Animal Science Papers and Reports vol. 35 (2017) no. 3, 301-316
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Effects of novel odours on the mating behaviour in mice*

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(Accepted November 3, 2016)

The aim of this study was to examine whether and how different odorants placed on the bodies of female mice, but having no reward value for the males, affect courtship and mating behaviour of male mice towards females in oestrus and thus emitting female pheromones. In this manner, certain consequences of concurrent activation of the main olfactory system and the vomeronasal system were investigated. Four different odorants (white musk, lavender, peppermint and valerian) were used for swabbing female mice in oestrus. Using a total of 160 sexually naive outbred mice of both sexes, divided for each of 4 odorants into controls (not swabbed with odorant) and two experimental groups, in the experimental group I the females observed previously as controls were swabbed with one of the 4 odorants, while in the experimental group II, new naive females were swabbed with one of the 4 odorants. The females were observed in individual cages for 30 min. each, together with a respective sexually naive male. The latency between introduction of a male into a cage with a previously swabbed female and initiation of courtship and mating behaviours by males (sniffing, circling, misdirected mounting, copulation failures, successful copulation) was recorded. Latency to the occurrence of all sexual behaviours was significantly longer in experimental groups compared to controls. Latency to initiation of courtship behaviour, especially sniffing and circling, was shorter towards females swabbed with peppermint odour than for other odorants, indicating no aversion to this odour. However, the peppermint odour completely inhibited copulation. It is concluded that alien volatile odours with no reward value nevertheless exert differentiated suppressing effects on female mice pheromones inducing courtship and mating behaviour. Thus, it is hypothesized that the activation of the main olfactory system suppresses the accessory vomeronasal system.

*This study was funded within the internal grant STAT/TADJEZ/2015/01 of the Institute of Genetics and Animal Breeding of Polish Academy of Sciences, Jastrzębiec, Poland
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KEY WORDS: alien odorants / courtship / main olfactory system / mating behaviour / mice / pheromones vomeronasal system

It is well established that in terrestrial vertebrates two anatomically separate but physiologically complementary chemosensory organs: the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) which both play important roles in the biology of animals. [e.g. Cooper and Burghardt 1990, Keller *et al*. 2009, Baum and Cherry 2015, Stowers and Kuo 2015]. Localization of food, sexual partners, avoiding predators [Apfelbach *et al.* 2005, Sarrafchi *et al*. 2013], marking of territory [Hurst 1990], communication between neonatal and parents, social interactions [Brown 1985], as well as proceptivity and receptivity recognition in sexual partner and mate selection [Lenington 1983], are the most important examples for the role of olfaction in animal biology. Chemical signals carried by odour molecules can contain information concerning an individual's sex, age, health status, phase of the ovarian cycle, major histocompatibility complex and much more [Beauchamp and Yamazaki 2003].

Another issue in olfaction concerns spontaneous aversion or attraction to odorants which are not natural chemosignals for animals and have no reward value in their previous experience. The efficacy and mechanisms of suppressing of some natural chemosignals by alien odours used as repellents or attractants is a subject needing more research.

Pheromones acting as chemosignals for communication within a species are traditionally divided into (1) releaser pheromones directly affecting the central nervous system and causing an immediate behavioural response, and (2) priming pheromones which stimulate the neurohormonal system, alter hormonal activity and physiological processes and, in consequence, modulate or change an animal's behaviour after some time [Hummer and McClintock 2009, Keller *et al*. 2009, Beny and Kimchi 2014, Haga-Yamanaka *et al*. 2014, Baum and Cherry 2015]. Additionally, the term signaller pheromones has been introduced for chemosignals that induce behavioural or physiological changes depending on the identity of the emitter or sender [Keller *et al.* 2009].

There are numerous examples of using pheromones as attractants and kairomones as repellents in conservation biology and pest control [Mullen 1992, McNeil 1991, McDaniel *et al*. 2000, Schmidt and Kowalczyk 2006]. Mice, for instance, demonstrate stress and anxiety reactions in response to cat odour [Berton *et al*. 1998, Roy *et al*. 2001, Takahashi *et al.* 2005, Apfelbach *et al.* 2005].

In mice, signalling molecules are excreted not only in urine but also in other body fluids including tears, saliva and milk [Mucignat and Caretta 2014]. Chemical signalling in animals is very complex and still not fully elucidated, as there is no common principle that underlies the use of a molecule as a signal [Mucignat and Caretta 2014]. Due to the fact that different kinds of chemoreceptors belonging to the main olfactory epithelium, the vomeronasal organ, the Grűneberg ganglion and Masera's organ, are present in the oronasal cavities, the response of the animal to an odour or odour mixture may depend on a highly integrated signal processing.

Recent literature [e.g. Baum and Cherry 2015, Martin-Sanchez et al. 2015, Fortes-Marco *et al*. 2015, Haga-Yamanaka *et al*. 2014, Beny and Kimchi 2014] has indicated that the ethological and physiological significance of odorants as rewards is still to be elucidated. In the opinion of Fortes-Marco *et al.* [2015], synthetic compounds (e.g. predator-related kairomones, alarm pheromones or other intense odorants) are useful in studies on emotional behaviours of rodents and their neurobiological basis. In some cases, intense odorants that are unlikely to act as chemosignals can elicit behavioural reactions similar to those of true chemosignals.

Keller *et al*. [2009], in a review of the literature, argue that the main and the accessory olfactory systems may function synergistically in sustaining some pheromone-dependent behaviours and that the relative roles played by both systems in detecting chemosignals and in regulating chemosensory-dependent behaviours is a central problem in olfaction.

The aim of this work was to examine if and how different odorants put on female mice bodies, processed by the main olfactory system and having no previous reward value for the animals, affect courtship and mating behaviour of male mice towards females in ooestrus emitting female pheromones processed by the males' vomeronasal system.

Material and methods

Animals

A total of 160 outbred Swiss-Webster laboratory mice maintained at the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec, were used for the study. All mice were approximately 5 months old. The oestrus stage in females was assessed according to the scale given in Table 1. Additionally, oestrus stage was identified by examination of vulva mucus using a microscope, in a standard 4-day cycle, based on the presence of characteristic cells. For behavioural observations females being in their 4th or 5th stage of oestrus were used for observations. All males used for observations of their mating behaviour were sexually naive, having had no previous contact with females.

Table 1. Oestrus stages as assessed by appearance of the vulva

Oestrus stage	Appearance of the vulva
1	no red colour - dioestrus
2	slight redness
3	visible redness
4	strong redness and slight swelling of the vulva
5 E	very strong redness, strong swelling and visible mucus in the vulva - oestrus phase

For each of four odorants used, the mice were divided into three groups: a control group (C), experimental group I (E1) and experimental group II (E2). Thus the total number of mice used (160) consisted of four C groups (C) each of 8 naive females + 8 naive males, and of four E1 groups each of 8 females from C groups + 8 new naive males, and of four E2 groups each of 8 new naive females + 8 new naive males.

Odorants used

The whole body and especially the region of the external genital organ of females from experimental groups was swabbed, directly before behavioural observations, with essential oils of four odorants: white musk, lavender, mint or valerian. The four kinds of essential oils applied in this experiment have different specific characteristics, as follows:

White musk (WM) is a synthetic equivalent of natural musk. It is characterized by a very strong smell of and long-term persistence. White musk is used in the perfume industry to emulate the scent of deer musk or other natural musk. Synthetic musks can be divided into three major classes — aromatic nitro musks, polycyclic musk compounds, and macrocyclic musk compounds [Sommer, 2004]. The first two groups have broad uses in industry, ranging from cosmetics to detergents. It was assumed that the white musk should demonstrate strong suppressing properties in the present experiment.

Lavender oils (LA) have antiparasitic, antibacterial, anti-inflamatory and analgesic properties [Moon *et al*. 2006, Inouye *et al*. 2001, Hajhashemi *et al*. 2003], and are also used as fragrances for bath products. Some kinds of lavender yield a similar essential oil, but have higher levels of terpenes including camphor. Lavender has been for centuries used as a repellent against moths, ants, snails and mice. In the present experiment, the smell of lavender was expected to act as a deterrent.

Peppermint oil (PE) contains menthol (40.7%) and methone (23.4%). Other components, including menthyl acetate, 1,8-cineole, limonene, beta-pinene and beta-caryophyllene, have been revealed by gas chromatography [Schmidt et al. 2009]. Peppermint is known to repel mice and this reaction was expected in the present experiment.

Valerian oil (VA) has been studied extensively using analytical methods. It contains numerous compounds that may contribute to sedative, antiseptic, anticonvulsant effects and has been used for migraine treatment and pain relief. The most important compounds detected in valerian are: different alkaloids including chatinine, shyanthinine, valerianine and valerine [Shahidi and Naczk 2004], gamma-aminobutyric acid (GABA) [Yuan et al. 2004], iridoids including valepotriates like isovaltrate and valtrate [Shahidi and Naczk 2004], isovaleric acid [Houghton 1997], sesquiterpenes including valerenic acid [Yuan *et al*. 2004], hydroxyvalerenic acid and acetoxyvalerenic acid [Willis and Shohet 2009], as well as flavones including hesperidin, 6-methylapigenin and linarin [Marder *et al*. 2003, Fernandez *et al*. 2004]. Valerian oil constituents are presumed to interact with the GABA receptor [Boullata

and Nace 2000]. However, many studies remain inconclusive and all require clinical validation. For example, valerian also contains isovaltrate, which has been shown to be an inverse agonist for adenosine A1 receptor and may have the sedative effects expected from an agonist, rather than from an inverse agonist, at this particular binding site. As to affecting animal behaviour, valerian contains the cat attractant actinidine [Janot *et al*.1979], which may mimic the odour of cat urine. According to some anecdotal reports valerian is also attractive to rats and has been used to bait traps. Thus, it could be supposed that valerian would be attractive to mice in the present experiment.

Test methods

Prior to the experiment, preliminary observations were carried out using 3 male-female pairs in 3 separate cages observed over 2 hours to establish the most relevant behaviours and behavioural sequences to be recorded. Taking into account the occurrence of the main behaviours and the time of evaporation of odorants used, or suppressing them by an animal's own odour, e.g. urine, a period of 30 min was found to be appropriate for real tests. For each of four odorants, a control group and two experimental groups consisted of 8 female mice, kept in individual cages, were used. To each female in oestrus stage 4-5, according to the adopted scale, a sexually naive male was put for 30 minutes. The behaviour of each male-female pair was recorded visually and the latency of the first occurrence of the following behaviours of males was recorded: (1) sniffing of the female without any attempt to mount, (2) circling the partner without attempt to mount, (3) misdirected mounts towards the head of the female, (4) correctly directed mounts but failure to copulate, (5) successful copulations with ejaculation. The observation period ended with the first successful copulation.

The females of the control group were not swabbed with any odorant. For each odorant, females in experimental group I were taken from the respective control group and, before putting new males in with them, they were swabbed with the odorant. After completing the observations on experimental group I, the observations were replicated for each odorant using experimental group II, where new females and new males were observed.

Statistical analysis

To assess the significance of differences between control and experimental groups in latency to demonstrate particular behaviours, as well as for differences between odours, the non-parametric Mann-Whitney test was used, whereas for differences in number of pairs which copulated during the observation session, the chi-square was used. The Bonferroni correction was used for multiple testing.

Ethical statement

All procedures were accepted by the 3rd Local Commission for ethics in Animal Experimentation, Warsaw, Poland. All odorants used in the present experiment were safe for humans and animals.

Results and discussion

All mice in the present experiment, both males and females in control and experimental groups, urinated during the 30 min observation sessions. Generally males from the control groups devoted more time sniffing females and interacting with them than sniffing bedding in the cage contaminated with urine. In contrast, the male of the experimental groups devoted more time to sniffing cage bedding than sniffing females or in other sequences of sexual behaviour.

Latency to first sniffing females

Sniffing females by males was the first behaviour in the behavioural sequence after males had been placed in the female's cage. For all odours the latency in males beginning to sniff females was longer, especially in experimental group E2 compared to controls. These differences in latency were significant at $P<0.05$ for musk, mint and valerian and at $P<0.01$ for lavender (Fig. 1). Lavender odour caused longer latency to the first sniffing than the other odorants ($P<0.05$, Fig. 1).

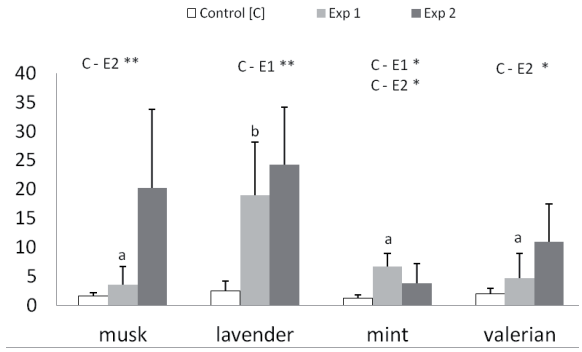

Fig. 1. Latency in min. to first sniffing. *P<0.05; **P<0.01. Bars denoted with different letters differ significantly at P<0.05.

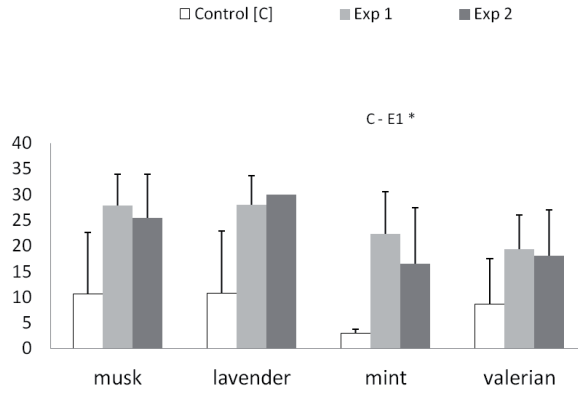

Fig. 2. Latency in min. to first circling. *P<0.05.

Latency to first circling

Circling of males and females around each other followed the sniffing. The latency to the first circling was shorter in the control groups compared to the experimental groups for each odour. However, only the difference between the control and the E1 group for mint was significant at $P<0.05$ (Fig. 2). No significant differences between experimental groups E1 and E2 in the latency to first circling were observed.

Latency to first misdirected mounting

After circling, the males attempted to mount females beginning usually with misdirected mounting towards the head of the female. The latency to first misdirected mounting was shorter in controls than in experimental groups for each odour. However, the difference was statistically significant ($P<0.05$) only for WM odour (Fig. 3). For unknown reasons, the latency to the first misdirected mounting was as much as twice shorter in controls for WM and PE odours compared to LA and VA controls, while this difference and the differences between E1 and E2 groups for each odour were non-significant (Fig. 3).

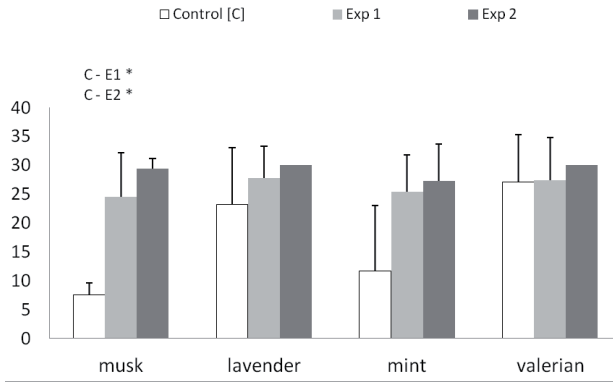

Fig. 3. Latency in min. to first misdirected mounting. *$P<0.05$.

Latency to failed copulation

After some misdirected mountings, the males mounted correctly but failed to copulate probably due to being disturbed by the odour of the females or for other reasons. The latency for this behaviour was shorter in control groups for all odours, compared to experimental groups, although being significant ($P<0.05$) only for PE odour (Fig. 4). As for other behaviours, the latency to copulation failures was non-significantly but markedly shorter in PE controls than in controls for the other odours (Fig. 4). In LA odour the experimental groups demonstrated the latency near 30 min with no variation which means that almost no attempts to copulate took place within the observation session (Fig. 4).

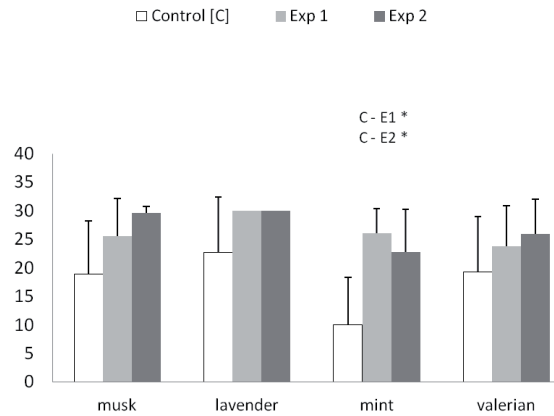

Fig. 4. Latency in min. to failed copulation. *P<0.05.

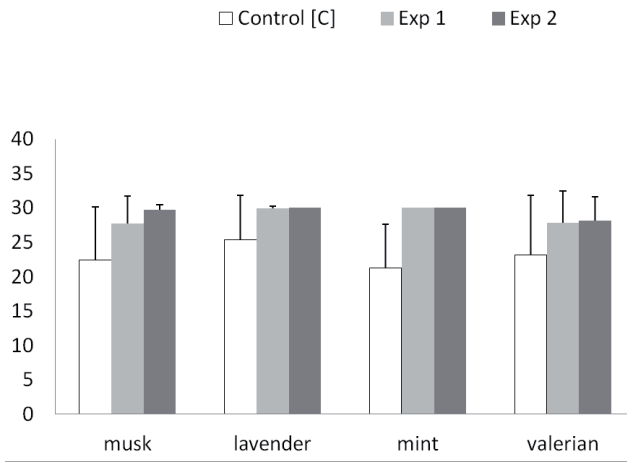

Fig. 5. Latency in min. to successful copulation.

Latency to successful copulation

The controls for all odours had a non-significantly shorter latency to completed copulation than the experimental groups swabbed with the odours (Fig. 5). The differences between odours were found non-significant. Almost no successful copulations were observed in experimental groups swabbed with the odorants (Fig. 5).

Number of copulating pairs

During the observation session, out of 8 control pairs for each odour, 3-7 pairs completed copulation (Fig. 6). The Chi-square test revealed significant differences (P<0.05) in the number of control pairs copulating between lavender control (3 pairs)

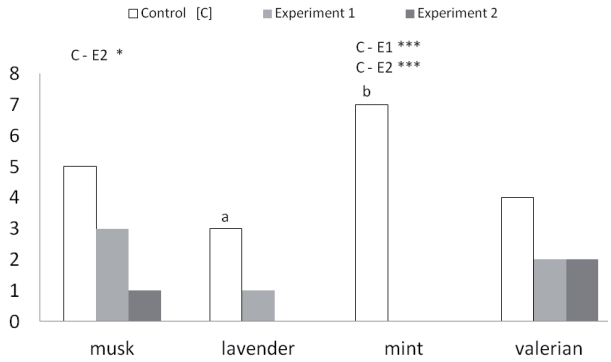

Fig. 6. Number of copulation pairs. *P<0.05; ***P<0.001. Bars denoted with different letters differ significantly at P<0.05.

and mint control (7 pairs). The differences between control pairs for each odour cannot be attributed to the odour effect since the control females were nor swabbed with any odour. The only possible explanation was that the observation period of 30 min was probably too short, and presumably at a longer observation period more control pairs would copulate. The difference in number of copulating pairs between overall controls irrespective of the odour and experimental groups 1 was significant (P<0.001), as well as between overall controls and experimental groups 2 (P<0.001), whereas the difference between experimental groups I and II was not significant. The swabbing of females with mint odour prevented males from successful copulation since no pairs copulated in experimental groups E1 and E2 (Fig. 6), which makes differences between controls for mint group and experimental groups E1 and E2 significant at P<0.001. Also no pairs copulated in experimental group E2 after females were swabbed with lavender oil (Fig. 6), although the difference to the control was non-significant due to a low number of control pairs that copulated (3 pairs). In the case of musk odour, the higher number of control pairs that copulated (5 pairs) caused the difference between control and experimental group E2 to be significant (Fig. 6, P<0.05).

The observed effects of swabbing of female mice in oestrus with alien odours in delaying or inhibiting typical mating behaviours in males raises issues regarding the deterrent effect of alien odour, suppression of species-specific female pheromones by the alien odour, and the odorant itself acting as an additional attractant to the males.

Increasing research on pheromones and on the role of the vomeronasal organ in the past 30 years has prompted some authors to propose a new nomenclature to distinguish between odour detection by nasal olfaction involving MOE, and by vomerolfaction, detecting so-called vomodours involving VNO [Cooper and Burghardt 1990]. Whereas the distinction between the MOE and VNO in terms of their different repertoires of receptors and signal transductions has been discussed [Cooper and Burghardt 1990, Keller et al. 2009, Baum and Cherry 2015, Stowers and Kuo 2015], it remains unclear how MOE and VNO compete as far as behavioural consequences of non-pheromonal vs.

pheromonal odorants are concerned. Beny and Kimchi [2014] stated that pheromones could be detected both by the VNO and the olfactory system; however, pheromones have intrinsic rewarding significance and trigger innate hardwired social behaviour responses, whereas other odorants, having primarily neutral reward value, induce an approach or avoidance response. This happens through conditioning that comes into effect by association with other stimuli that possess intrinsic properties. Stowers and Kuo [2015] listed candidate mouse pheromones both in male and female urine. As all females used in the present experiment were in oestrus, male pheromones accelerating puberty or inducing oestrus are of secondary importance for this study, whereas most relevant would be female pheromones inducing male courtship. Haga-Yamanaka et al. [2014] by profiling the calcium response of individual VNO neurons, identified two groups of vomeronasal receptors (V1Rs) that respond to a female pheromone. These authors found that receptors belonging to the V1re clade recognize gender identifying cues, and the receptors that are multiple members of the V1rj clade are receptors for sulphated estrogens. The latter compounds are classified by Stowers and Kuo [2015] as candidate female pheromones present in female urine that attract males.

Stowers and Kuo [2015] distinguish two classes of olfactory signals: associative and specialized. Whereas for associative olfactory signals the response depends on experiences an individual has gained with an odour in terms of its pleasant or aversive consequences, the specialized olfactory signals activate a subset of neurons generating the same pre-set behaviour, irrespective of individual experience. On this distinction proposed by Stowers and Kuo [2015], we could assume that odorants used in the present experiment are associative odours, while pheromones in female urine in the litter or on genital area of females' body are specialized odours. On the other hand it must be emphasized that males used in the present experiment had no previous experience with the odorants, either positive or negative, so no pleasant or aversive associations should be produced as to these odours and the responses of the males was spontaneous.

Another issue, though not the aim of this study, should be discussed, which is the significant difference in latency to particular sequences of mating behaviour demonstrated by control males used for each odorant. It could be speculated that the effects of male pheromones on females' sexual behaviour during courtship could contribute to the differences between control groups. Male mice produce in their tear glands exocrine gland secreting peptide 1 (ESP1), which is transferred to the female's vomeronasal organ through physical contact during courtship [Wyatt 2014, Haga *et al.* 2010, Kimoto *et al*. 2005]. The ESP1 provokes the lordosis behaviour in females, thus facilitating copulation. It could be speculated that either individual differences in production of the ESP1 by control males, or individual differences in control females in their sensitivity to the ESP1, contributed to the differences between control pairs used for particular odorants as to their mating behaviour. Wyatt [2014] has indicated the role of proteins and peptides as pheromone signals and chemical signatures across invertebrates and vertebrates species. Proteins and peptides, not being volatile but rather being soluble, act like pheromones if they are received by

the recipient during a close physical contact to the sender or the communication takes place indirectly via proteins left on scent marks in, for instance, urine [Wyatt 2014]. Although not investigated in the present study, the role of mouse protein pheromones, such as darcin, one of the major urinary proteins (MUPs) expressed in male urine which attracts females [Hurst and Beunon 2013, Roberts *et al.* 2014, Wyatt 2014], cannot be neglected when discussing the present results.

We assume that odorants used in the present experiment are volatile, which means that they can be perceived by mice at a distance without physical contact. If so, the relative differences in the latency of a particular behavioural sequence in mouse pairs, where the female was swabbed with particular odorant, can tell us whether the odour exerted a deterring, suppressing or attracting effect. A shorter latency to start sniffing in experimental groups compared to the controls would indicate that an odorant has an attracting effect. Since none of the odorants showed shorter latency to start sniffing than its respective control, no true attractiveness of the investigated odorants could be claimed. A longer latency to start sniffing, circling around females, misdirected mounting, copulation failures, and successful copulation in experimental pairs of mice used for an assay of a particular odorant, may suggest deterring and pheromone suppressing effects of the odorant. If this assumption is correct, the lavender odour showing the longest latency to start sniffing would have the most deterring effect of all odorants used here. However, the lavender odour seems not to have an absolute suppressing effect since one pair of the E1 group completed copulation (Fig. 6). The peppermint odour was not deterring for male mice since the latency to start sniffing was the shortest, but this odour seems to suppress female pheromones since no successful copulation was observed in either experimental group E1 or E2.

Although statistically not always significant, generally the differences in latency to demonstrate sexual behaviours by males in experimental groups E1 and E2 could be attributed to the fact that E1 females had some experience in contact with males, since they were previously used as controls, whereas the E2 females were sexually naive. It is worth mentioning that the peppermint odour, in contrast to other odorants used, caused shorter latency to sniffing and to mounting, but ended with a failure in experimental group E2 compared to E1.

Courtship and mating are believed to be genetically programmed, hardwired innate behaviours although they are dependent on the animal's experience and environment [Roberts *et al.* 2010, Beny and Kimchi 2014]. If so, these behaviours could be expected not to be affected by volatile odorants processed by the main olfactory system that are not pheromones and have neutral reward value based on the mice's earlier experience. Beny and Kimchi [2014] listed pheromonal signals received by the vomeronasal organ that have either a positive, neutral or negative reward value in relation to sexual behaviours of mice. Out of many pheromonal substances, for the present study most relevant may be those secreted by females and having signalling or attracting effects on males. Those are for instance: 17β – diol disulphate (E1050), which has a positive reward value for males and signals to males that a female is in oestrus [Haga-

Yamanaka *et al*. 2014, Isogai *et al*. 2011, Nodari *et al*. 2008], exocrine gland secreting peptide1 (ESP1), which increases female receptivity but has neutral reward value to males [Haga *et al.* 2010, Kimoto *et al.* 2005] and proteins and peptides MHC class1, which enhance attractiveness of female mice of different strains [Spehr *et al*. 2006, Zufall and Leinders-Zufall 2007] and have positive rewarding value.

It can be speculated that odorants used in the present experiment differentially suppress all or some of the above mentioned pheromones. According to Haga-Yamanaka *et al*. [2014], neither gender-specific cues, nor pheromones like sulphated estrogens acting alone, are sufficient to promote courtship. The latter authors claim that, in pheromone-triggered mating behaviour, integrated action of different female cues is necessary. For example when two of the above-mentioned cues are applied together, they can induce a robust courtship behaviour.

Beny and Kimchi [2014] stated that pheromones have been thought to have intrinsic rewarding significance, whereas other odorants generally possess a neutral reward value. These authors argue that responses to both pheromones and other odorants can be modified by experience. Our study demonstrates that odorants, of neutral reward value and processed by the main olfactory system, can have different consequences for courtship and mating depending on the kind of odorants, without involving previous experience and/or learning. Lanuza *et al*. [2014] showed that the effect of darcin, a male mice pheromone that attracts females, can be modulated by the physiological and health status both of the sender and receiver. With regards to our study, it could be hypothesized that alien odours of females were perceived by males as signs of illness or not species-specific, which in consequence delayed or inhibited courtship, although such effects were not equal for all odorants used. Baum and Cherry [2015] collected evidence that the main olfactory system detects volatile odorants that function as pheromones to facilitate mate recognition in terms of attraction to volatile opposite-sex pheromones. Our study, on the other hand, demonstrates that alien odours processed by the main olfactory system and having neutral reward value as based on no experience or learning, may differentially delay or inhibit courtship and mating. As all males used in the present experiment were sexually naive and had no previous contact with the odorants used, no positive or negative reward value could be expected and the differences between odorants can be attributed to an intrinsic aversion or attractiveness of particular odorants for mice. As to human sense of smell, none of the odorants used evokes a spontaneous unpleasant olfactory impression. To the contrary, they are often used as fragrances in the perfume industry.

Attention should be paid to our finding that peppermint odour delayed sniffing females in oestrus and precopulatory behaviours of males, while totally inhibiting copulation. This is interesting in view of the results of earlier studies by O'Connell and Meredith [1984], showing that chemical lesions of the main olfactory system blocked investigative behaviour of male hamsters towards vaginal discharge of oestrus females, though actual mating was not disrupted. O'Connell and Meredith [1984] found that vaginal discharge of hamster females in oestrus contains both volatile and

nonvolatile chemical signals that collectively elicit both male attraction to females and male mating behaviour. According to these authors, males were attracted by female odour and engaged in mating behaviour when only the volatile components of vaginal discharge were available, and behaviours were further enhanced when both volatile and nonvolatile components of discharge were provided. The authors hypothesized that the main olfactory system is preferentially involved with processing those volatile chemical signals in vaginal discharge that denote female attractiveness, whereas the accessory olfactory system is preferentially involved with processing volatile and nonvolatile chemical signals that evoke subsequent steps in male sexual behaviour. Applying the above-mentioned hypothesis to our experiment it could be supposed that the peppermint odour does not decrease females' attractiveness but inhibits the activation of the accessory olfactory system. On the other hand, the other odours, especially lavender, tended to decrease females' attractiveness whereas these odours did not totally inhibit copulation and thus did not inhibit the accessory olfactory system.

The learning effects of scenting oestrus rat females with alien non-pheromonal odorants on male preferences were studied by Kippin *et al*. [1998]. These authors found that male rats that initially mated with oestrus females scented with almond later preferred almond-scented females when given a choice between almond-scented and not-scented females.

It is difficult to speculate on chemical relationships between volatile compounds present in odorants used in our study and non-volatile or volatile pheromonal compounds eliciting courtship and mating behaviour in mice. However, we suggest that because peppermint odour inhibits or delays copulation in mice, having relatively less impact on pre-copulatory behaviours, it may be used in future experiments on semiochemical communication between sexes and on reproductive behaviours of mice and other species. This could provide a means of avoiding use of drastic methods that interfere with the animals' physiology, such as bulbectomy or surgical removal of the vomeronasal organ.

Our study shows that volatile compounds present in natural odorants and activating the main olfactory system, even with no reward value for mice, can differently interact with pheromones that activate chiefly the vomeronasal system but probably also the main olfactory system. This resulted in a latency or inhibition of courtship and mating behaviour in laboratory mice. Recently Baum and Cherry [2015] postulated that there is a need to determine whether mating-induced activation of the accessory olfactory system paired with concurrent activation of the main olfactory system by volatile pheromonal odorants is always required to give reward salience to those volatile chemosignals. On the basis of our results, it could be supposed that interaction between main and accessory olfactory systems exists, at least towards non-pheromonal chemiosignals, without any reward salience.

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