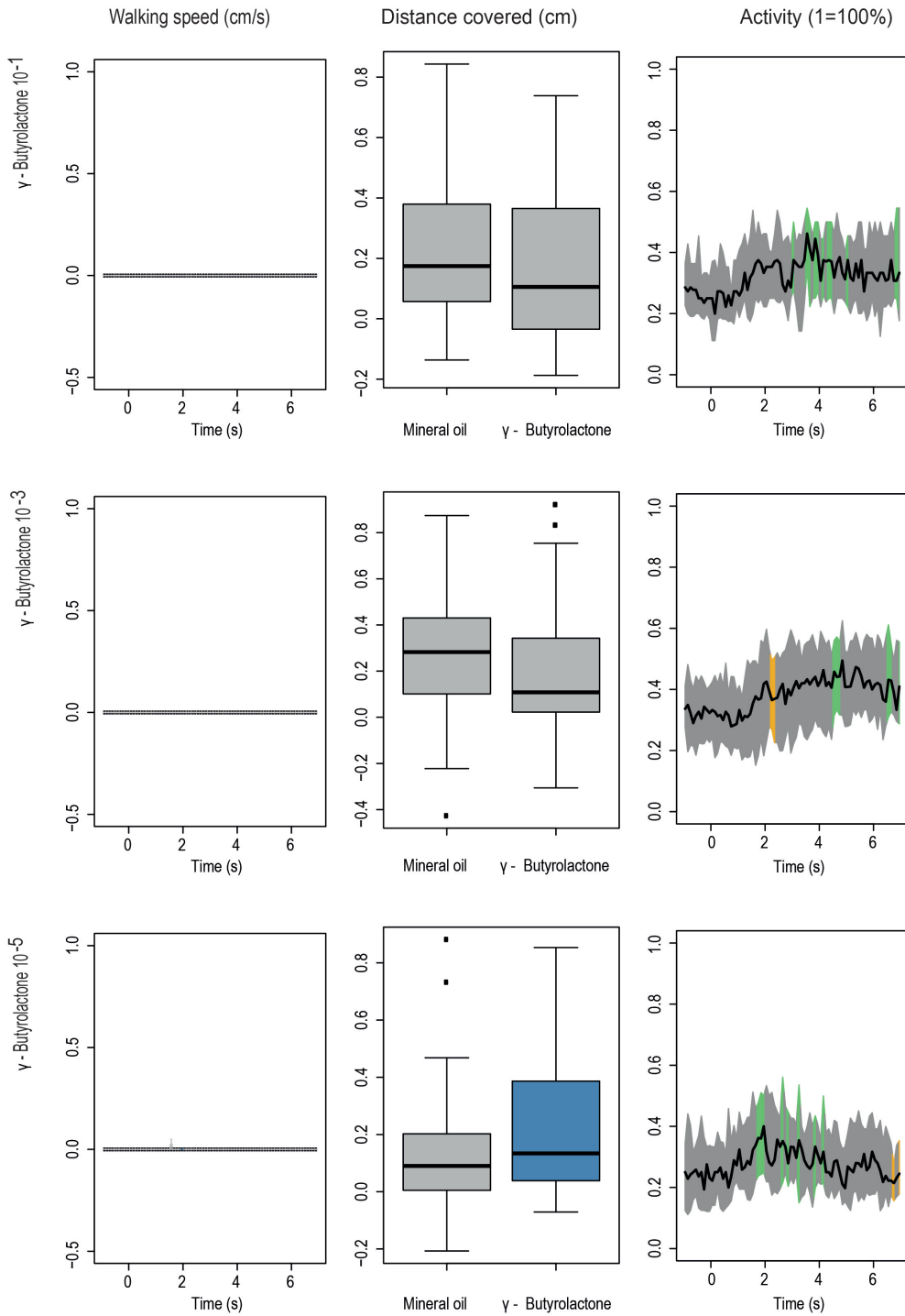
Figure A.16.: Response of Orco -/- flies to three different concentrations of β**-Caryophyllene.** For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
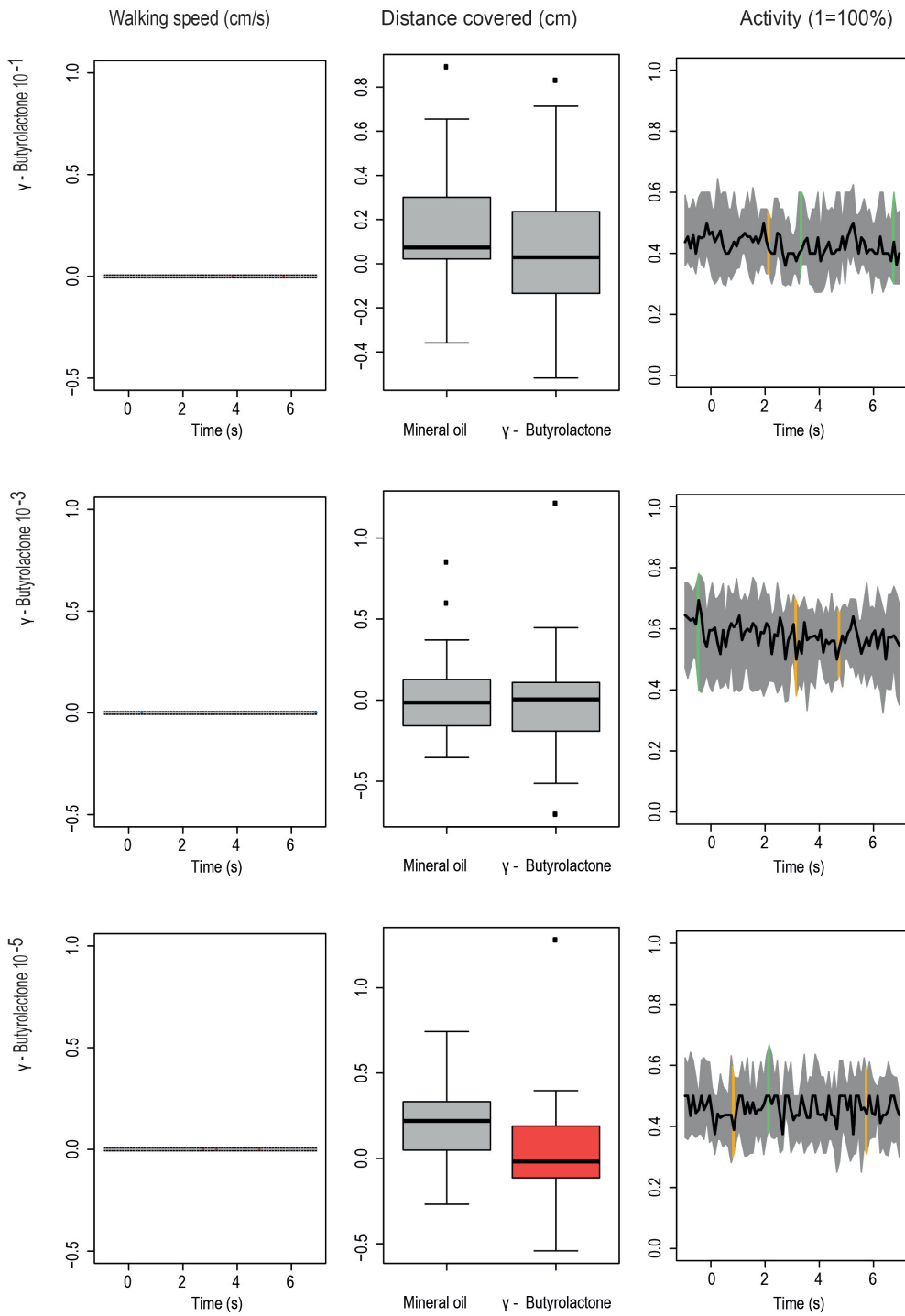
Figure A.17.: Response of wild type flies to three different concentrations of *n*-Propyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30; for the concentration of 10^{-3} n=15.
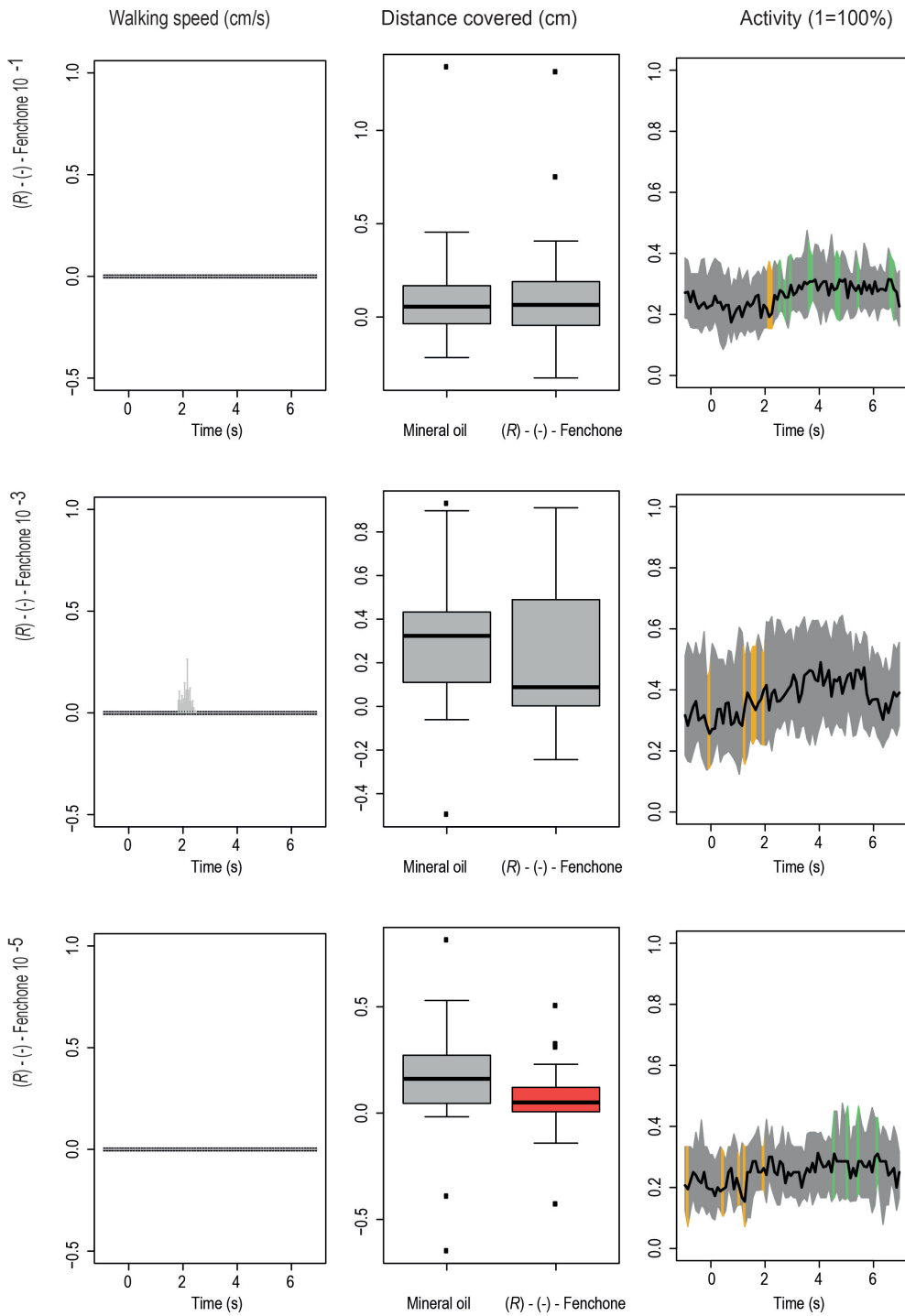
Figure A.18.: Response of Orco -/- flies to three different concentrations of n-Propyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30; for the concentration of 10^{-3} n=15.

Figure A.19.: Response of wild type flies to three different concentrations of γ-Butyrolactone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
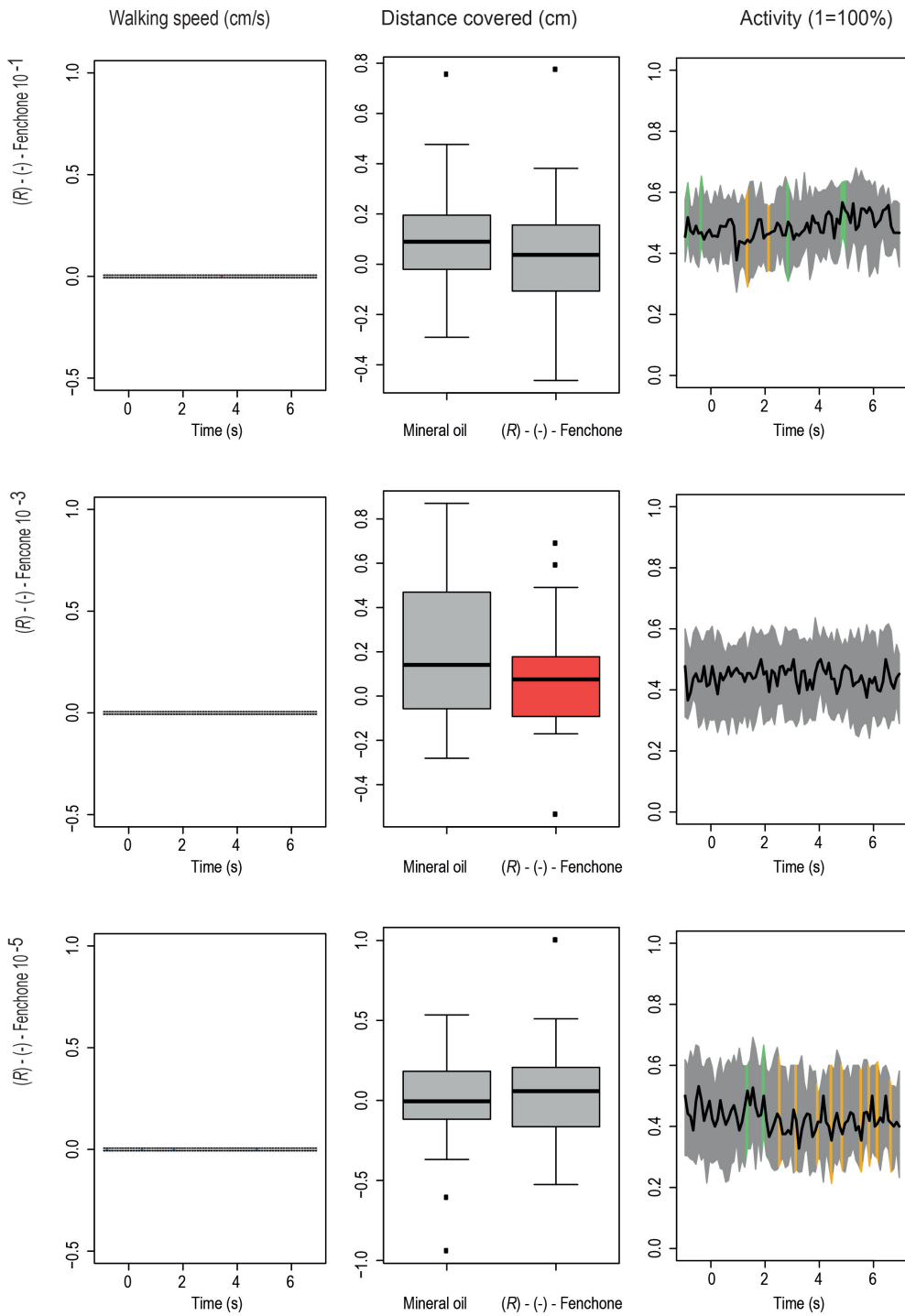
Figure A.20.: Response of Orco -/- flies to three different concentrations of γ-Butyrolactone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
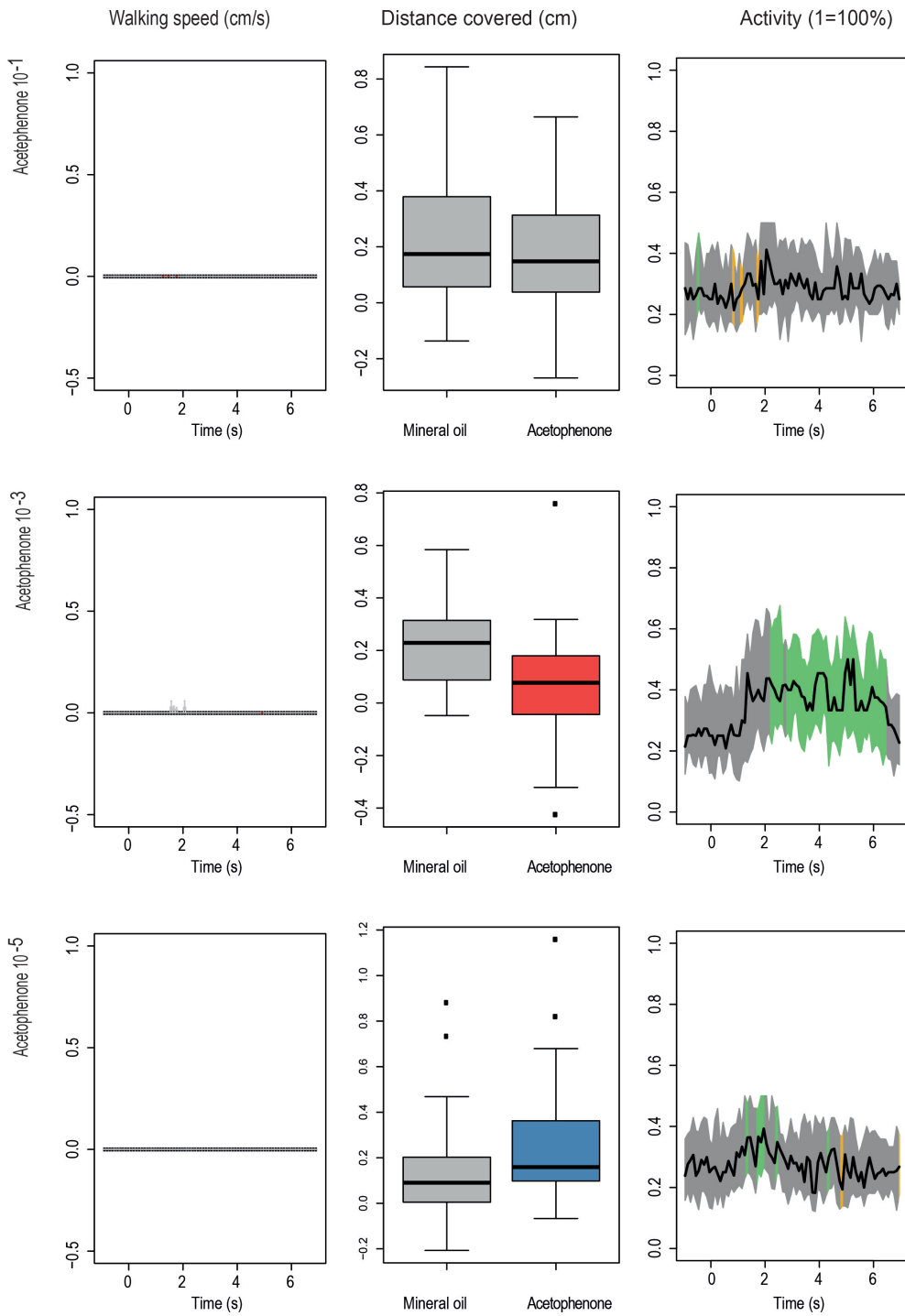
Figure A.21.: Response of wild type flies to three different concentrations of (*R*)-(-)-Fenchone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.22.: Response of Orco -/- flies to three different concentrations of (R)-(-)-Fenchone For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
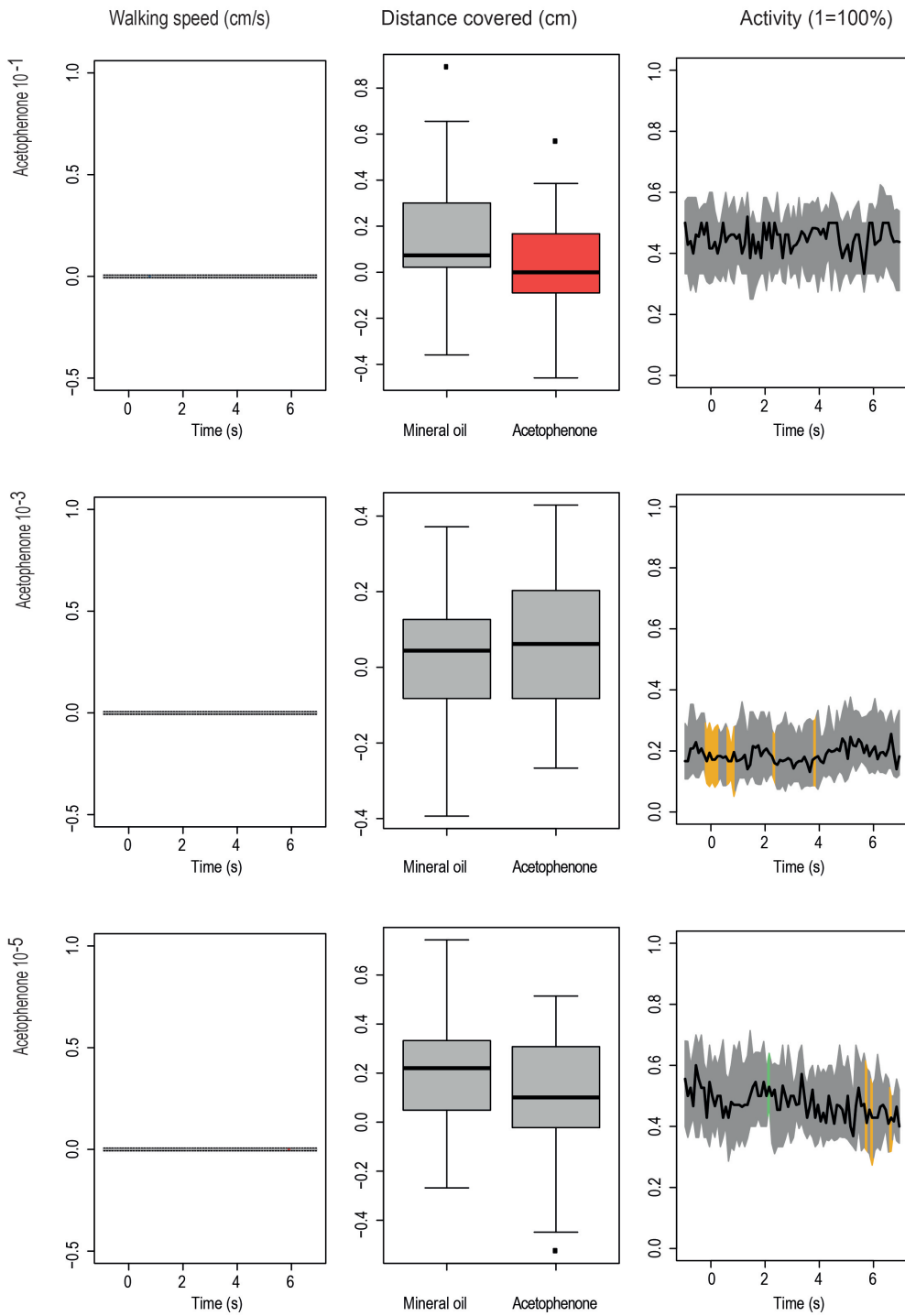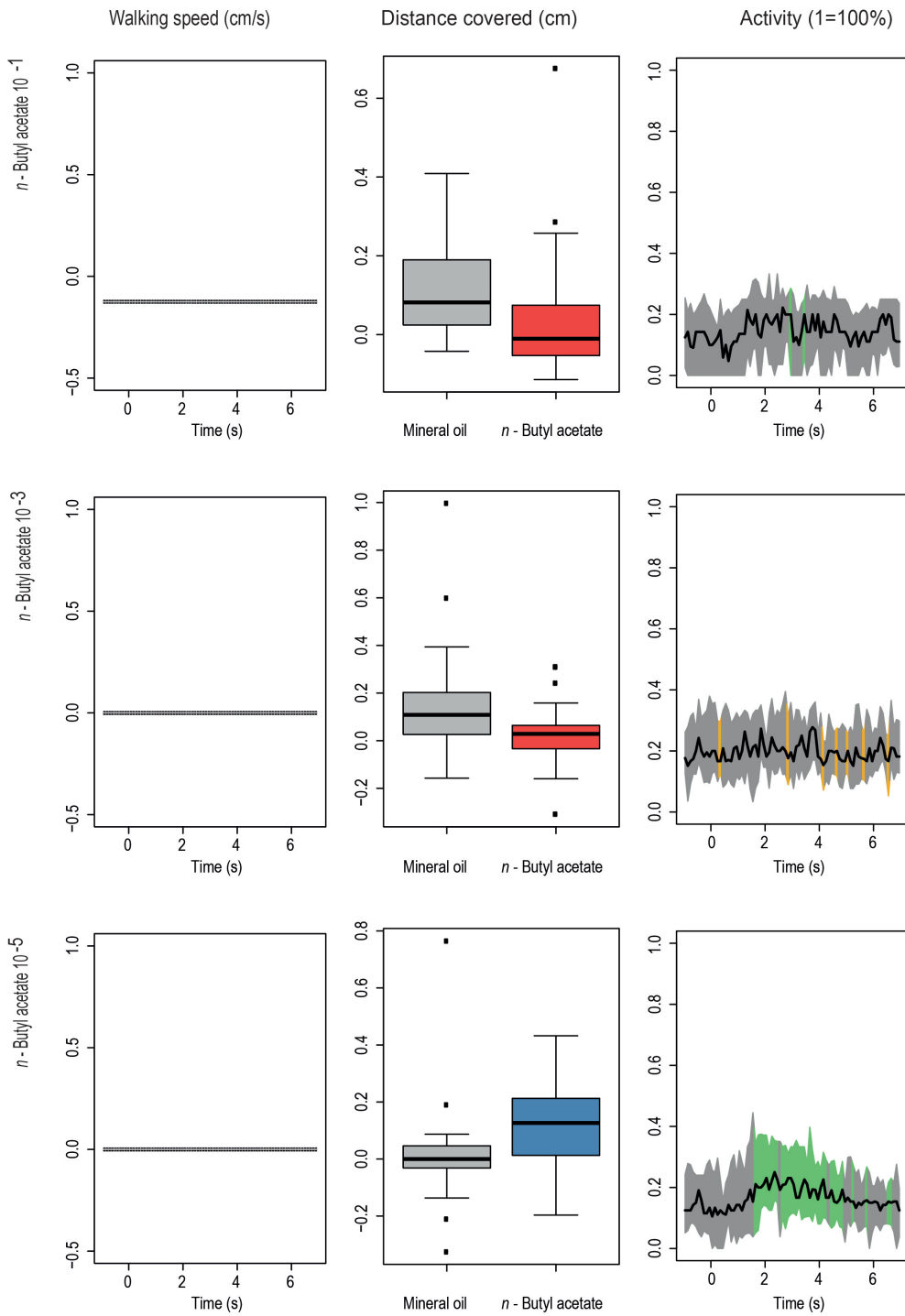
65

Figure A.23.: Response of wild type flies to three different concentrations of Acetophenone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.24.: Response of Orco -/- flies to three different concentrations of Acetophenone. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
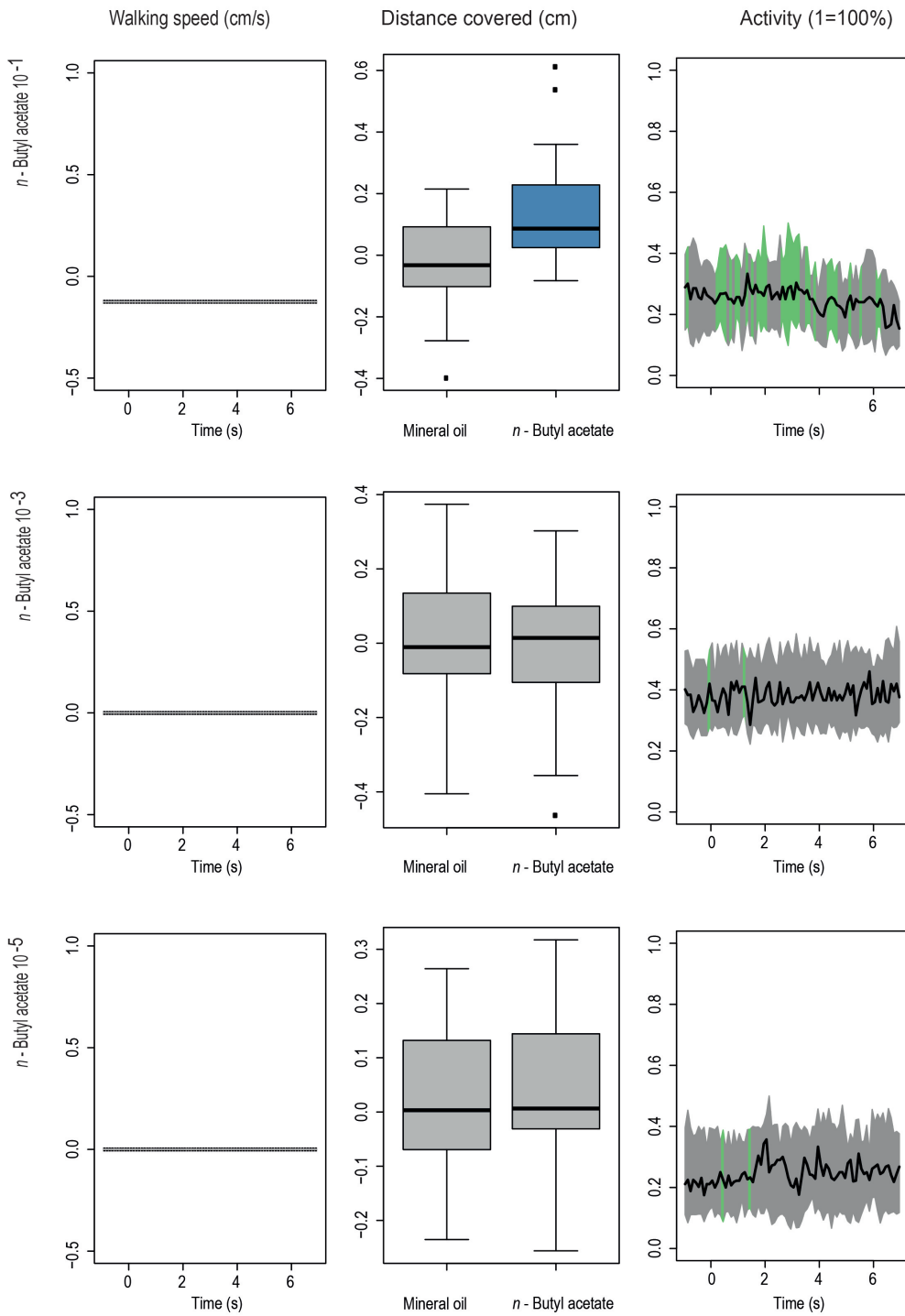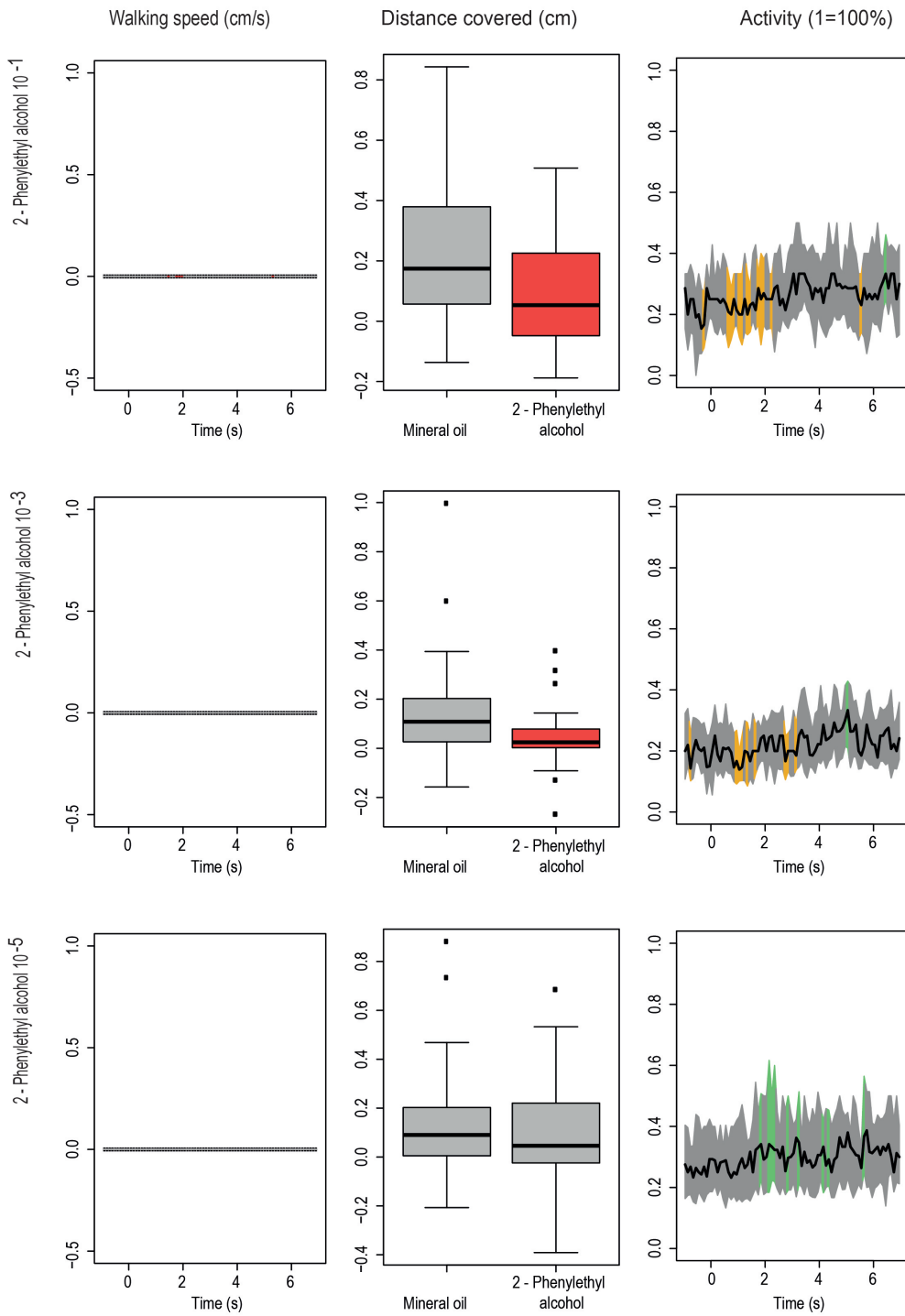
Figure A.25.: Response of wild type flies to three different concentrations of *n*-Butyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.26.: Response of Orco - /- flies to three different concentrations of *n*-Butyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.27.: Response of wild type flies to three different concentrations of 2-Phenylethyl alcohol. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.28.: Response of Orco -/- flies to three different concentrations of 2-Phenylethyl alcohol. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
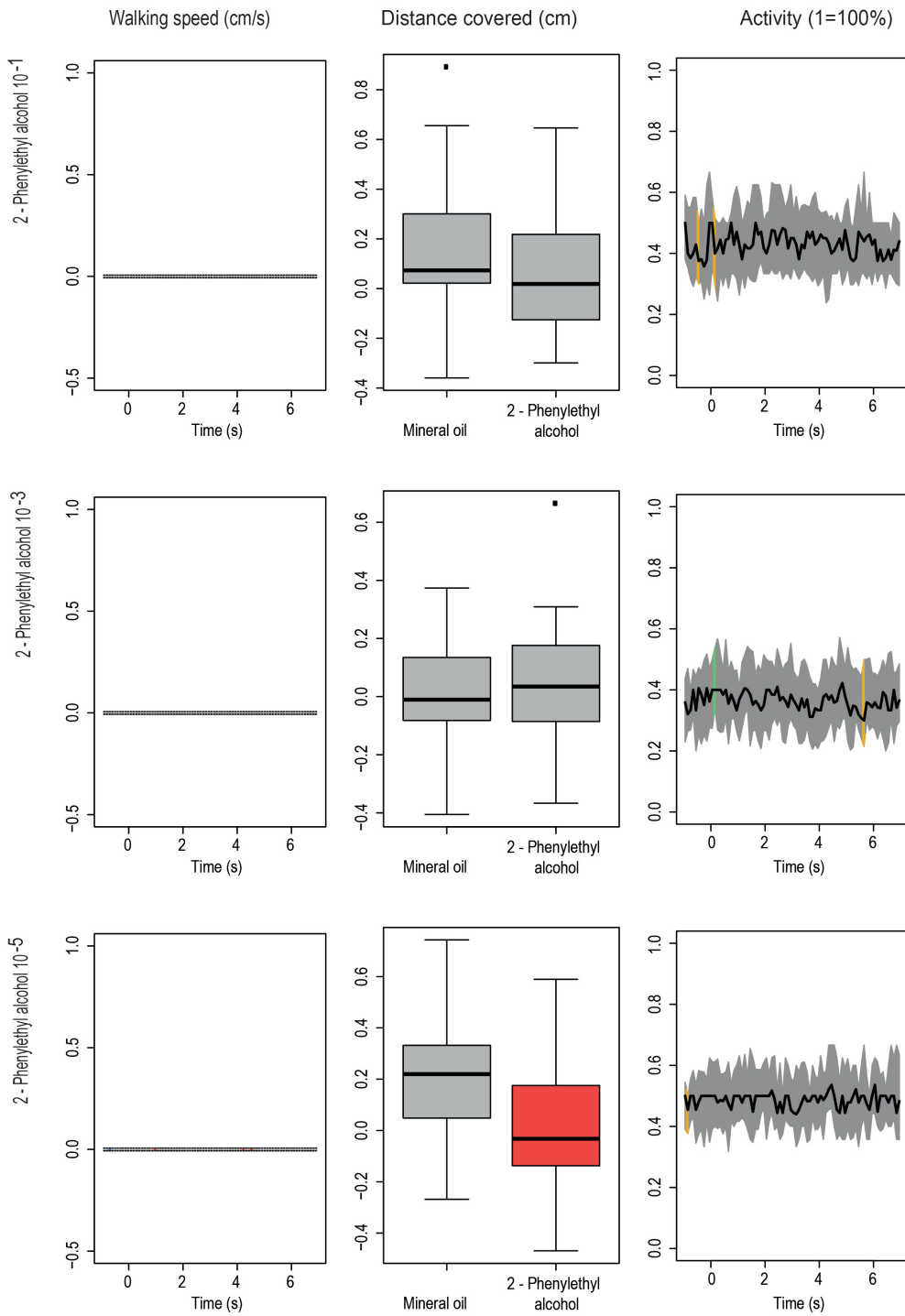
Figure A.29.: Response of wild type flies to three different concentrations of *n***-Heptyl acetate.** For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.30.: Response of Orco -/- flies to three different concentrations of *n*-Heptyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
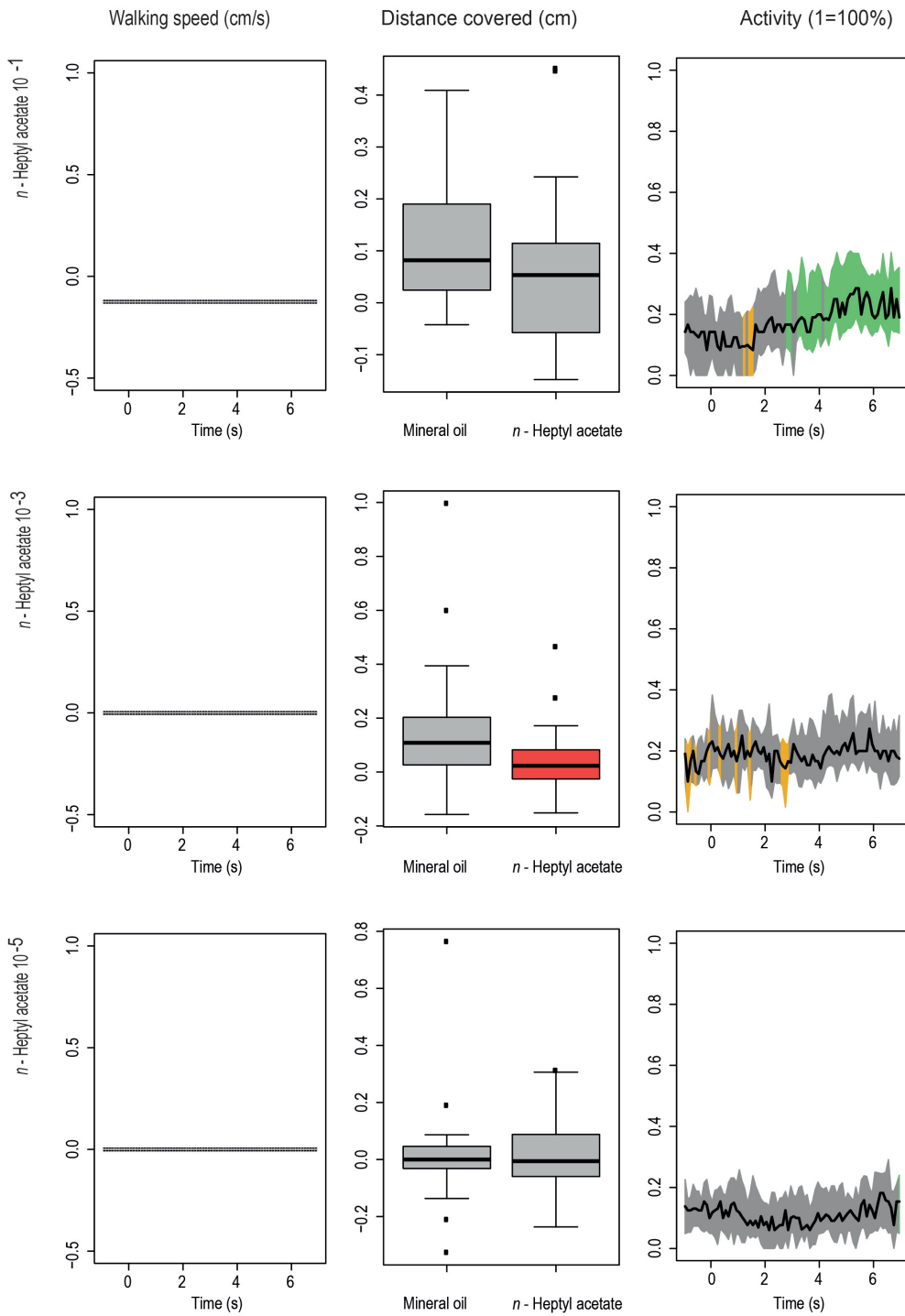
Figure A.31.: Response of wild type flies to three different concentrations of *n*-Pentyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.32.: Response of Orco -/- flies to three different concentrations of *n*-Pentyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
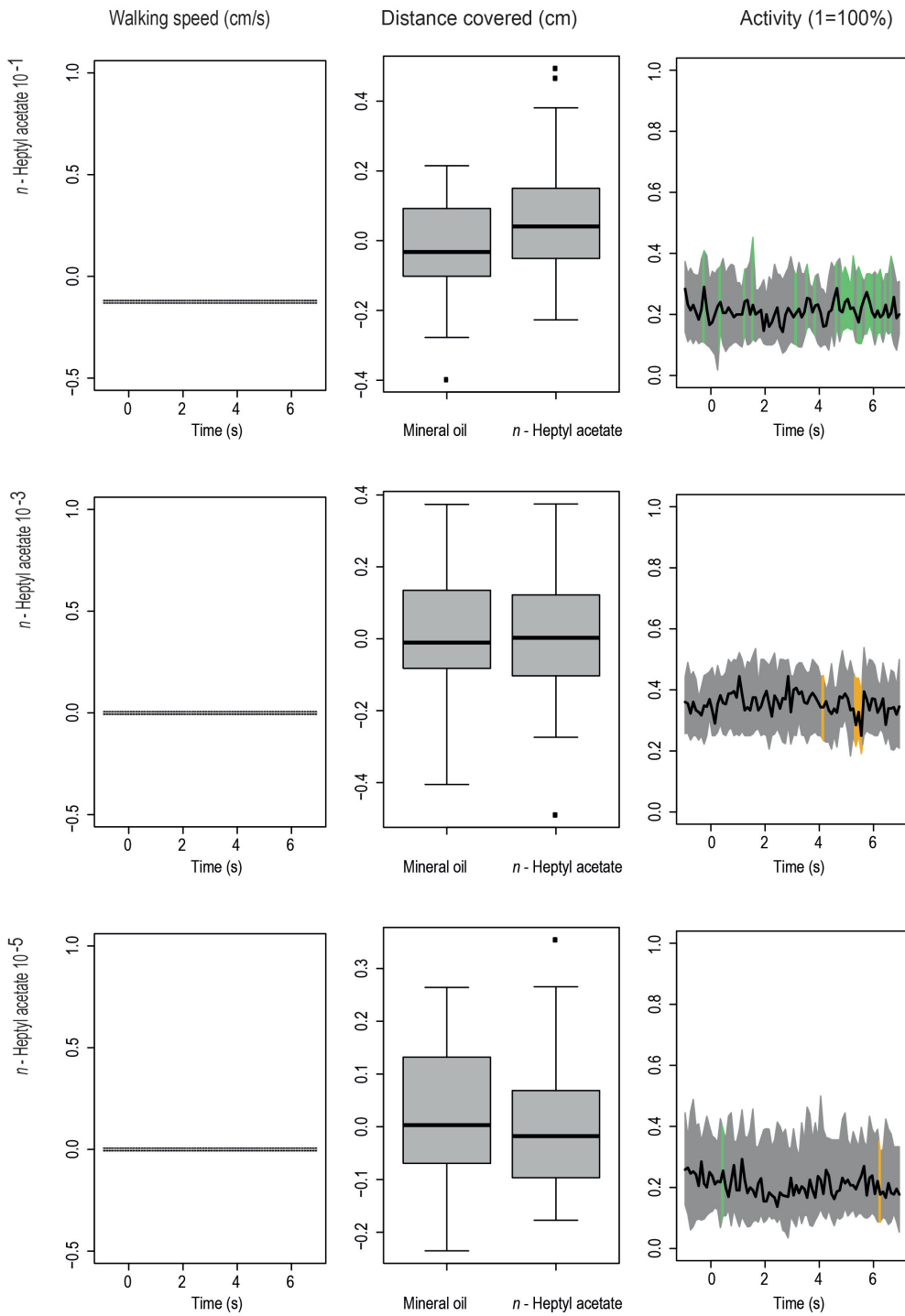
Figure A.33.: Response of wild type flies to three different concentrations of n-Hexyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
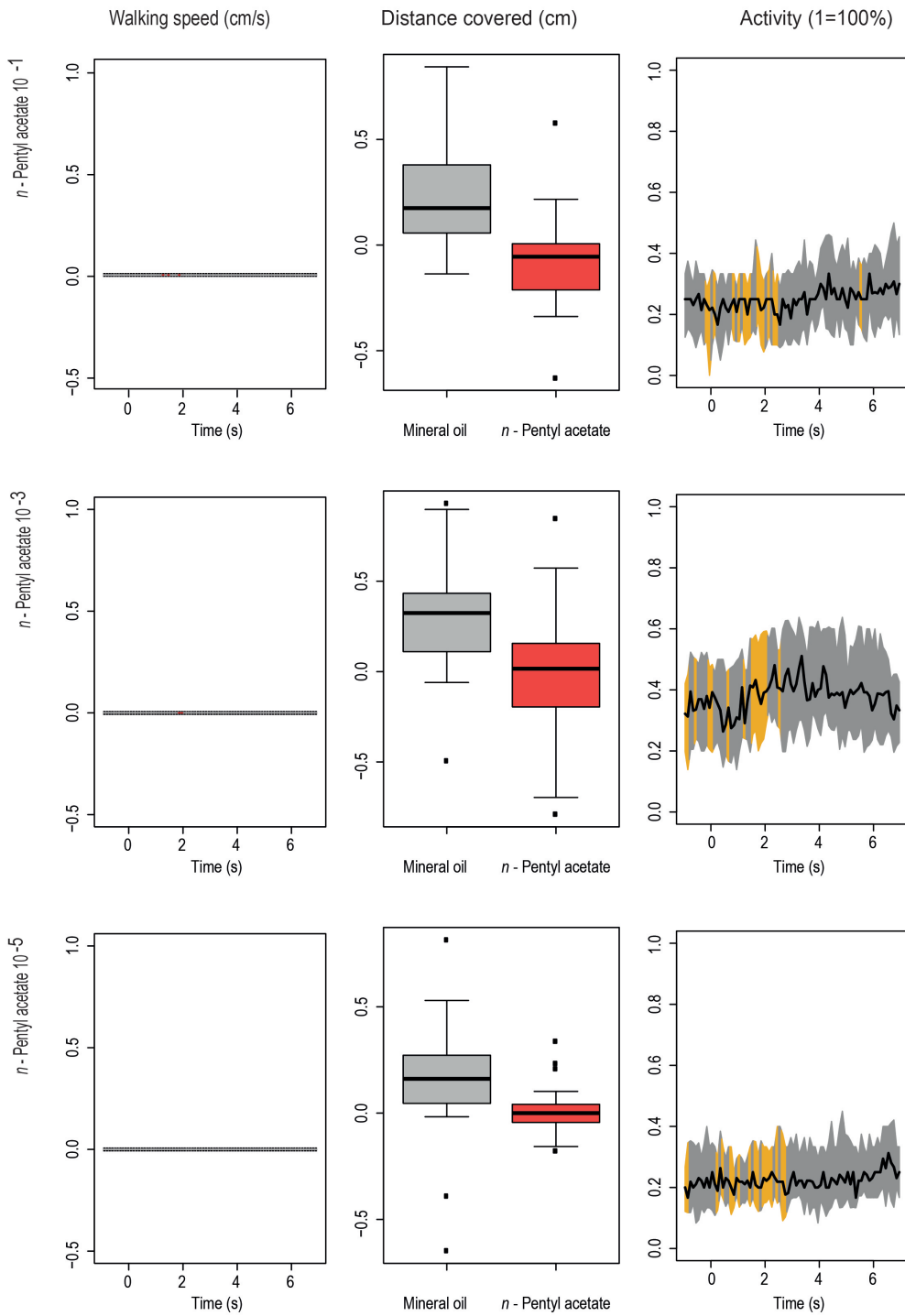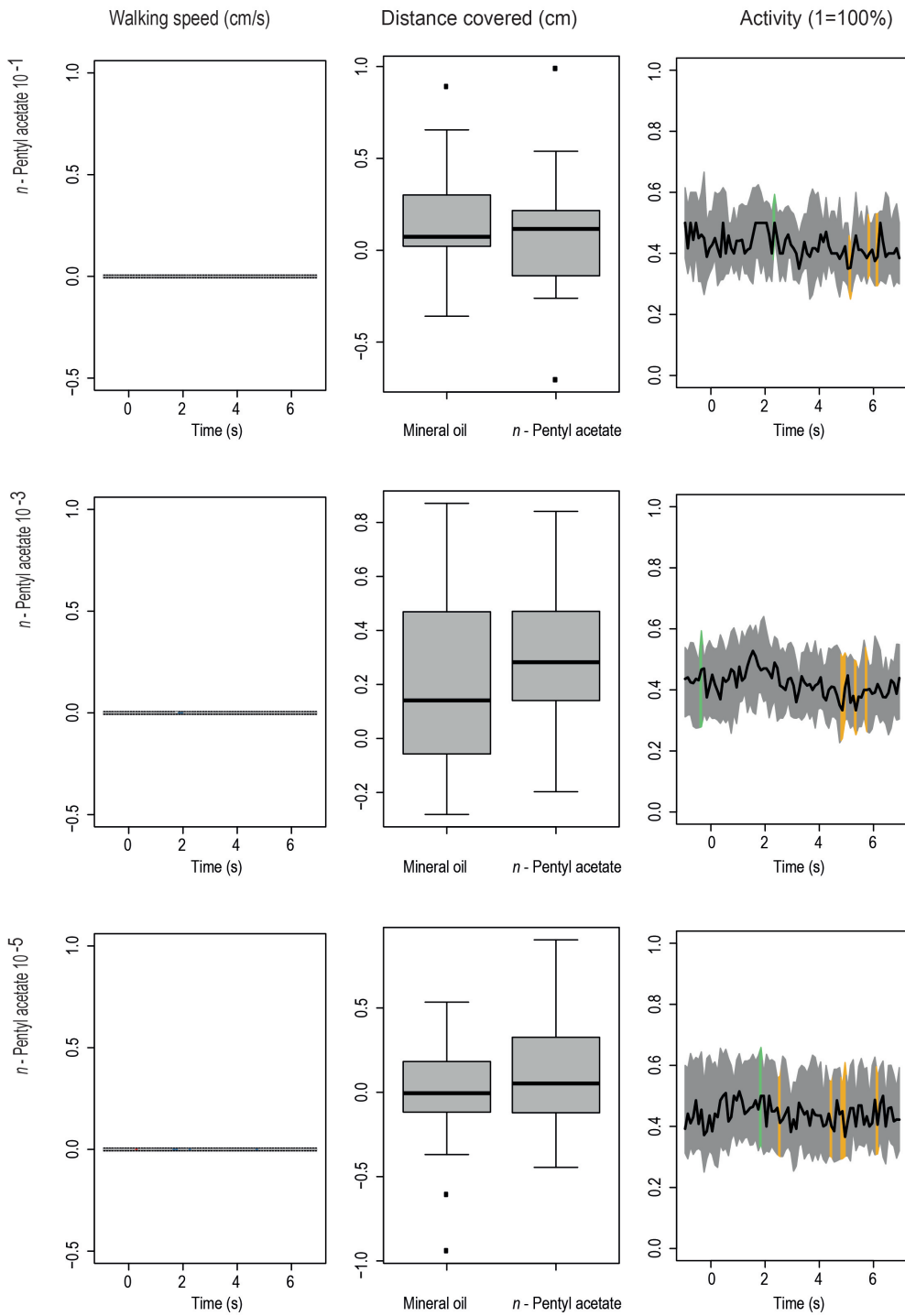
76
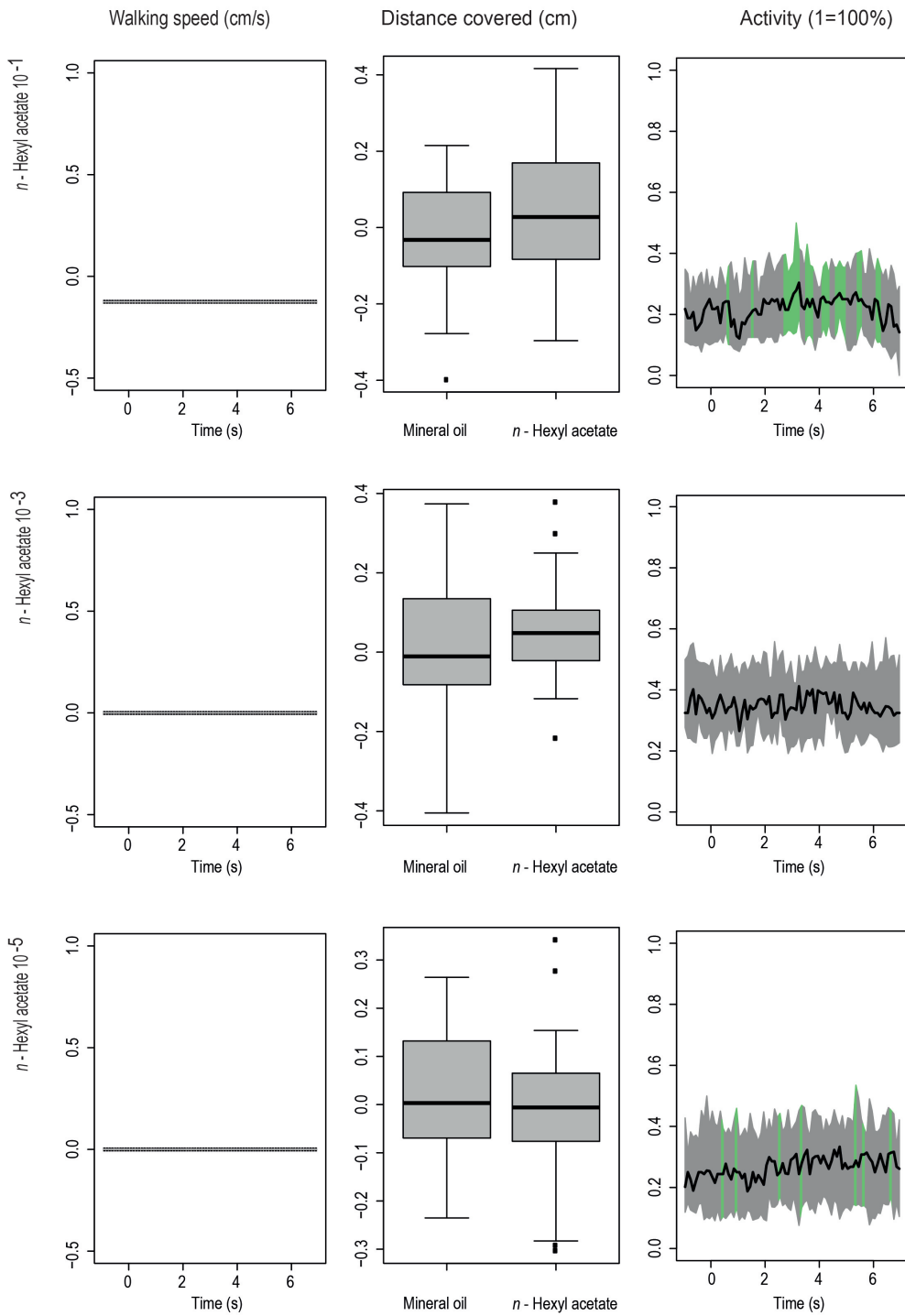
Figure A.34.: Response of Orco -/- flies to three different concentrations of *n*-Hexyl acetate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
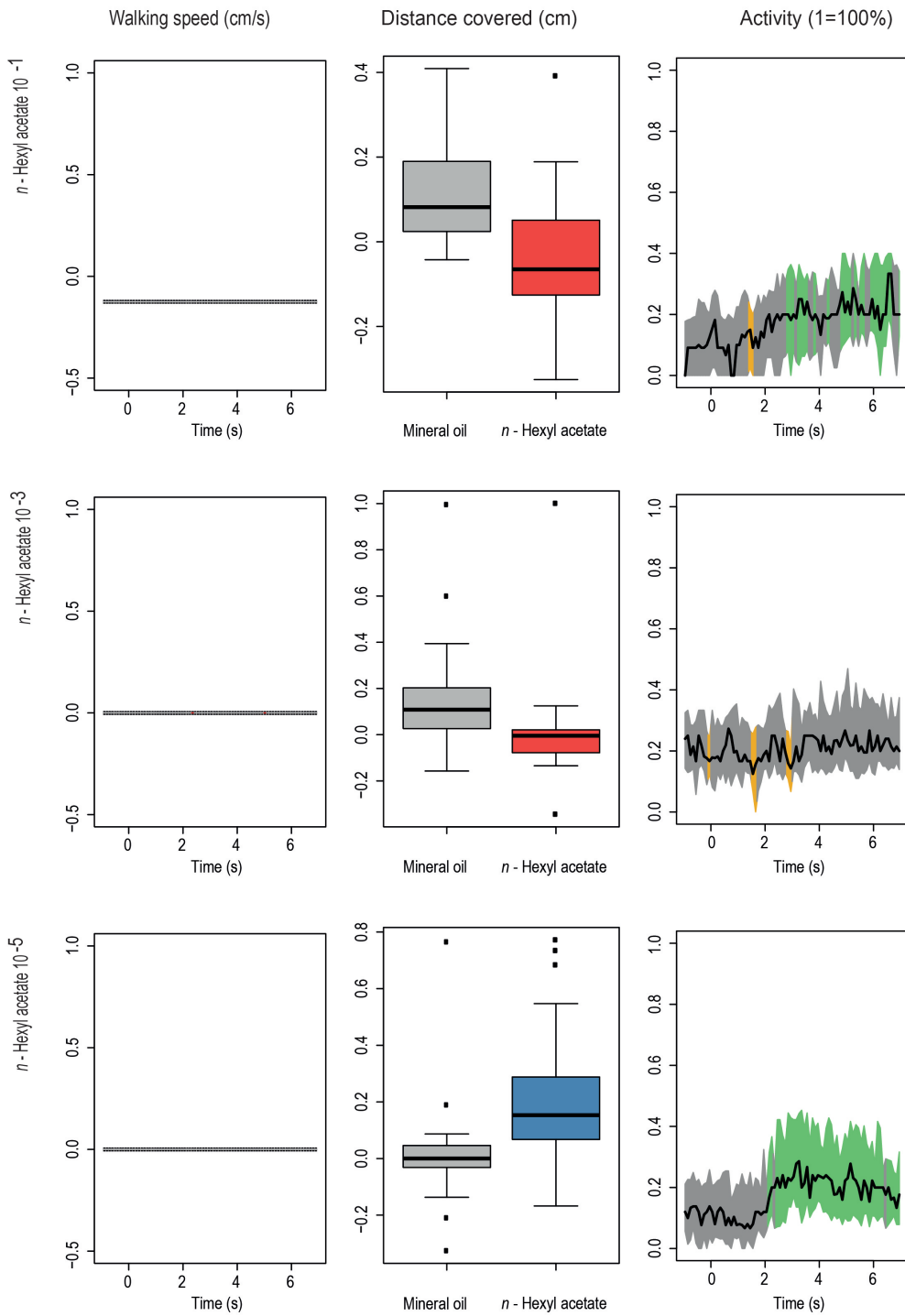
Figure A.35.: Response of wild type flies to three different concentrations of Methyl salicylate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.

Figure A.36.: Response of Orco -/- flies to three different concentrations of Methyl salicylate. For detailed explanation about the plots, color codes used, and statistics, refer to caption of Figure 3.1.. n=30.
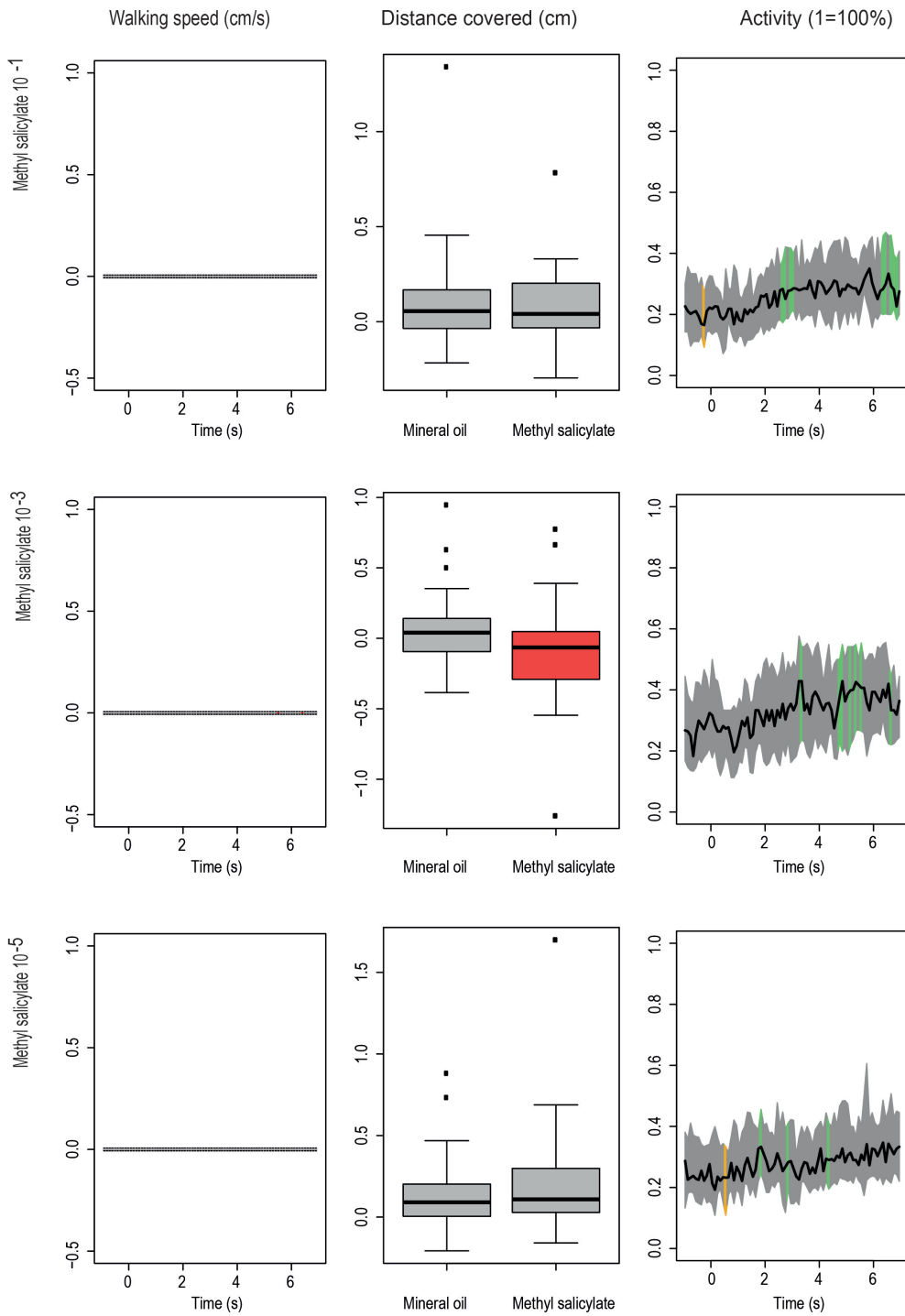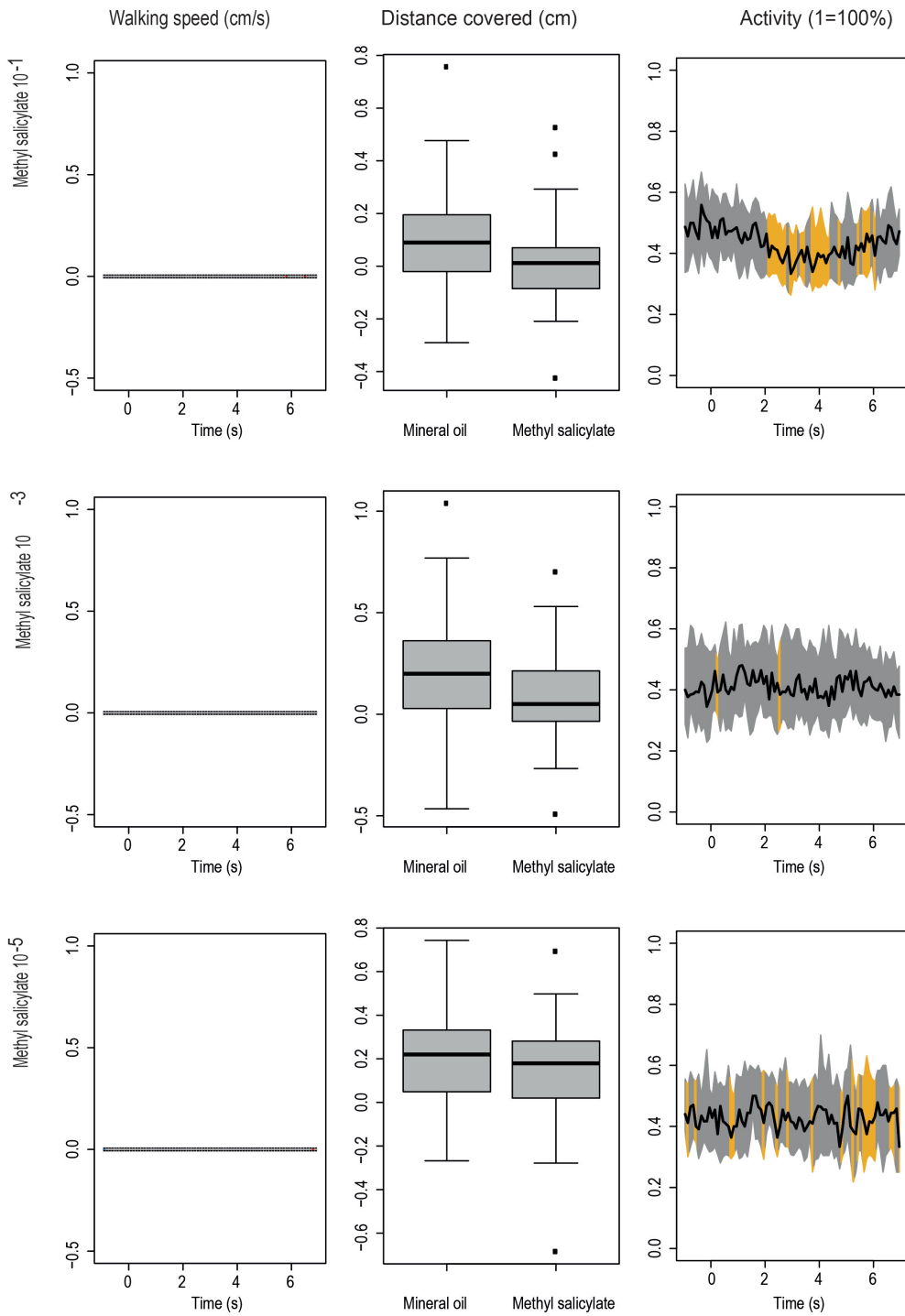
B. Physicochemical properties and medians of tested odorants

Odorant	Median	$\log P_{oct/wat}$	MS [g/L]	MW [g/mol]	VP [Torr]	BP [°C]
Methyl salicylate	-0.065210	2.523	3.20	152.15	0.0700	222.0
n-Hexyl acetate	-0.004480	2.823	1.90	144.21	1.3900	171.5
n-Pentyl acetate	0.015847	2.314	3.90	130.18	3.9300	149.9
n-Heptyl acetate	0.022863	3.333	0.85	158.24	0.5100	191.6
2-Phenylethyl alcohol	0.024482	1.504	20.00	122.16	0.0741	218.2
n-Butyl acetate	0.028870	1.804	8.50	116.16	11.5000	126.6
Acetophenone	0.077045	1.674	2.40	120.15	0.2990	202.9
(R)-(-)-Fenchone	0.088517	2.089	1.10	152.23	0.4630	193.5
γ-Butyrolactone	0.108187	-0.632	70.00	86.09	0.2700	204.0
β-Caryophyllene	0.234571	6.416	6.9×10^{-6}	204.35	0.0128	268.4
n-Octyl acetate	0.274534	3.842	0.40	172.26	0.1940	210.3
3-(methylthio)-1-propanol	0.298626	0.417	132.00	106.19	0.1560	189.3
n-Propyl acetate	0.330976	1.295	18.00	102.13	35.2000	101.4
Ethyl propionate	0.610767	1.295	18.00	102113	44.5000	95.9
2,3-Butanedione	0.625958	-1.340	169.00	86.09	62.3000	88.0
Ethyl acetate	0.699962	0.785	39.00	88.11	112.000	73.9
Geranyl acetate	0.814517	3.904	0.57	196.29	0.0256	247.5
Methyl acetate	0.827614	0.276	81.50	74.08	368.000	44.0

Table B.1.: Selected physicochemical properties of tested odorants. Median= Medians of the walking distance covered of wild type flies at an odorant concentration of 10^{-3}. MS= Mass solubility, MW= Molecular weight, VP= Vapor pressure, BP= Boiling point.

Bibliography

ACHE, B. W., YOUNG, J. M., Olfaction: Diverse species, conserved principles: *Neuron*, 48(3), 2005, 417–430.

AI, M., MIN, S., GROSJEAN, Y., LEBLANC, C., BELL, R., BENTON, R., SUH, G. S. B., Acid sensing by the Drosophila olfactory system: *Nature*, 468(7324), 2010, 691–695.

BADEKE, E., *Influence of the Drosophila Adipokinetic Hormone on Starvation Effects in Olfactory Perception*: Diploma thesis, 2012, Friedrich-Schiller-Universitaet, Jena.

BARTELT, R. J., SCHANER, A. M., JACKSON, L. L., Cis-vaccenyl acetate as an aggregation pheromone in Drosophila melanogaster: *Journal of Chemical Ecology*, 11(12), 1985, 1747–1756.

BECHER, P. G., FLICK, G., ROZPEDOWSKA, E., SCHMIDT, A., HAGMAN, A., LEBRETSON, S., LARSSON, M. C., HANSSON, B. S., PISKUR, J., WITZGALL, P., BENGTSSON, M., Yeast, not fruit volatiles mediate Drosophila melanogaster attraction, oviposition and development: *Functional Ecology*, 26, 2012, 822–828.

BENTON, R., SACHSE, S., MICHNICK, S. W., VOSSHALL, L. B., Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo: *Plos Biology*, 4(2), 2006, 240–257.

BENTON, R., VANNICE, K. S., GOMEZ-DIAZ, C., VOSSHALL, L. B., Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in Drosophila: *Cell*, 136(1), 2009, 149–162.

DE BRUYNE, M., CLYNE, P. J., CARLSON, J. R., Odor coding in a model olfactory organ: The Drosophila maxillary palp: *Journal of Neuroscience*, 19(11), 1999, 4520–4532.

DE BRUYNE, M., FOSTER, K., CARLSON, J. R., Odor coding in the Drosophila antenna: *Neuron*, 30(2), 2001, 537–552.

BUDICK, S. A., DICKINSON, M. H., Free-flight responses of Drosophila melanogaster to attractive odors: *Journal of Experimental Biology*, 209(15), 2006, 3001–3017.

CHERTEMPS, T., FRANCOIS, A., DURAND, N., ROSELL, G., DEKKER, T., LUCAS, P., MAIBECHE-COISNE, M., A carboxylesterase, Esterase-6, modulates sensory physiological and behavioral response dynamics to pheromone in Drosophila: *Bmc Biology*, 10(56), 2012.

CLYNE, P. J., WARR, C. G., CARLSON, J. R., Candidate taste receptors in Drosophila: *Science*, 287(5459), 2000, 1830–1834.

COUTO, A., ALENIUS, M., DICKSON, B. J., Molecular, anatomical, and functional organization of the Drosophila olfactory system: *Current Biology*, 15(17), 2005, 1535–1547.

ELMORE, T., IGNELL, R., CARLSON, J. R., SMITH, D. P., Targeted mutation of a Drosophila odor receptor defines receptor requirement in a novel class of sensillum: *Journal of Neuroscience*, 23(30), 2003, 9906–9912.

FERVEUR, J. F., STORTKUHL, K. F., STOCKER, R. F., GREENSPAN, R. J., Genetic feminization of brain structures and changed sexual orientation in male Drosophila: *Science*, 267(5199), 1995, 902–905.

FOELIX, R. F., STOCKER, R. F., STEINBRECHT, R. A., Fine-structure of a sensory organ in the arista of Drosophila melanogaster and some other dipterans: *Cell and Tissue Research*, 258(2), 1989, 277–287.

GUERRIERI, F., SCHUBERT, M., SANDOZ, J. C., GIURFA, M., Perceptual and neural olfactory similarity in honeybees: *Plos Biology*, 3(4), 2005, 718–732.

HADDAD, R., KHAN, R., TAKAHASHI, Y. K., MORI, K., HAREL, D., SOBEL, N., A metric for odorant comparison: *Nature Methods*, 5(5), 2008, 425–429.

HALLEM, E. A., CARLSON, J. R., Coding of odors by a receptor repertoire: *Cell*, 125(1), 2006, 143–160.

HEIMBECK, G., BUGNON, V., GENDRE, N., KELLER, A., STOCKER, R. F., A central neural circuit for experience-independent olfactory and courtship behavior in Drosophila melanogaster: *Proceedings of the National Academy of Sciences of the United States of America*, 98(26), 2001, 15336–15341.

HEISENBERG, M., BORST, A., WAGNER, S., BYERS, D., Drosophila mushroom body mutants are deficient in olfactory learning: *Journal of Neurogenetics*, 2(1), 1985, 1–30.

HEKMAT-SCAFE, D. S., SCAFE, C. R., McKINNEY, A. J., TANOUYE, M. A., Genome-wide analysis of the odorant-binding protein gene family in Drosophila melanogaster: *Genome Research*, 12(9), 2002, 1357–1369.

HILDEBRAND, J. G., SHEPHERD, G. M., Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla: *Annual Review of Neuroscience*, 20, 1997, 595–631.

JAENIKE, J., Environmental modification of oviposition behavior in Drosophila: *American Naturalist*, 119(6), 1982, 784–802.

JONES, W. D., CAYIRLIOGLU, P., KADOW, I. G., VOSSHALL, L. B., Two chemosensory receptors together mediate carbon dioxide detection in Drosophila: *Nature*, 445(7123), 2007, 86–90.

KIM, M. S., REPP, A., SMITH, D. P., LUSH odorant-binding protein mediates chemosensory responses to alcohols in Drosophila melanogaster: *Genetics*, 150(2), 1998, 711–721.

KNADEN, M., STRUTZ, A., AHSAN, J., SACHSE, S., HANSSON, B. S., Spatial Representation of Odorant Valence in an Insect Brain: *Cell Reports*, 1(4), 2012, 392–399.

KWON, J. Y., DAHANUKAR, A., WEISS, L. A., CARLSON, J. R., The molecular basis of CO_2 reception in Drosophila: *Proceedings of the National Academy of Sciences of the United States of America*, 104(9), 2007, 3574–3578.

LARSSON, M. C., DOMINGOS, A. I., JONES, W. D., CHIAPPE, M. E., AMREIN, H., VOSSHALL, L. B., Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction: *Neuron*, 43(5), 2004, 703–714.

LASKA, M., TEUBNER, P., Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes: *Chemical Senses*, 24(3), 1999, 263–270.

MONTELL, C., A taste of the Drosophila gustatory receptors: *Current Opinion in Neurobiology*, 19(4), 2009, 345–353.

VAN DER GOES VAN NATERS, W., CARLSON, J. R., Receptors and neurons for fly odors in Drosophila: *Current biology*, 17(7), 2007, 606–612.

NEUHAUS, E. M., GISSELMANN, G., ZHANG, W. Y., DOOLEY, R., STORTKUHL, K., HATT, H., Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster: *Nature Neuroscience*, 8(1), 2005, 15–17.

ODELL, K. M. C., ARMSTRONG, J. D., YANG, M. Y., KAISER, K., Functional dissection of the Drosophila mushroom bodies by selective feminization of genetically defined subcompartments: *Neuron*, 15(1), 1995, 55–61.

PARK, S. K., SHANBHAG, S. R., WANG, Q., HASAN, G., STEINBRECHT, R. A., PIKIELNY, C. W., Expression patterns of two putative odorant-binding proteins in the olfactory organs of Drosophila melanogaster have different implications for their functions: *Cell and Tissue Research*, 300(1), 2000, 181–192.

ROBERTSON, H. M., WARR, C. G., CARLSON, J. R., Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster: *Proceedings of the National Academy of Sciences of the United States of America*, 100, 2003, 14537–14542.

SACHSE, S., RAPPERT, A., GALIZIA, C. G., The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code: *European Journal of Neuroscience*, 11(11), 1999, 3970–3982.

SCOTT, K., BRADY, R., CRAVCHIK, A., MOROZOV, P., RZHETSKY, A., ZUKER, C., AXEL, R., A chemosensory gene family encoding candidate gustatory and olfactory receptors in Drosophila: *Cell*, 104(5), 2001, 661–673.

SEMMELHACK, J. L., WANG, J. W., Select Drosophila glomeruli mediate innate olfactory attraction and aversion: *Nature*, 459(7244), 2009, 218–224.

SHANBHAG, S. R., MULLER, B., STEINBRECHT, R. A., Atlas of olfactory organs of Drosophila melanogaster - 1. Types, external organization, innervation and distribution of olfactory sensilla: *International Journal of Insect Morphology & Embryology*, 28(4), 1999, 377–397.

SHANG, Y. H., CLARIDGE-CHANG, A., SJULSON, L., PYPAERT, M., MIESENBOCK, G., Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe: *Cell*, 128(3), 2007, 601–612.

SILBERING, A. F., RYTZ, R., GROSJEAN, Y., ABUIN, L., RAMDYA, P., JEFFERIS, G., BENTON, R., Complementary function and integrated wiring of the evolutionarily distinct Drosophila olfactory subsystems: *Journal of Neuroscience*, 31(38), 2011, 13357–13375.

SINGH, R. N., NAYAK, S. V., Fine-structure and primary sensory projections of sensilla on the maxillary palp of Drosophila melanogaster Meigen (Diptera, Drosophilidae): *International Journal of Insect Morphology & Embryology*, 14(5), 1985, 291–306.

STECK, K., VEIT, D., GRANDY, R., BADIA, S. B. I., MATHEWS, Z., VERSCHURE, P., HANSSON, B. S., KNADEN, M., A high-throughput behavioral paradigm for Drosophila olfaction - The Flywalk: *Scientific Reports*, 3, 2012.

STENSMYR, M. C., GIORDANO, E., BALLOI, A., ANGIOY, A. M., HANSSON, B. S., Novel natural ligands for Drosophila olfactory receptor neurones: *Journal of Experimental Biology*, 206(4), 2003, 715–724.

STOCKER, R. F., The organization of the chemosensory system in Drosophila melanogaster: a review: *Cell and Tissue Research*, 275(1), 1994, 3–26.

VOSSHALL, L. B., HANSSON, B. S., A unified nomenclature system for the insect olfactory coreceptor: *Chemical Senses*, 36(6), 2011, 497–498.

VOSSHALL, L. B., WONG, A. M., AXEL, R., An olfactory sensory map in the fly brain: *Cell*, 102(2), 2000, 147–159.

WANG, Y. L., CHIANG, A. S., XIA, S. Z., KITAMOTO, T., TULLY, T., ZHONG, Y., Blockade of neurotransmission in Drosophila mushroom bodies impairs odor attraction, but not repulsion: *Current Biology*, 13(21), 2003, 1900–1904.

WICHER, D., SCHAFER, R., BAUERNFEIND, R., STENSMYR, M. C., HELLER, R., HEINEMANN, S. H., HANSSON, B. S., Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels: *Nature*, 452(7190), 2008, 1007–1111.

WILSON, R. I., LAURENT, G., Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe: *Journal of Neuroscience*, 25(40), 2005, 9069–9079.

YAO, C. A., IGNELL, R., CARLSON, J. R., Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna: *Journal of Neuroscience*, 25(37), 2005, 8359–8367.

ZHU, J. W., PARK, K. C., BAKER, T. C., Identification of odors from overripe mango that attract vinegar flies, Drosophila melanogaster: *Journal of Chemical Ecology*, 29(4), 2003, 899–909.