Department of Physics, Chemistry and Biology

Master Thesis

Behavioural responses of mice to predator odour components

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Sammanfattning/Abstract:

Having means to detect and avoid potential predators is a necessity for prey species. Most mammalian prey species are able to detect odours emitted by predators and to adapt their behaviour accordingly. These odour cues are therefore considered to act as semiochemicals. Predator odours consist of several dozen different odourants. In order to assess if single odourants elicit aversive behavioural reactions, predator-naïve CD-1 mice were presented with six odourants which are part of body-borne odours of different mammalian predator species. A two-compartment chamber was used in order to assess place-preference, motor activity and faecal excretions when the animals were simultaneously presented with a predator odourant and a blank control. Further trials were performed to assess whether the odourant concentrations had an influence on the behaviours. The only odourant that elicited a significant aversion was 3-methyl-1-butanethiol, a compound found in the anal gland secretion of skunks, when presented at a factor of 100 above the olfactory detection threshold of mice. Two other concentrations of 3-methyl-1-butanethiol did not elicit significant behavioural changes. Based on the present study, only one out of six selected predator odourants elicited a significant aversive response in CD-1 mice. This suggests that more than one odour component, or perhaps even the full mixture of odourants, may be necessary for CD-1 mice to respond to a predator odour with aversive behaviour.

Nyckelord/Keyword:

Predator, odour, olfaction, mouse, prey, neophobia, kairomone, avoidance, behaviour

Contents

1 Abstract

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2 Introduction

In order to avoid predation, prey species have developed behavioural and sensory adaptations. Most mammalian prey species are therefore able to detect predator emitted odours and display adaptive behavioural responses e.g. avoidance or freezing (Apfelbach et al. 2005). Thus, odours emitted by predators act as kairomones. These are semiochemicals secreted by an organism (in this case: the predator) which cause interspecific interaction beneficial for the perceiving organism (in this case: the prey), without gain for the emitter (Sbarbati and Osculati 2006). Studies found that volatile sulphur-containing metabolites, results of a protein-rich diet, are characteristic for the odour of urine, faeces and anal-gland secretions of mammalian predators (Mason et al. 1994; Nolte et al. 1994). In contrast, herbivorous mammals do not excrete these sulphur-containing compounds, which permits prey species to distinguish between predator and non-predator odours (Fendt 2006; Belton et al. 2007). Nonetheless, little is known whether single components of predator odour are sufficient to evoke repellent effects in prey animals or whether the complex mixture of

compounds in predator odour is needed to evoke avoidance responses. Some studies reported behavioural effects in prey species caused by single predator odour components (Sullivan and Crump 1984; Woolhouse and Morgan 1995), whereas other studies found only weak effects or found no effect of single sulphur-containing odourants at all (Epple et al. 1995).

Furthermore, prey species are not only able to detect predator odours and adapt accordingly, but also to determine the concentration (weak or strong) of these odours. This allows the species to react differently upon encountering a strong and supposedly "fresh" or "close by", or a weak and supposedly "old" or "far away" odour (Sullivan and Crump 1984; Takahashi et al. 2005; Vasudevan and Vyas 2013).

Not only odour emitted from predators can affect the prey's behaviour, but also new environment and unknown objects can elicit anxiety-like behaviour. This phenomenon is called neophobia. The fear of the unknown is a widespread phenomenon in several different species (Sloan Wilson et al. 1994; Jain et al. 2012; Brown et al. 2013; Costa et al. 2014). It has also been shown that odour cues play an important role for neophobia (Royet and Pager 1982; Clark and King 2008; Kimball et al. 2009).

Depending on the anxiety-inducing situation, mice (*Mus musculus*, Linnaeus 1758) have a variety of behaviours at their disposal. Some of them are, but are not limited to: ultra-sonic vocalization, aversion, increased defecation, freezing and decreased activity (Archer 1973).

The aims of the present study were to determine whether single components of predator odour elicit anxiety-like behaviour in predator-naïve mice, to compare the behavioural response of mice to predator odourants with those of to a fruity control odour, and to assess the effects of the odour concentration on the behavioural response.

3 Material and methods

3.1 Animals

A total of 50 adult male CD-1 mice at the age of about twelve weeks were used in the study. The CD-1 strain is an outbred strain and therefore favourable for this study since its gene pool is more similar to the wild type than that of inbred strains. All mice were laboratory-born and thus predator-naïve. The experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* (national Institute of Health Publication no.86-23, revised 1985) and conform to Swedish laws on

animal welfare. They have been approved by the local ethics committee (Linköpings djurförsöksetiska nämnd, Dnr. 76/12).

The mice were housed in individual rodent cages (40 x 25 x 15 cm) and had *ad libitum* access to food and water. The cages contained woodchips as bedding material, cardboard rolls for enrichment and paper strips as nesting material. The room containing the mice had a temperature of 22 ± 1 °C and a diurnal rhythm of twelve hours light and darkness (starting at 7:30 a.m. and p.m., respectively).

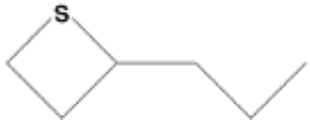
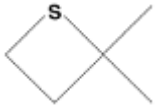
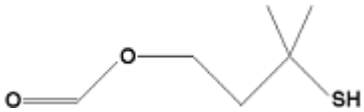
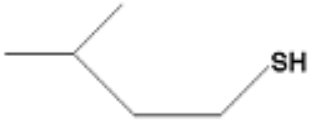
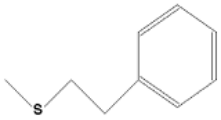
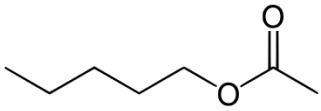
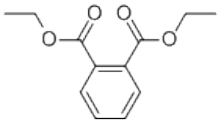
3.2 Odour stimuli

The mice were exposed to seven different odourants (

Table *1*). Six of them are sulphur-containing compounds found in the body odour or secretions of natural predators of the mouse and one is found in fruits. All odourants were presented at a concentration which is a factor of 100 above the olfactory detection threshold of mice (Sarrafchi et al. 2013).Three of the odours were additionally presented at concentrations which are a factor of 10 and 1000 above the detection threshold. The odourants were diluted with the near-odourless solvent diethyl phthalate (

Table *1*). The solvent was also used as the "BLANK" stimulus in every test. All odourants were obtained from Sigma-Aldrich (St. Louis MO) and had a nominal purity of at least 99 %.

Table 1. *The different odourants used in the study, including the solvent.*

Systematic name	Molecular structure	CAS #
2-propylthietane		70678-49-8
2,2-dimethylthietane		55022-72-5
3-mercapto-3-methylbutan-1-ol		34300-94-2
3-mercapto-3-methyl-1-butyl-1-formate		50746-10-6
3-methyl-1-butanethiol		541-31-1
Methyl-2-phenylethyl sulfide		5925-63-3
n-pentyl acetate		628-63-7
Diethyl phthalate		84-66-2

2-propylthietane (2-PT) is found in the anal gland secretion of stoat (*Mustela erminea*, Linnaeus 1758), ferret (*Mustela putorius furo*, Linnaeus 1758), mink (*Mustela vison*, Schreber 1777), Siberian weasel (*Mustela sibirica*, Palas 1773) and steppe polecat (*Mustela eversmanii*, Lesson 1827)

(Brinck et al. 1983; Zhang et al. 2002). The olfactory detection threshold of mice for this odourant has been reported to be at 0.03 ppm (parts per million) (Sarrafchi et al. 2013).

2,2-dimethylthietane (2,2-DT) is found in the anal gland secretion of stoat (*Mustela erminea*), ferret (*Mustela putorius furo*), mink (*Mustela vison*), Siberian weasel (*Mustela sibirica*) and steppe polecat (*Mustela eversmanii*) (Brinck et al. 1983; Zhang et al. 2002). The olfactory detection threshold of mice for this odourant has been reported to be at 0.000003 ppm (Sarrafchi et al. 2013).

3-mercapto-3-methylbutan-1-ol (3-M-3-MB-1-O) has been found in cat (*Felis catus*, Linnaeus 1758) and bobcat (*Lynx rufus*, Schreber 1777) urine (Mattina et al. 1991; Miyazaki et al. 2006). The olfactory detection threshold of mice for this odourant has been reported to be at 0.000003 ppm (Sarrafchi et al. 2013).

3-mercapto-3-methylbutyl-1-formate (3-M-3-MF) has been found in cat (*Felis catus*) urine (Miyazaki et al. 2006). The olfactory detection threshold of mice for this odourant has been reported to be at 0.000003 ppm (Sarrafchi et al. 2013).

3-methyl-1-butanethiol (3-M-1-BE) has been found in the anal gland secretion of striped skunk (*Mephitis mephitis*, Schreber 1776), hooded skunk (*Mephitis macroura*, Lichtenstein 1832) and spotted skunk (*Spilogale putorius*, Linnaeus 1758) (Wood et al. 2002). The olfactory detection threshold of mice for this odourant has been reported to be at 0.000003 ppm (Sarrafchi et al. 2013).

Methyl-2-phenylethyl sulphide (M-2-PES) is found in urine of female and male red fox (*Vulpes vulpes*, Linnaeus 1758) (Jorgenson et al. 1978). The olfactory detection threshold of mice for this odourant has been reported to be at 0.000003 ppm (Sarrafchi et al. 2013).

N-pentyl acetate (FRUITY) has been found in a variety of fruit odours (Burdock 2005). The olfactory detection threshold of mice for this odourant has been reported to be at 0.0000089 ppm (O'Connell et al. 1983).

Diethyl phthalate (BLANK) is a synthetic, near odourless liquid which is, due to its chemical properties, often used as a solvent for fragrances. Furthermore, it is also used as a plasticizer (Api 2001).

3.3 Experimental set-up

The mice were kept in a room that was separate from the experimental room, thus they were individually collected and brought to the experimental room for testing. Both rooms provided the same ambient temperature. Each mouse was individually put into the testing arena, a modified standard mouse cage (40 x 25 x 15 cm), subdivided into two equally sized compartments by a vertical plexiglass wall attached to the lid, with a semi-circular opening at the bottom which allows a mouse to switch between the compartments (Figure 1). The two-compartment chamber had a perforated floor, and under the floor of each compartment it had a petri dish with a filter paper impregnated with either 100 µL of odourant or 100 µL blank stimulus (solvent) (Figure 2). The test arena was placed in a light tent in order to distribute the light evenly and reduce the risk of biases for one of the compartments due to possible differences in light intensity. Additionally, the lights in the testing room were dimmed.

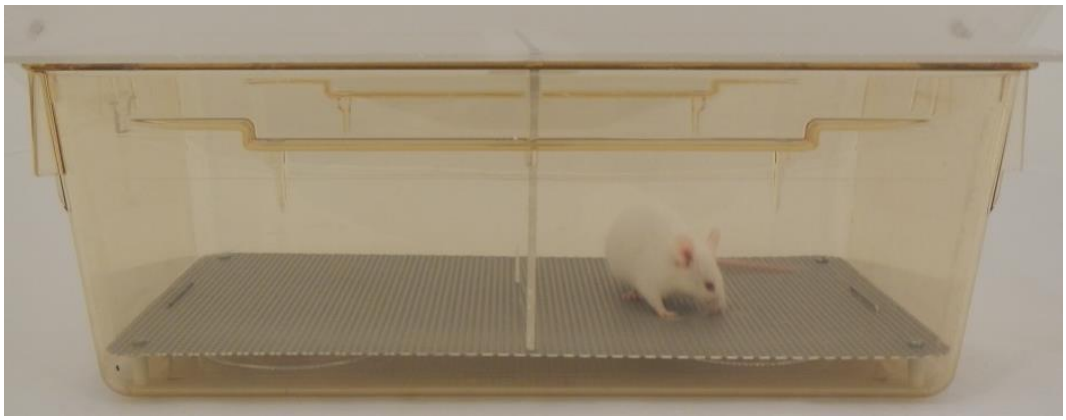


Figure 1: *Side view of the test arena with dividing wall in the middle, perforated floor on the bottom, and plexiglas lid on top.*

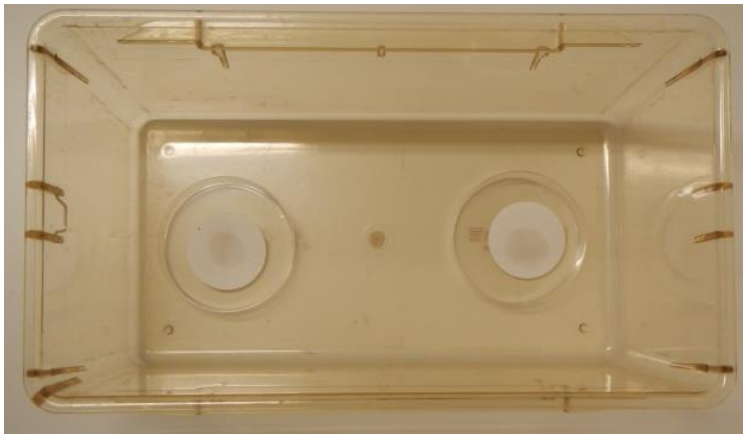


Figure 2: *Top view of the test arena with the impregnated petri dishes in position and the perforated floor removed.*

After every test, each part of the test arena was cleaned with ethanol to eliminate odour contaminations for the next test. Consequently, three cages were used in a rotating scheme to allow each cage enough time to dry after cleaning. Finally, after 21 testing days every cage was additionally thoroughly cleaned with odourless soap and ethanol to minimize the risk of remaining odours.

3.4 Behavioural procedure

In every test, one side of the two-compartment chamber contained the blank stimulus and the other side one of the seven odourants. Two predator odours and n-pentyl acetate were tested on the same set of ten mice. A new group of ten mice was used for each set of odourants. All mice were exposed to the odourants six times each, three times with the odourant being under the left compartment and three times under the right. The placement of the odourant alternated pseudo-randomly from day to day and the odourants were used on a pseudo-random daily rotation scheme. Only one test session of ten minutes was performed per mouse and day.

The tests with odourants were preceded by three days with the blank stimulus on both sides to habituate the mice to the test arena and to exclude the possibility of spontaneous side preference in the animals. This resulted in a total of 21 testing days per two predator odourants. After these 21 days, ten new mice were tested on two of the other predator odourants plus the fruity odour.

After five mice were tested on a given day, the petri dishes and filter papers were exchanged for new ones to ensure the same odourant concentration

for each test. In the case of urination onto the filter paper, the petri dishes were immediately exchanged.

The time spent in each compartment was recorded continuously for ten minutes as an indicator of aversion or preference for one of the odours. The number of switches between the compartments was recorded, as an indicator of the overall activity level of the mouse. Additionally, the number of faecal pellets dropped during the test per compartment was recorded, as an indicator of the animal's anxiety level.

3.5 Data analysis

The two-tailed binomial test was used to assess whether the proportion of individuals spending more time in proximity to an odourant or in proximity to the blank stimulus differed from chance. The Wilcoxon signed-rank test was used to determine whether the time spent in an odourised compartment differed from the time spent in the blank compartment. Furthermore it was used to determine differences between the three odours within a set of ten mice regarding the number of switches and the number of dropped pellets. The Spearman signed-rank test was used in order to assess possible correlations between the number of switches between compartments across the six test sessions per odourant. All analyses and figures were obtained with Microsoft Excel 2010 and R (R Core Team 2014).

4 Results

4.1 2,2-dimethylthietane and methyl-2-phenylethyl sulphide

When presented with 2,2-DT, the number of trials in which mice spent more time in the odour compartment compared to mice spending more time in the BLANK compartment was 29:31. Similarly, the number of trials for FRUITY vs. BLANK and M-2-PES vs. BLANK were 29:31 and 27:33, respectively. None of these three ratios differed significantly from chance (binomial test, n=60; 2,2-DT: $z=0.129$, $p>0.05$; FRUITY: $z=0.129$, $p>0.05$; M-2-PES: $z=0.645$, $p>0.05$).

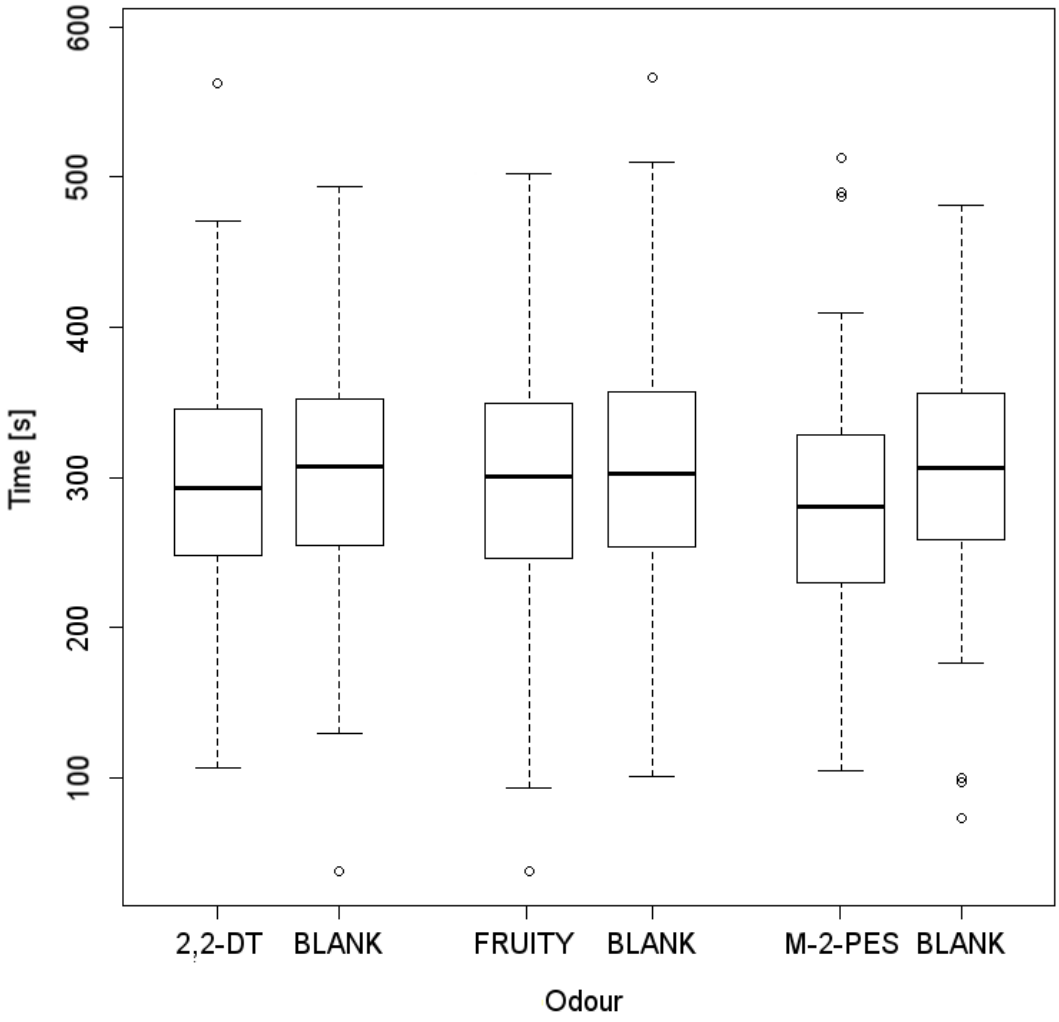


Figure 3: *Time spent in the different odour compartments compared to its matching BLANK stimulus. Each boxplot consists of the following elements: median value (bold horizontal line), 0.75 and 0.25 percentiles (upper and lower box limits, respectively), and highest and lowest values (upper and lower whisker ends, respectively). Outliers are marked as circles.*

With both predator odourants and the fruity odour, the average time spent in the odourised compartment was not significantly different from the time spent in the BLANK compartment (Figure 3) (Wilcoxon test, n=60; 2,2-DT $p>0.05$, V=1001; FRUITY $p>0.05$, V=933.5; M-2-PES $p>0.05$, V=1075.5).

The mice performed a significantly lower average number of switches between the two compartments when presented with 2,2-DT (median ± median absolute deviation; 29 ± 6) compared to the BLANK stimulus alone (B/B) (30.5 ± 5.5)(Wilcoxon test, n=30, $p=0.0037$, V=352). Similarly, when exposed to FRUITY (26.5 ± 6.5) and M-2-PES (26 ± 7), the mice also showed a significantly lower number of switches compared to B/B

(Wilcoxon test, n=30, p=0.0136, V=332; p=0.01, V=316.5, respectively). No other significant differences were found (Wilcoxon test, n=60; 2,2-DT (26 ± 6) vs. M-2-PES (24 ± 6) p>0.05, V=873; 2,2-DT (26 ± 6) vs. FRUITY (25 ± 6) p>0.05, V=864; FRUITY (25 ± 6) vs. M-2-PES (24 ± 6) p>0.05, V=699).

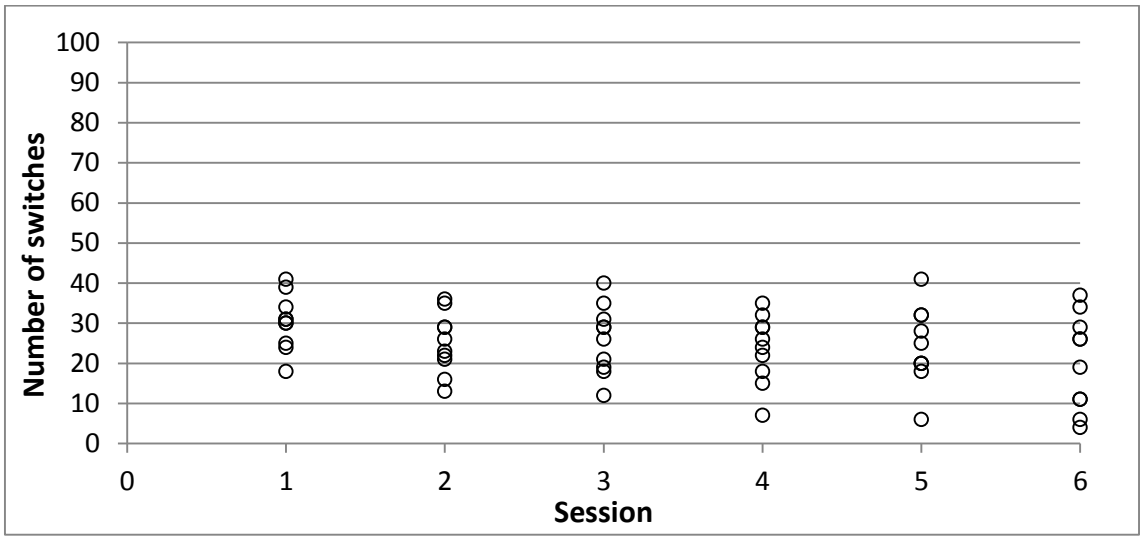


Figure 4: *Number of switches during the test session with 2,2-DT. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for 2,2-DT (Spearman correlation test, p=0.0418, S=45475.94, rho=-0.2635) (Figure 4).

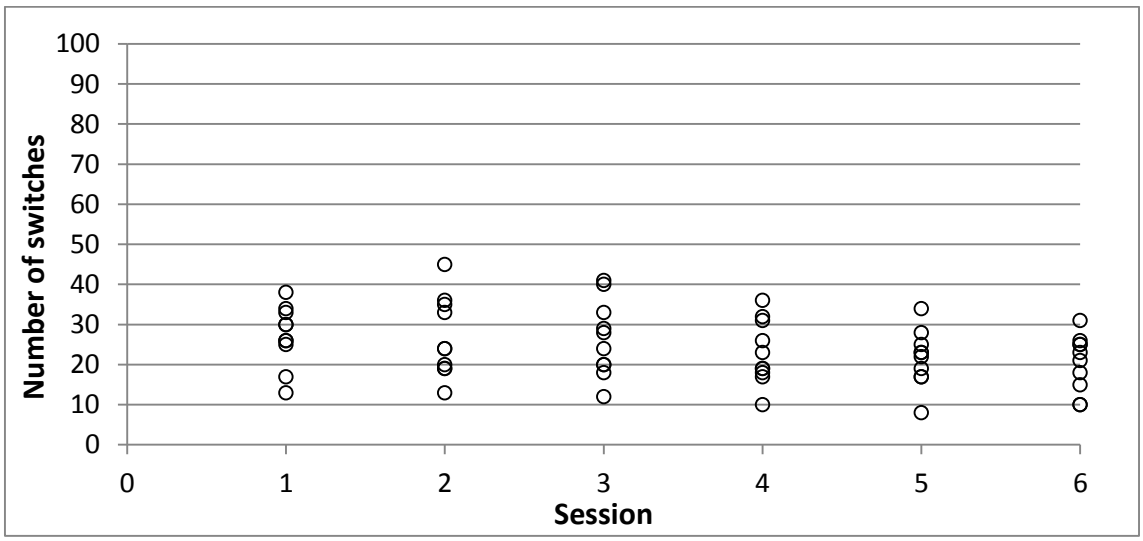


Figure 5: *Number of switches during the test session with M-2-PES. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for M-2-PES (Spearman correlation test, p=0.0184, S=46914.18, rho=-0.3035) (Figure 5).

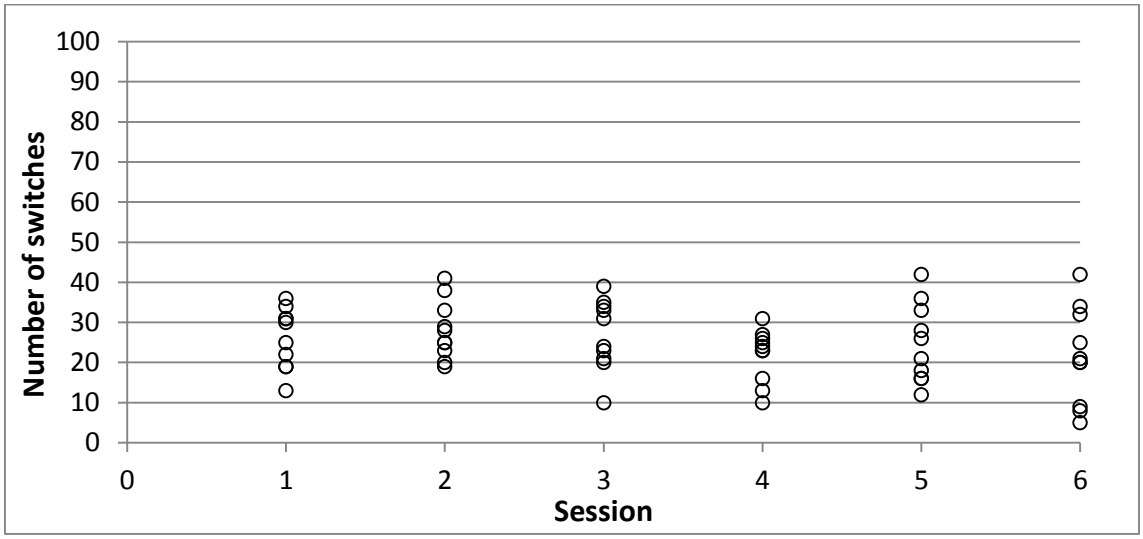


Figure 6: *Number of switches during the test session with FRUITY. Each circle represents one mouse.*

No significant correlation between the number of switches and the test session was found for FRUITY (Spearman correlation test, p=0.15, S=42760.92, rho=-0.1881) (Figure 6).

The mice did not show significant differences in the number of fecal pellets excreted when exposed to the different odours (Wilcoxon test, n=30, B/B (5.5 ± 1.5) vs. 2,2-DT (7 ± 1) p>0.05, V=130; B/B (5.5 ± 1.5) vs. M-2-PES (6 ± 1.5) p>0.05, V=199.5; B/B (5.5 ± 1.5) vs. FRUITY (5 ± 1) p>0.05, V=237; n=60, 2,2-DT (6 ± 1) vs. M-2-PES (5 ± 1) p>0.05, V=903; 2,2-DT (6 ± 1) vs. FRUITY (5 ± 1) p>0.05, V=716; FRUITY (5 ± 1) vs. M-2-PES (5 ± 1) p>0.05, V=638).

4.2 2-propylthietane and 3-mercapto-3-methylbutyl-1-formate

When presented with 2-PT, the number of trials in which mice spent more time in the odour compartment compared to mice spending more time in the BLANK compartment was 24:36. Similarly, the number of trials for M-3-M-3-MF vs. BLANK was 29:31. When exposed to the FRUITY odour, the ratio was 35:25. None of these three ratios differed significantly from

chance (binomial test, n=60; 2-PT: z=1.42, p>0.05; FRUITY: z=1.162, p>0.05; 3-M-3-MF: z=0.129, p>0.05).

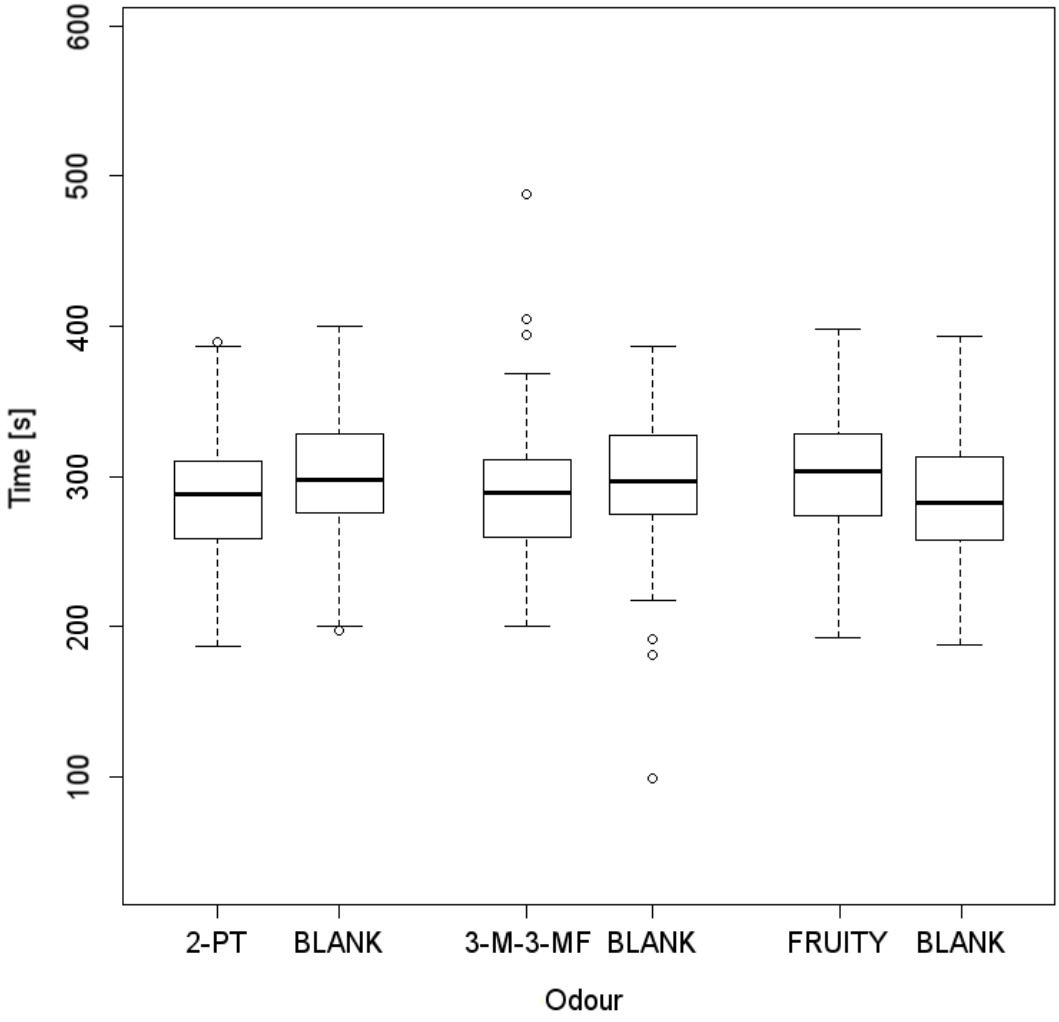


Figure 7: *Time spent in the different odour compartments compared to its matching BLANK stimulus.*

With both predator odourants and the fruity odour, the average time spent in the odourised compartment was not significantly different from the time spent in the BLANK compartment (Figure 7) (Wilcoxon test, n=60; 2-PT p>0.05, V=1124.5; 3-M-3-MF p>0.05, V=1016; FRUITY p>0.05, V=691.5).

The mice did not show significant differences for the number of switches with the different odours (Wilcoxon test, n=30, B/B (42.5 ± 5.5) vs. 2-PT (41 ± 11) p>0.05, V=237; B/B (42.5 ± 5.5) vs. 3-M-3-MF (42 ± 9) p>0.05, V=253.5; B/B (42.5 ± 5.5) vs. FRUITY (39 ± 9) p>0.05, V=299; n=60, 2-PT (36 ± 8) vs. 3-M-3-MF (39 ± 10) p>0.05, V=749.5; 2-PT (36 ± 8) vs.

FRUITY (37.5 ± 7.5) p>0.05, V=698; 3-M-3-MF (39 ± 10) vs. FRUITY (37.5 ± 7.5 p>0.05, V=659.5).

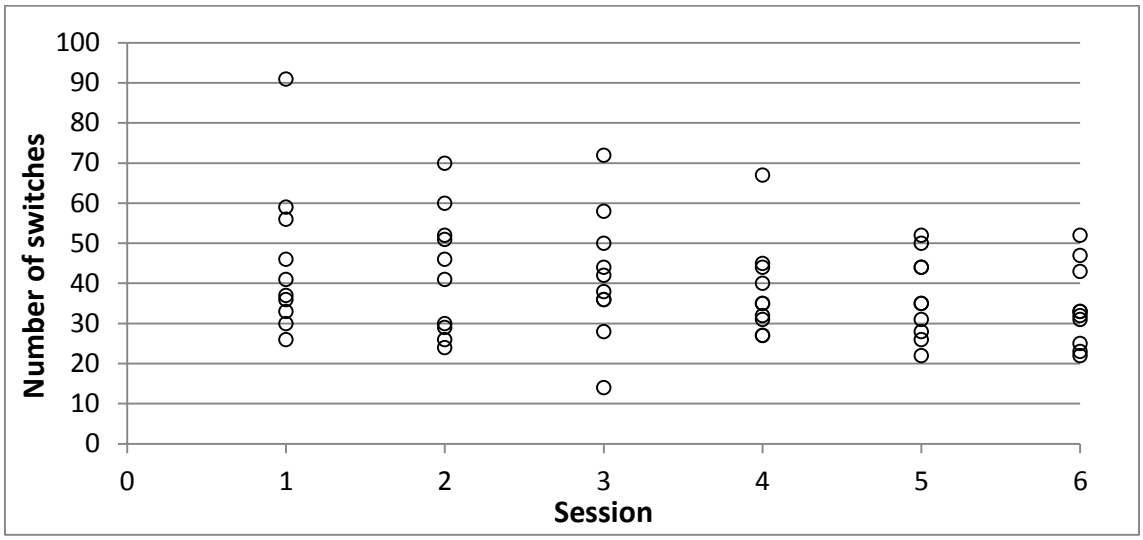


Figure 8: *Number of switches during the test session with 2-PT. Each circle represents one mouse.*

No significant correlation between the number of switches and the test session was found for 2-PT (Spearman correlation test, p=0.0592, S=44807.93, rho=-0.245) (Figure 8).

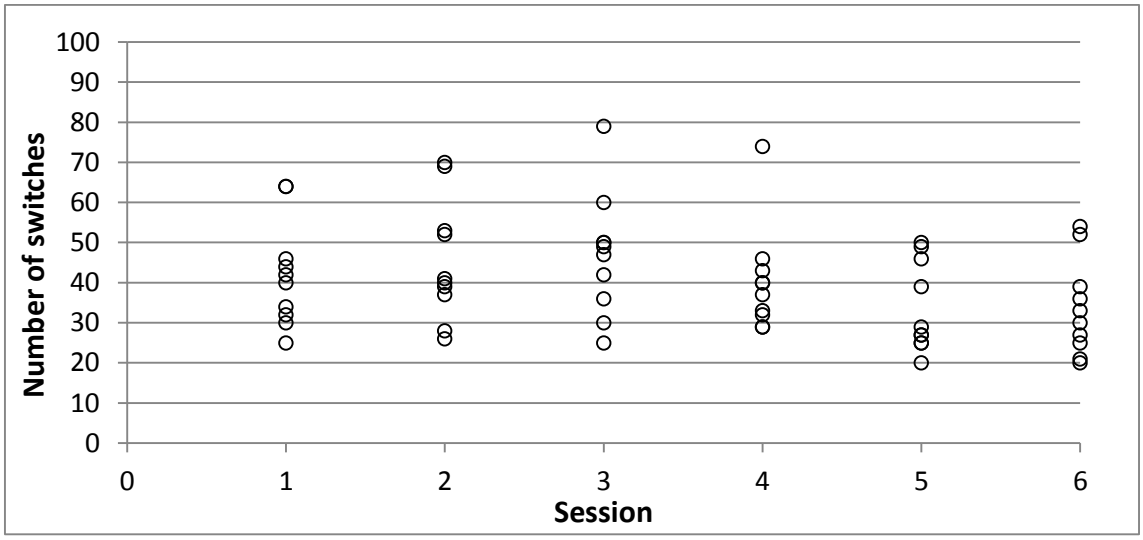


Figure 9: *Number of switches during the test session with 3-M-3-MF. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for 3-M-3-MF (Spearman correlation test, p=0.0171, S=47032.47, rho=-0.3068) (Figure 9).

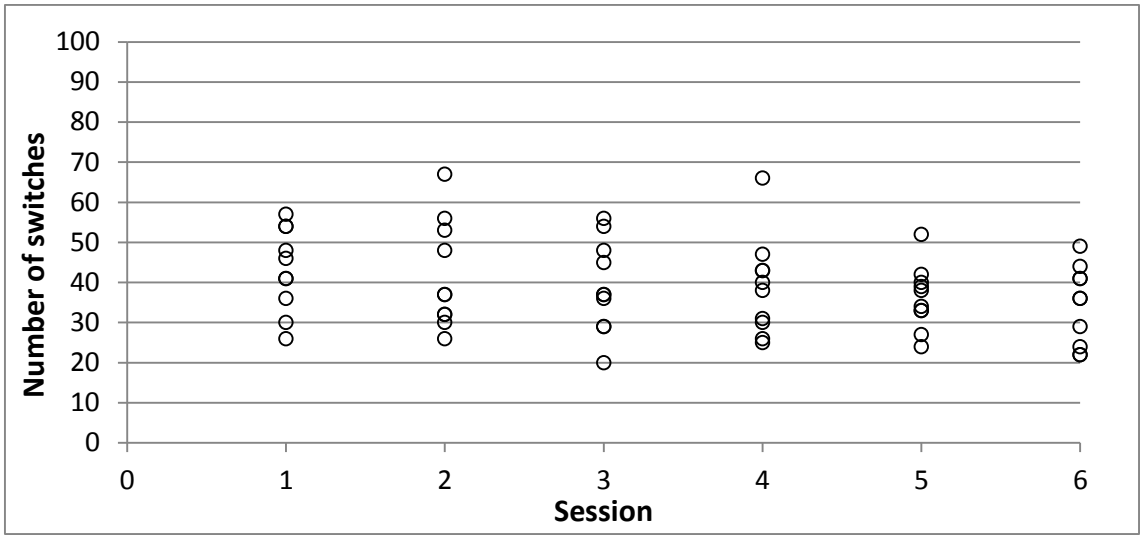


Figure 10: *Number of switches during the test session with FRUITY. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for FRUITY (Spearman correlation test, p=0.0448, S=45347.03, rho=-0.26) (Figure 10).

The mice did not show significant differences in the number of fecal pellets excreted when exposed to the different odours (Wilcoxon test, n=30, B/B (4 ± 2) vs. 2-PT (5 ± 1) p>0.05, V=212; B/B (4 ± 2) vs. 3-M-3-MF (5 ± 1) p>0.05, V=198; B/B (4 ± 2) vs. FRUITY (4.5 ± 1.5) p>0.05, V=163.5; n=60, 2-PT (5 ± 1) vs. 3-M-3-MF (5 ± 1) p>0.05, V=450.5; 2-PT (5 ± 1) vs. FRUITY (5 ± 1.5) p>0.05, V=761; 3-M-3-MF (5 ± 1) vs. FRUITY (5 ± 1.5) p>0.05, V=743.5).

4.3 3-methyl-1-butanethiol and 3-mercapto-3-methylbutan-1-ol

When presented with 3-M-1-BE, the number of trials in which mice spent more time in the odour compartment compared to mice spending more time in the BLANK compartment was 22:38. Similarly, the number of trials for FRUITY vs. BLANK and 3-M-3-MB-1-O vs. BLANK were 30:30 and 32:28, respectively. None of these three ratios differed significantly from chance (binomial test, n=60; 3-M-1-BE, z=1.936, p>0.05; 3-M-3-MB-1-O, z=0.387, p>0.05; FRUITY, z=-0.129, p>0.05).

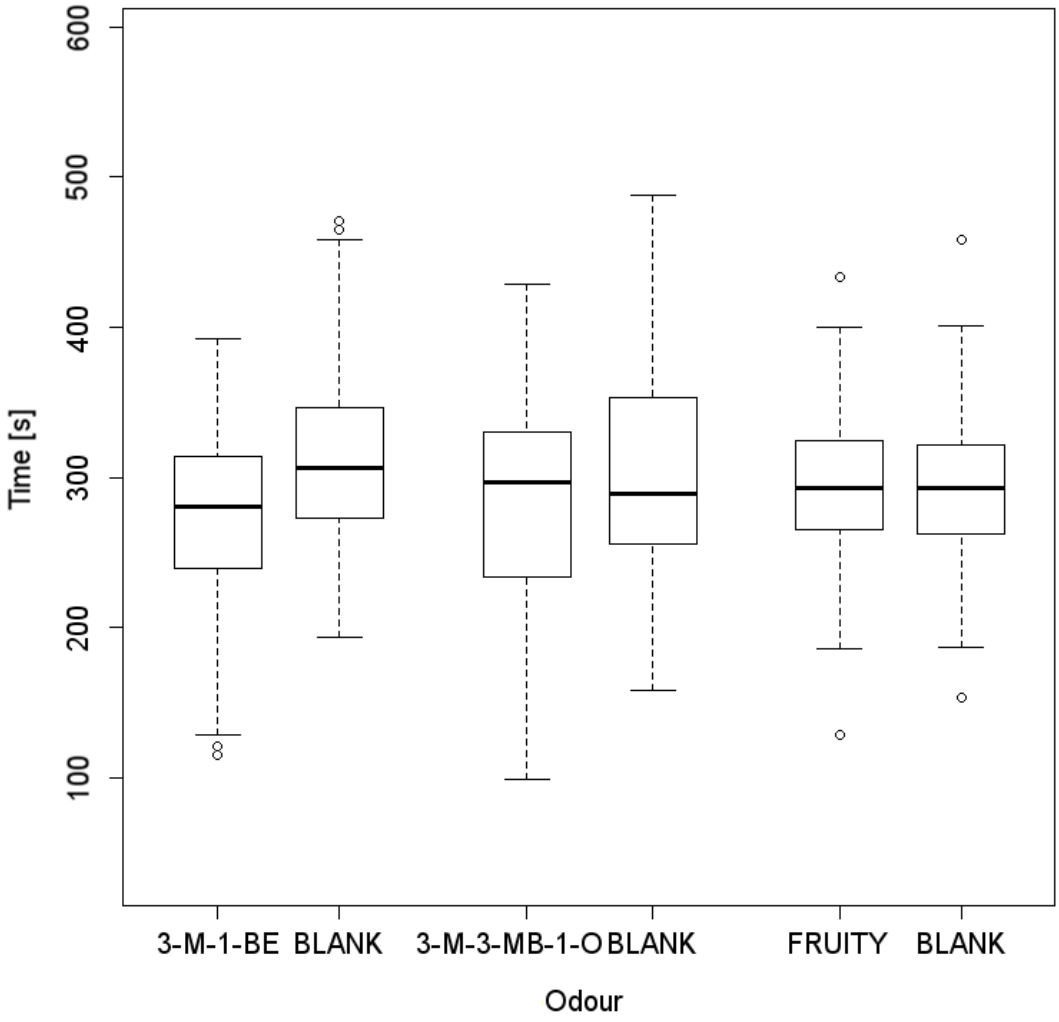


Figure 11: *Time spent in the different odour compartments compared to its matching BLANK stimulus.*

The mice spent significantly less time with the 3-M-1-BE stimulus than with the BLANK stimulus (Wilcoxon test, n=60, p=0.0229, V=1224.5). No other significant differences were found regarding the time spent with a stimulus compared to the time spent in the BLANK compartment (Wilcoxon test, n=60; 3-M-3-MB-1-O, p>0.05, V=985.5; FRUITY, p>0.05, V=895).

The mice showed a significantly higher number of switches for the B/B (43.5 ± 9) stimulus than for FRUITY (39.5 ± 12.5), 3-M-1-BE (37 ± 7) and 3-M-3-MB-1-O (37.5 ± 11) (Wilcoxon test, n=30,: p=0.006, V=366.5; p=0.0066, V=343.5; p=0.0025, V=380, respectively). No other significant differences were found (Wilcoxon test, n=60, 3-M-1-BE (33.5 ± 8) vs. 3-M-3-MB-1-O (31.5 ± 8.5) p>0.05, V=652.5; 3-M-1-BE (33.5 ± 8) vs.

FRUITY (35 ± 9) p>0.05, V=447; 3-M-3-MB-1-O (31.5 ± 8.5) vs. FRUITY (35 ± 9) p>0.05, V=599.5).

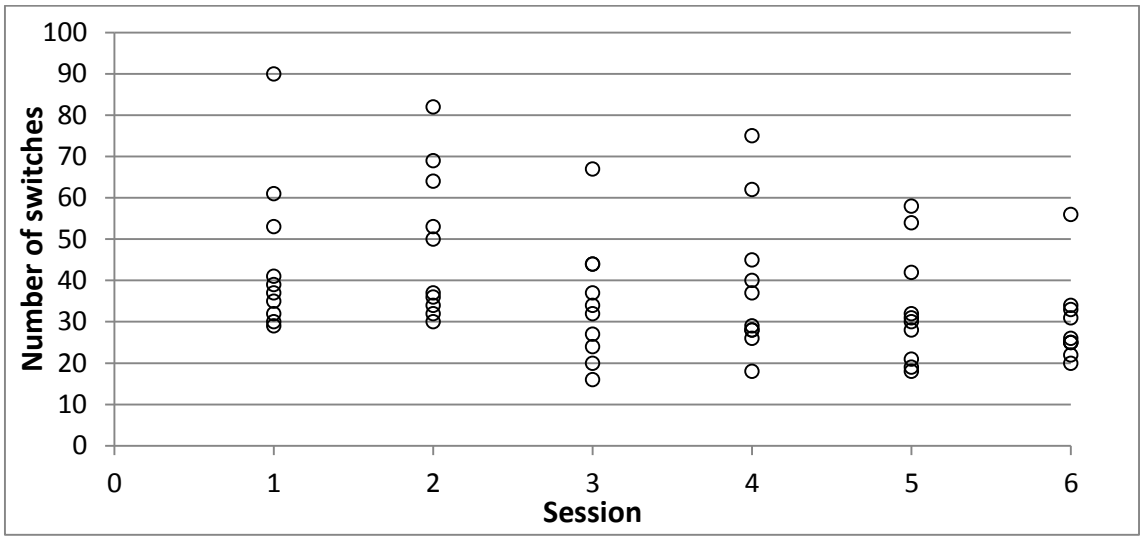


Figure 12: *Number of switches during the test session with 3-M-1-BE. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for 3-M-1-BE (Spearman correlation test, p=0.0016, S=50316.87, rho=-0.3981) (Figure 12).

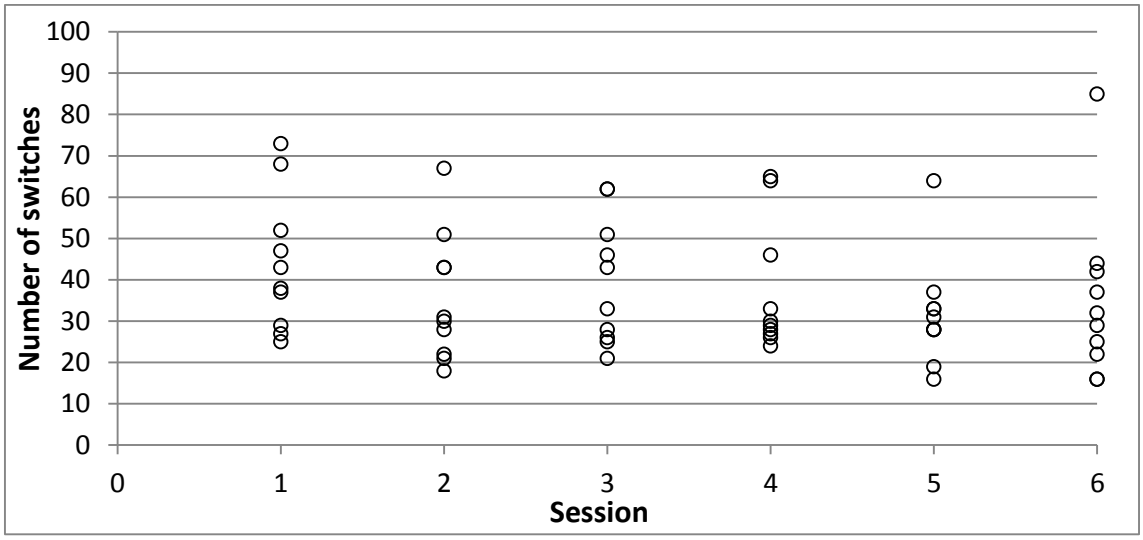


Figure 13: *Number of switches during the test session with 3-M-3-MB-1-O. Each circle represents one mouse.*

No significant correlation between the number of switches and the test session was found for 3-M-3-MB-1-O (Spearman correlation test, p=0.1056, S=43583.6, rho=-0.211) (Figure 13).

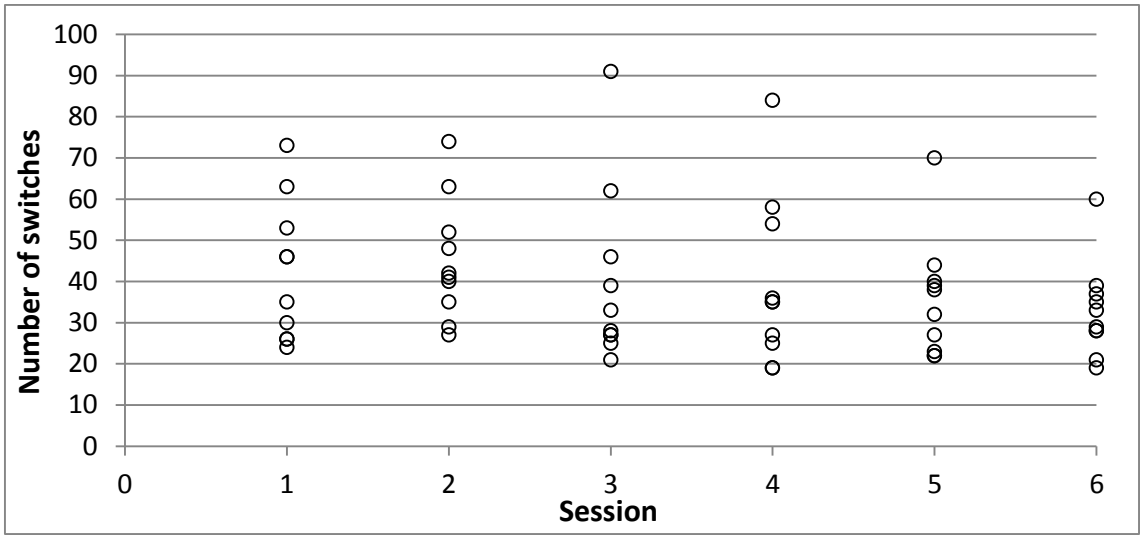


Figure 14: *Number of switches during the test session with FRUITY. Each circle represents one mouse.*

No significant correlation between the number of switches and the test session was found for FRUITY (Spearman correlation test, p=0.0708, S=44444.51, rho=-0.2349) (Figure 14).

The mice did not show significant differences in the number of fecal pellets excreted with the different odours (Wilcoxon test, n=30, B/B (5 ± 3) vs. 3-M-1-BE (5 ± 1) p>0.05, V=205; B/B (5 ± 3) vs. 3-M-3-MB-1-O (5 ± 2) p>0.05, V=223; B/B (5 ± 3) vs. FRUITY (4.5 ± 2.5) p>0.05, V=231; n=60, 3-M-1-BE (5 ± 2) vs. 3-M-3-MB-1-O (4.5 ± 2.5) p>0.05, V=652.5; 3-M-1-BE (5 ± 2) vs. FRUITY (4.5 ± 2.5) p>0.05, V=447, 3-M-3-MB-1-O (4.5 ± 2.5) vs. FRUITY (4.5 ± 2.5) p>0.05, V=599.5).

4.4 3-methyl-1-butanethiol, n-pentyl acetate and methyl-2-phenylethyl sulphide (factor of ten above the olfactory detection threshold)

When presented with 3-M-1-BE, the number of trials in which mice spent more time in the odour compartment compared to mice spending more time in the BLANK compartment was 24:36. Similarly, the number of trials for FRUITY vs. BLANK and M-2-PES vs. BLANK were 30:30 and 26:34, respectively. None of these three ratios differed significantly from chance (binomial test, n=60; 3-M-1-BE, z=1.42, p>0.05; M-2-PES, z=0.904, p>0.05, FRUITY, z=-0.129, p>0.05).

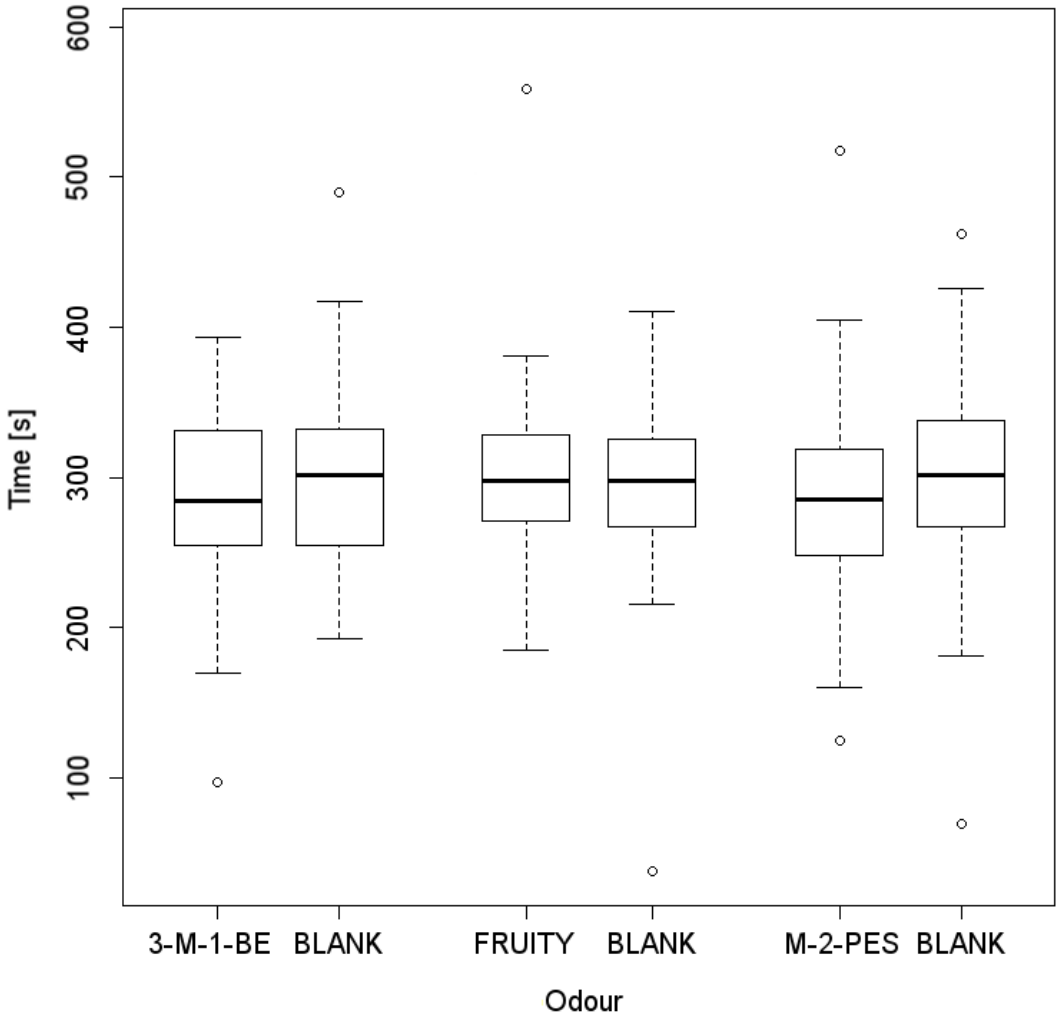


Figure 15: *Time spent in the different odour compartments compared to its matching BLANK stimulus.*

With both predator odourants and the fruity odour, the average time spent in the odourised compartment was not significantly different from the time spent in the BLANK compartment (Figure 15) (Wilcoxon test, n=60; 3-M-1-BE $p>0.05$, V=985.5; M-2-PES $p>0.05$, V=1083; FRUITY $p>0.05$, V=894).

Significant differences for the number of switches were found between B/B (57.5 ± 13.5) and 3-M-1-BE (46.5 ± 5.5) (Wilcoxon test, n=30, p=0.0004, V=382), B/B (57.5 ± 13.5) and M-2-PES (48 ± 8) (Wilcoxon test, n=30, p=0.0081, V=340.5), and between 3-M-1-BE (46 ± 9.5) and FRUITY (49 ± 7.5) (Wilcoxon test, n=60, p=0.0028, V=1017). No other significant differences were found (Wilcoxon test, n=30, B/B (57.5 ± 13.5) vs. FRUITY (51.5 ± 6); n=60, 3-M-1-BE (46 ± 9.5) vs. M-2-PES (47 ± 6.5)

p>0.05, V=989.5; M-2-PES (47 ± 6.5) vs. FRUITY (49 ± 7.5) p>0.05, V=613.5).

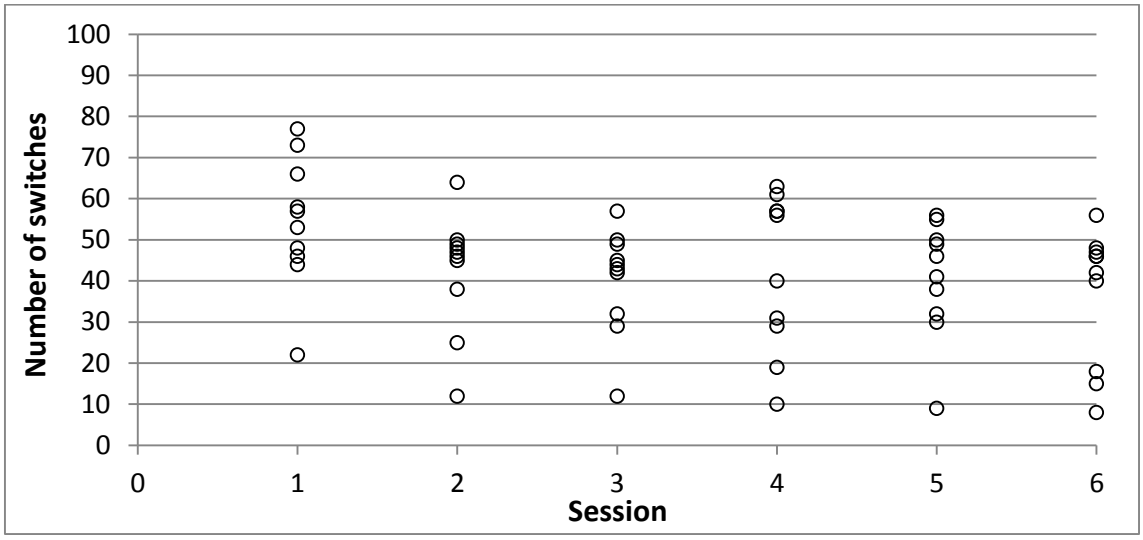


Figure 16: *Number of switches during the test session with 3-M-1-BE. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for 3-M-1-BE (Spearman correlation test, p=0.0317, S=45985.98, rho=-0.2777) (Figure 16).

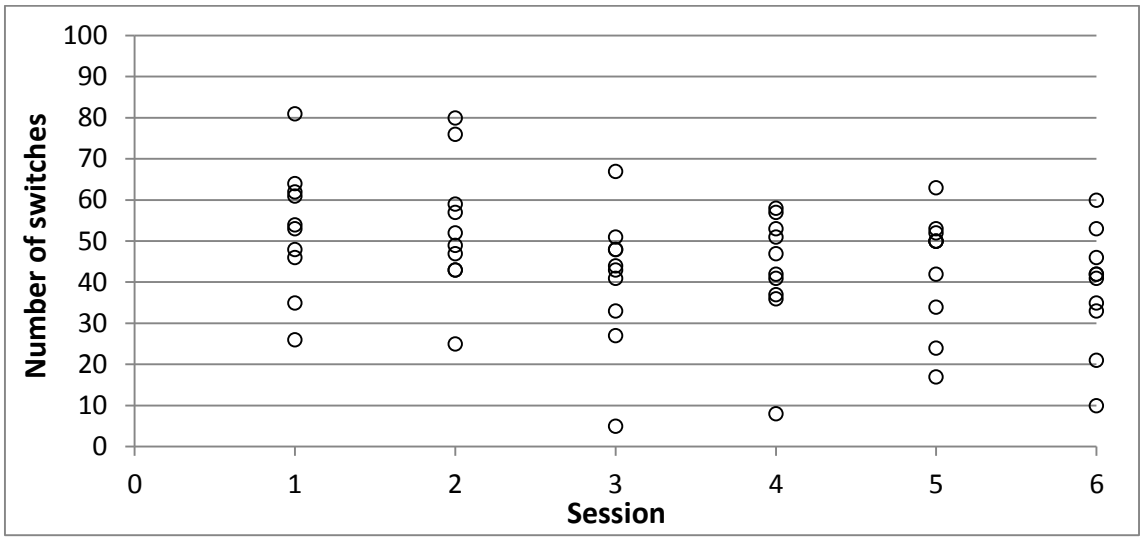


Figure 17: *Number of switches during the test session with M-2-PES. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for M-2-PES (Spearman correlation test, p=0.014, S=47354.09, rho=-0.3158) (Figure 17).

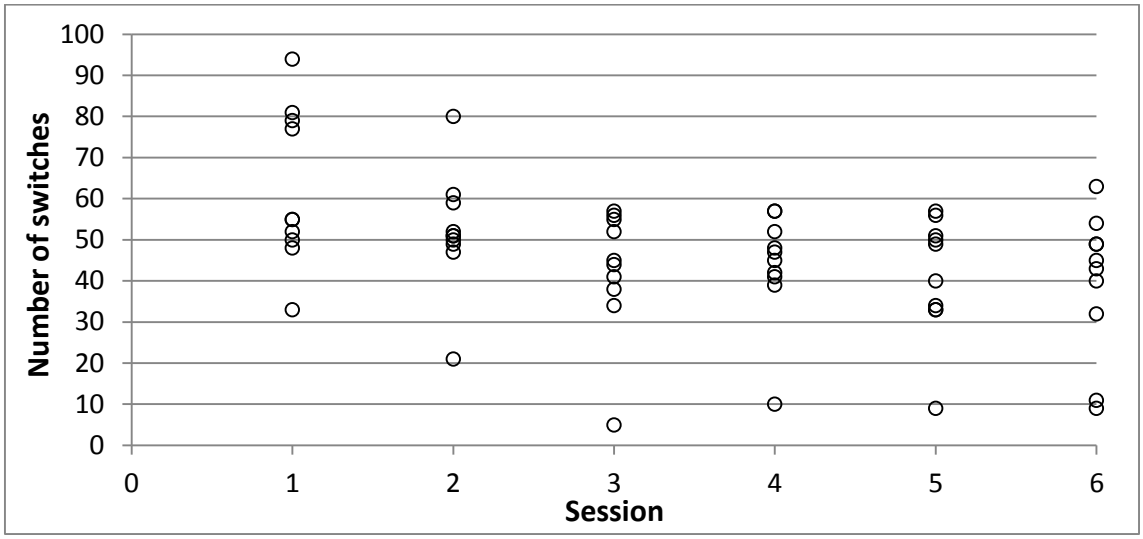


Figure 18: *Number of switches during the test session with FRUITY. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for FRUITY (Spearman correlation test, p=0.002, S=50086.24, rho=-0.3917) (Figure 18).

Significant differences for the number of fecal pellets were found between FRUITY (5 ± 2) and M-2-PES (4 ± 1), and between FRUITY (5 ± 2) and 3-M-1-BE (4 ± 2) (Wilcoxon test, n=60, M-2-PES p=0.0248, V=443.5; 3-M-1-BE p=0.0388, V=789). No other significant differences were found (Wilcoxon test, n=30, B/B (3.5 ± 1.5) vs. 3-M-1-BE (4 ± 1.5) p>0.05, V=186; B/B (3.5 ± 1.5) vs. M-2-PES (4 ± 1.5) p>0.05, V=153; B/B (3.5 ± 1.5) vs. FRUITY (5 ± 2) p>0.05, V=317; n=60, 3-M-1-BE (4 ± 2) vs. M-2-PES (4 ± 1) p>0.05, V=727).

4.5 3-methyl-1-butanethiol, n-pentyl acetate and methyl-2-phenylethyl sulphide (factor of 1000 above the olfactory detection threshold)

When presented with 3-M-1-BE, the number of trials in which mice spent more time in the odour compartment compared to mice spending more time in the BLANK compartment was 23:37. Similarly, the number of trials for FRUITY vs. BLANK and M-2-PES vs. BLANK were 22:38 and 31:29,

respectively. None of these three ratios differed significantly from chance (binomial test, n=60, 3-M-1-BE, z=1.678, p>0.05; M-2-PES, z=0.129, p>0.05, FRUITY, z=1.936, p>0.05).

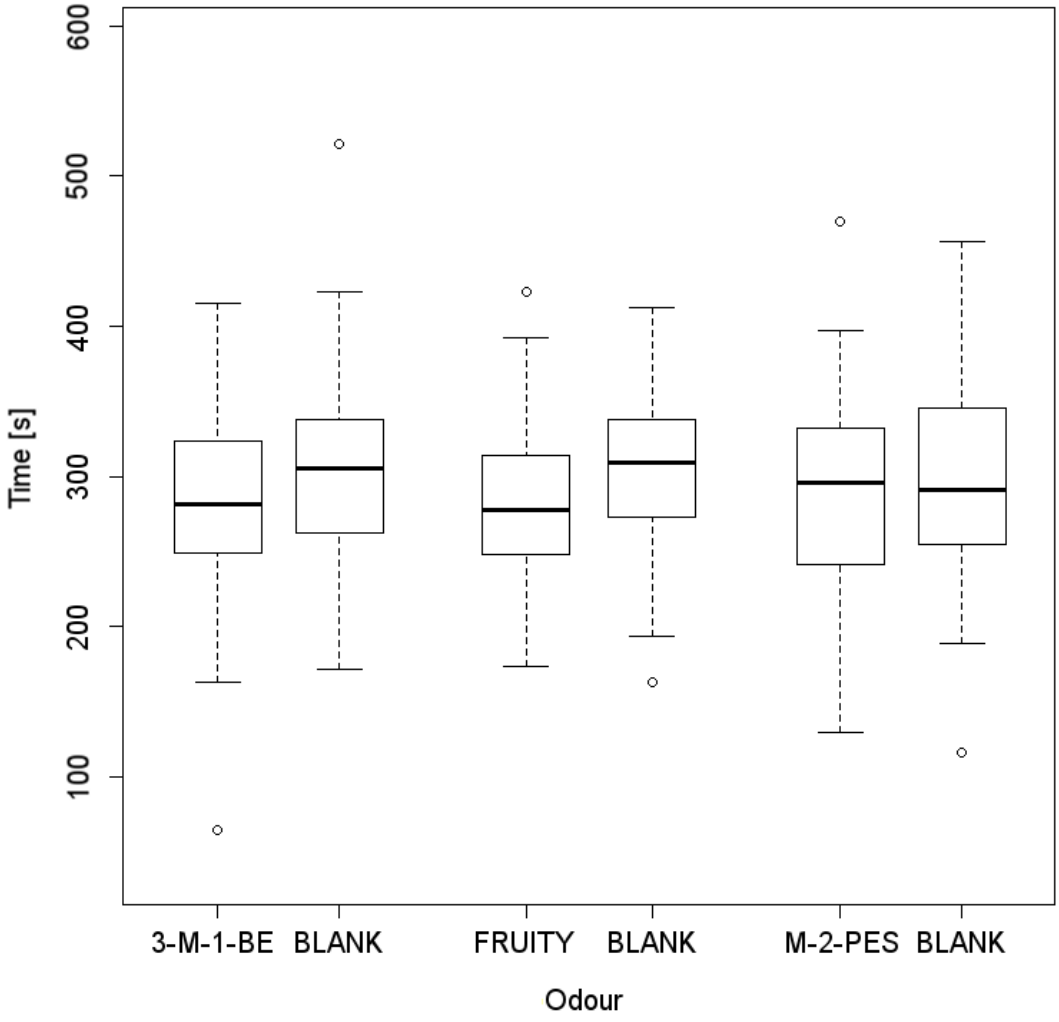


Figure 19: *Time spent in the different odour compartments compared to its matching BLANK stimulus.*

With both predator odourants and the fruity odour, the average time spent in the odourised compartment was not significantly different from the time spent in the BLANK compartment (Figure 19) (Wilcoxon test, n=60; 3-M-1-BE p>0.05, V=1075; M-2-PES p>0.05, V=992; FRUITY p>0.05, V=1108).

Significant differences for the number of switches were found between B/B (49.5 ± 9.5) and 3-M-1-BE (41 ± 6) (Wilcoxon test, n=30, p=0.0044, V=371.5), M-2-PES (38 ± 8.5) and 3-M-1-BE (37.5 ± 7.5) (Wilcoxon test, n=60, p=0.006, V=1062), and between 3-M-1-BE (37.5 ± 7.5) and

FRUITY (40.5 ± 8) (Wilcoxon test, n=60, p=0.0038, V=1152.5). No other significant differences were found (Wilcoxon test, n=30, B/B (49.5 ± 9.5) vs. M-2-PES (45.5 ± 9.5) p>0.05, V=237; B/B (49.5 ± 9.5) vs. FRUITY (44 ± 7) p>0.05, V=300; n=60, M-2-PES (38 ± 8.5) vs. FRUITY (40.5 ± 8) p>0.05, V=771.5).

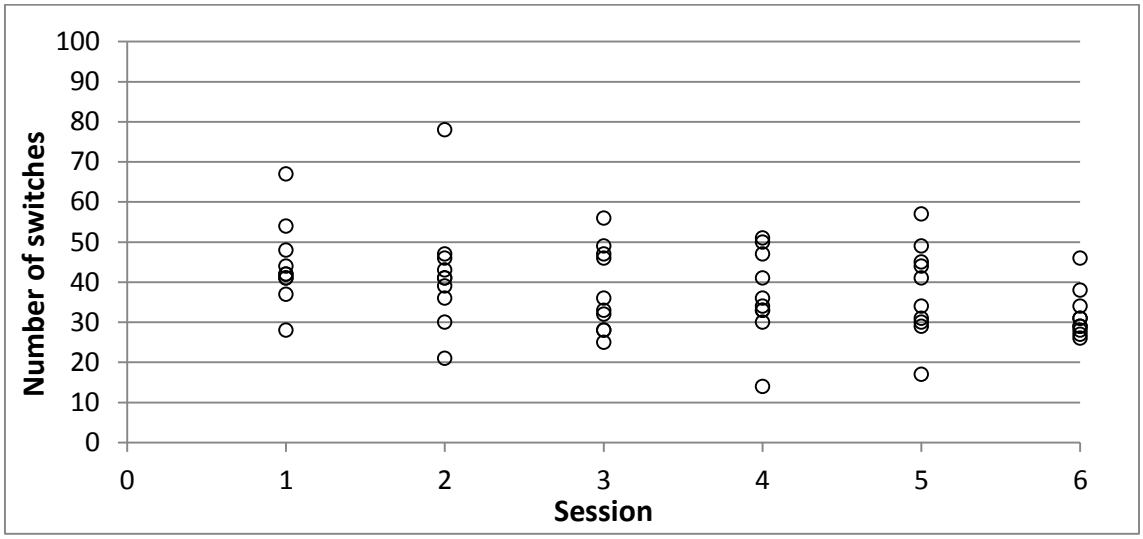


Figure 20: *Number of switches during the test session with 3-M-1-BE. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for 3-M-1-BE (Spearman correlation test, p=0.0089, S=48050.26, rho=-0.3351) (Figure 20).

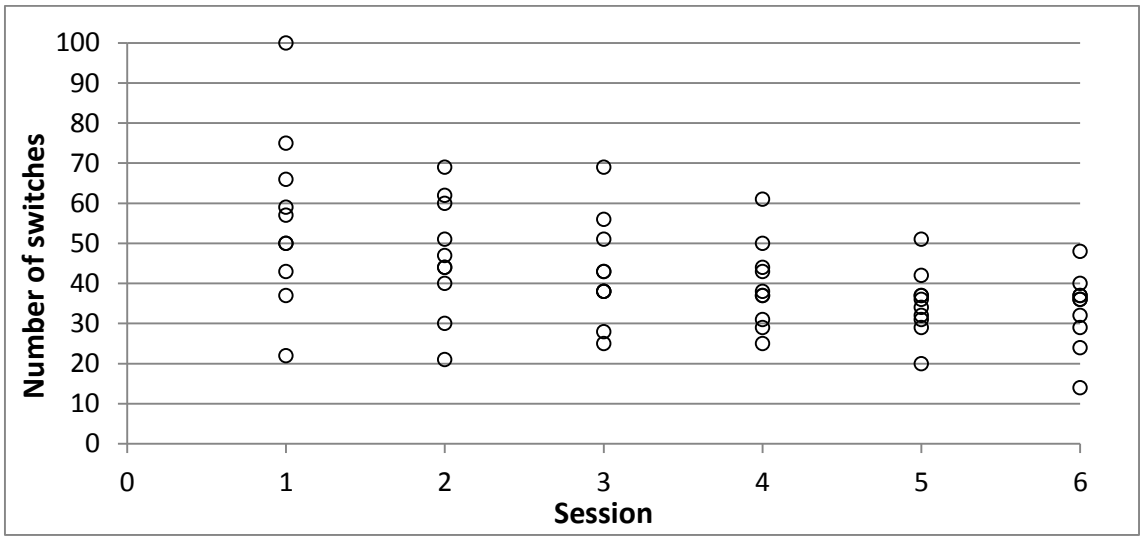


Figure 21: *Number of switches during the test session with M-2-PES. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for M-2-PES (Spearman correlation test, p=4.646 x 10^{-5}, S=54004.44, rho=-0.5005) (Figure 21).

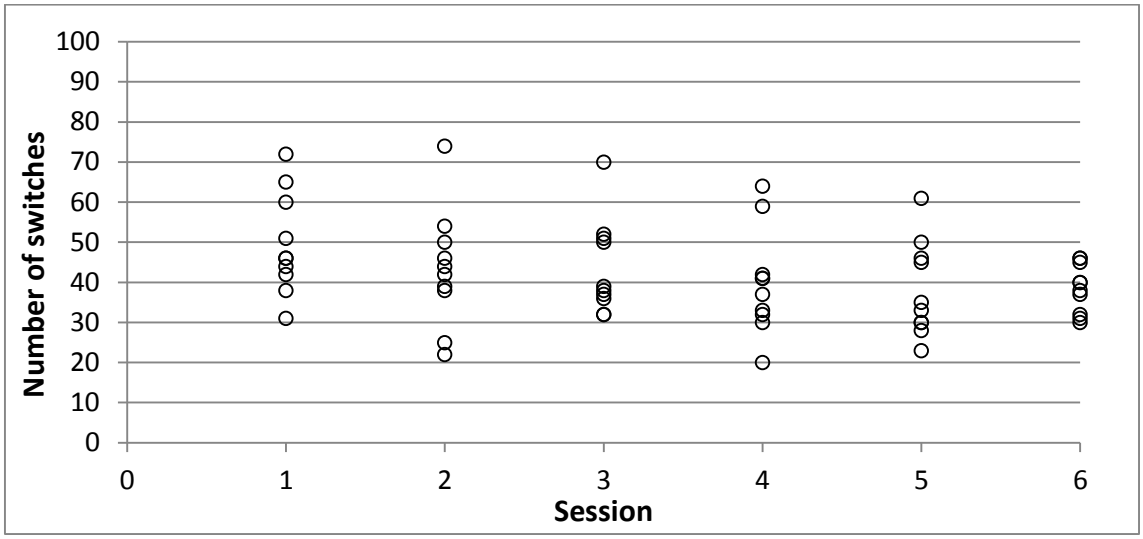


Figure 22: *Number of switches during the test session with FRUITY. Each circle represents one mouse.*

A significant negative correlation between the number of switches and the test session was found for FRUITY (Spearman correlation test, p=0.018, S=46954.63, rho=-0.3047) (Figure 22).

A significant difference for the number of fecal pellets was found between M-2-PES (5 ± 1.5) and 3-M-1-BE (6 ± 1) (Wilcoxon test, n=60, p=0.0091, V=369). No other significant differences were found (Wilcoxon test, n=30, B/B (6 ± 2) vs. 3-M-1-BE (6 ± 1) p>0.05, V=163.5; B/B (6 ± 2) vs. M-2-PES (5 ± 1.5) p>0.05, V=212; B/B (6 ± 2) vs. FRUITY (5± 1) p>0.05, V=174.5; n=60, 3-M-1-BE (6 ± 1) vs. FRUITY (5 ± 1) p>0.05, V=493; M-2-PES (5 ± 1.5) vs. FRUITY (5 ± 1) p>0.05, V=640.5).

5 Discussion

The results of the present study show that mice presented with 3-M-1-BE, an odourant found in the anal gland secretion of three skunk species, spent significantly less time in the odourised compartment compared to the BLANK compartment. In contrast, the ratios of compartment preference did not differ significantly from chance for any of the other predator odour components used in the present study.

Decreased activity is considered to be a sign of increased stress (Archer 1973). The present results show that in 11 out of 15 cases, the odours elicited a significantly lower number of switches than the BLANK stimulus alone. Only 2-PT, 3-M-3-MF (both at a factor of 100 above the detection threshold), M-2-PES (factor of 1000 above detection threshold) and FRUITY (in one session at a factor of 100 above detection threshold, at a factor of ten above the threshold and at a factor of 1000 above the threshold) did not cause such a decrease in this measure of overall activity.

However, a comparison with the BLANK stimulus, which was used in both compartments of the test arena during the first three test sessions, might be misleading. Experiments in rats showed an initial increase in motor activity followed by a decrease (Stretch 1960), therefore the present results might only represent an acclimatisation to the experimental set-up. This phenomenon could be reduced by habituating the mice to the test environment prior to their first contact with odours and by prolonging the testing time with the BLANK stimulus to six days. It should be noted, that the first batch of mice had additional three days of habituation, resulting to a total of six days, to the experimental set-up and the BLANK odour prior to the data collection. Nevertheless, these mice also showed a significantly lower number of switches between the compartments for the odourants compared to the BLANK stimulus. Furthermore, it seems like reduced motor activity is a key aspect when prey animals are presented with predator odour (Hegab et al. 2014b). This leads to the conclusion that the significant differences in the number of switches are unlikely to be caused by a lack of habituation.

Two different aspects of habituation might explain the present results. The significant reduction in the number of switches during the critical tests compared to the BLANK stimulus hints towards a habituation to the test situation, and the statistically significant negative correlation between the test session and the number of switches between the compartments might show a habituation to the odourants. In 11 out of 15 cases significant decrease in the number of switches across sessions was found.

2-PT, a component found in in the anal gland secretion of stoat, ferret, mink, Siberian weasel and steppe polecat, did not elicit any behavioural change. Previous studies have shown a significant aversive reaction of field voles (presented with weasel faeces) (Bolbroe et al. 2000), rats (presented with 2-PT alone)(Heale and Vanderwolf 1994; Perrot-Sinal and Petersen 1997; Bramley et al. 2000) and possums (presented with 2-PT alone) (Woolhouse and Morgan 1995). However, greater mouse-eared-bats did

not show aversive behaviour (Driessens and Siemers 2010). This leads to the conclusion, that 2-PT might not cause avoidance behaviour in CD-1 mice.

In the present study, mice did not show behavioural changes when exposed to 2,2-DT, which is found in the anal gland secretion of stoat, ferret, mink, Siberian weasel and steppe polecat. However, previous results show that this odour elicit aversive behaviour in field voles (presented with weasel faeces) (Bolbroe et al. 2000), snowshoe hares (2,2-DT alone) (Sullivan and Crump 1984), possums (2,2-DT alone) (Woolhouse and Morgan 1995) and slight aversive behaviour in roof rats (2,2-DT alone) (Burwash et al. 1998). Beavers have been shown to not react to the odour component (Epple et al. 1995) as well as northern pocket gophers (Sullivan et al. 1988). Based on the results obtained here, 2,2-DT does not seem to elicit avoidance behaviour in CD-1 mice.

3-M-3-MB-1-O and 3-M-3-MF, found in cat and bobcat urine, have not been assessed for their repellent properties. Nevertheless, bobcat urine elicited defensive behaviour in rats (Fendt 2006). Based on the present study, 3-M-3-MB-1-O and 3-M-3-MF do not cause avoidance behaviour in CD-1 mice.

The behaviour of prey species exposed to 3-M-1-BE has not been assessed, yet. In this study, 3-M-1-BE elicited significant avoidance behaviour when presented at a factor of 100 above their olfactory detection threshold. At the same concentration, the odour also caused a significantly lower number of switches compared with the FRUITY odour. However, 3-M-1-BE did not elicit behavioural changes at a higher or lower concentration. Further experiments are needed to assess the behavioural differences connected with the concentration change.

It has been shown that M-2-PES does not elicit aversive behaviour in rats (Vernet-Maury et al. 1984) and snowshoe hares (Sullivan and Crump 1986). According to the present study, M-2-PES does not seem to cause avoidance behaviour in CD-1 mice.

The FRUITY odour did not elicit significant behavioural changes for concentrations up to a factor of 1000 above their olfactory detection threshold. This shows that significant behavioural changes are unlikely to be caused by odour-based neophobia in this type of study.

The low number of tested individuals may have affected the results in such a way that individual differences among the mice might have had a strong

influence. Furthermore, a few outliers who showed freezing behaviour and only rarely switched between the compartments might have influenced the results.

Unfortunately, only a small number of studies evaluated the effects of specific odour components instead of a complex mixture, such as urine or faeces. Due to this fact, it is difficult to draw conclusions whether the odour components used in this study really do not have a behavioural significance for CD-1 mice or if the lack of significant differences is caused by the small sample size. This is further complicated by the fact, that predator odours often consist of several dozen different odourants (Jorgenson et al. 1978; Brinck et al. 1983; Mattina et al. 1991; Wood et al. 2002; Zhang et al. 2002) and no study has systematically assessed whether a single odourant is sufficient to elicit aversive behaviour or if a mixture of several is needed. Furthermore, only two of the predator odourants were tested at different concentrations. So far only studies with TMT (Wallace and Rosen 2000), a sulphur-containing compound in the anal gland secretion of the red fox, and cat faeces (Hegab et al. 2014a) have assessed the relation between aversive behaviour and odour concentration. Both studies came to the conclusion, that increased odour concentrations are positively correlated with the intensity of aversive behaviour. It is therefore possible that the remaining odourants of this study may elicit a behavioural change in CD-1 mice when presented at a different concentration.

Since general stress-related behaviour (Lindzey et al. 1964; Archer 1973; Belzung and Griebel 2001) as well as responses when presented with predator odour (Dell'Omo et al. 1994) seem to differ depending on the chosen mouse strain, it is also important to note that the insights gained in this study might only be valid for CD-1 mice and may differ significantly in other mouse strains.

Finally, a study found that CD-1 mice can be separated into two behavioural phenotypes with high and low reaction towards TMT (Hebb et al. 2004). To the best of my knowledge, no similar experiments have been performed for the odours used in the present study

In conclusion, the present study provides evidence for the possibility that single mammalian predator odourants might not be sufficient to elicit avoidance behaviour in CD-1 mice. This suggests that several odourants, or perhaps even the full mixture of predator odour components, may be needed in order to elicit aversive behaviour in mice.

5.1 Societal & ethical considerations

The experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* (national Institute of Health Publication no.86-23, revised 1985) and conform to Swedish laws on animal welfare. They have been approved by the local ethics committee (Linköpings djurförsöksetiska nämnd, Dnr. 76/12).

After careful consideration, I came to the conclusion that the present study does not have societal implications. Since experiments showed that laboratory mice display a significantly higher aversion towards predator odour than wild mice (Coulston et al. 1993), it is questionable if the study would have an effect on the practice of pest control.

6 Acknowledgement

I want to give special thanks to my supervisor Matthias Laska. He has been very supportive, a great help and guided me through the complete work. I am also thankful he arranged an extra training-course, so that I would be able to do this project. I also want to thank Siv Nilsson who gave me the theoretical part of the Laboratory Animal Science course. Finally, I want to thank Marie Eriksson who gave me the practical training in that course.

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