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Cattle olfaction: Dairy cows' interest in odors and factors affecting their odor exploration behavior

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ABSTRACT

Animals use their sense of smell in various situations, including foraging, selecting mates, and assessing predation risks. Consequently, odors are likely to affect farm animals in numerous handling and management practices. Cattle have a well-developed sense of olfaction that may play a larger role in their everyday life than is currently considered. The current body of research on cattle olfactory abilities is, however, surprisingly scarce. The aim of this study was to investigate if cows can detect and discriminate 4 odors of natural, nonsocial origin, and if any of the specific odors evoke more interest (measured as sniffing time) than others. We further aimed to assess if age, parity, and breed affected this. In addition, we investigated olfactory-exploration behavior (other than sniffing: licking, biting, flehmen, head movements, backing, snorting) of dairy cattle and ear positions to elucidate if certain behaviors and ear positions were restricted to certain odors. Twenty-eight cows (16 Swedish Holstein, 12 Swedish Red) were enrolled in a habituation-dishabituation test where they were tested in pairs on 4 natural odors (essential oils, nondiluted): cedarwood, lavender, orange, and peppermint. The test was conducted on individual animals in their home environment where each odor was presented 3 times in a row for 1 min each with an intertrial interval of 2 min. Following another 2-min interval without the first odor, the cow was presented with a different odor, with order of odor presentation balanced among animals. Duration of sniffing (muzzle in proximity to) the odor box, occurrence of licking or biting the odor box, and avoidance behavior (e.g., backing and head movements), and ear positions were recorded. Although the results showed a decrease in sniffing time over repeated presentations of the same odor, only the first-to-third presentation of cedarwood and first-to-second and first-to-third presentation of orange differed significantly. Only some dishabituation trials elicited a significant reinstatement of sniffing; hence, it is unclear if cows were able to discriminate all odors from each other. Testing cows in pairs potentially led to brief pre-exposure to odors, thereby affecting overall sniffing durations. More studies are thus needed to elucidate if cows can recognize but also discriminate the odors. Cows did not show a clear interest in any particular odor, though they numerically sniffed cedarwood the most and orange the least. Younger cows expressed more sniffing behavior than older cows regardless of odor, and younger cows also expressed axial ear positions for longer. Specific odors did not elicit more of any of the ear positions than others. Behaviors indicative of avoidance reactions (head movements, backing, snorting) were generally low for all odors, but Swedish Holstein cows expressed more backward ear positions than Swedish Red, highlighting the need for further studies including various cattle breeds. We encourage future studies on olfactory abilities and preferences, as well as refinement of methods to further adapt testing regimens for cattle olfaction.

Key words: bovine, welfare, smell, ear position, sensory enrichment

INTRODUCTION

The sense of smell plays an important role in the lives of animals, facilitating their survival (Brown and Macdonald, 1985; Wyatt, 2003). Animals use their sense of smell in various situations, including foraging, selecting mates, and assessing predation risks (Nielsen et al., 2015). For most mammals, including domestic species such as pigs (Schild and Rørvang, 2023) and horses (Briant et al., 2010; Jezierski et al., 2018), olfaction is a salient sensory modality in comparison to, for instance, vision. Consequently, odors are likely to affect numerous handling and management practices involving farm animals. The study of how farm animals use their sense of smell and how different odors influence their behavior is a relatively new field of research (Nielsen et al., 2015). Consequently, the current knowledge is limited.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

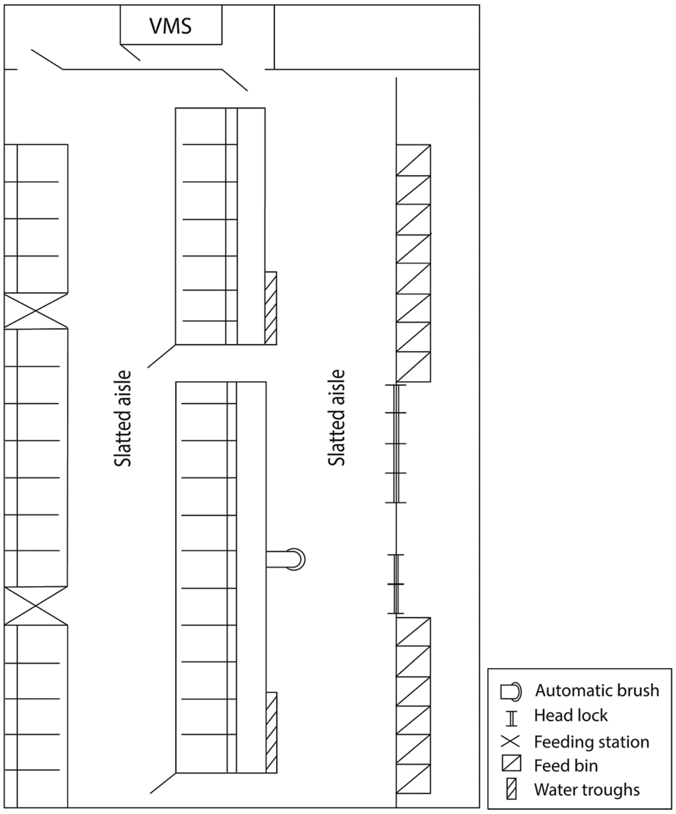

Figure 1. Top-view schematic illustration of the stable section in which the experimental group of cows was kept. Cubicles, slatted aisles, automatic brush, water troughs, the VMS, feeding stations, feed bins, and the headlocks are shown. The headlocks were used during testing of the cows, where 2 cows at a time were tested while in the headlocks.

Figure 2. The 5 headlocks placed in the middle of the stable aisle. Dimensions of the headlocks are given. The bedded packs can be seen adjacent to the headlocks and a blue feed bin can be seen to the right in the figure. During the experiments, 2 cows would be placed in headlocks and tested in pairs for social contact during testing. Testing in headlocks was done to standardize odor exposure times and distances from the odor samples, while also minimizing interference from other herd members.

Considering farm animals with a keen sense of smell or who are highly odor-dependent, pigs are most commonly thought of, but other species, such as horses, sheep, and cattle are also equipped with well-developed olfactory systems. Cattle have relatively large nostrils connected to a large surface area of olfactory epithelium (Phillips, 2002) containing a large array of olfactory receptors (Lee et al., 2013). The olfactory receptor genes are essentially coding for which olfactory receptors are expressed in the epithelium, and hence which odors are possible for the animal to detect. Lee et al. (2013) analyzed the olfactory sub-genome of cattle and found a total of 1,071 olfactory receptor-related sequences including 881 functional genes, 190 pseudogenes, and 352 partial sequences, clarifying that cattle in theory should be able to detect: fatty, sour, floral, woody, green, lily of the valley, vanilla, spearmint, caraway, sweet, hay-like, lemon, rancid, and spicy odors. Interestingly, the authors also conducted comparative analyses of olfactory receptor genes across cattle, pigs, humans, mice, and dogs, showing that 6.0% (n = 53) of the cattle functional olfactory receptor genes were species-specific. In comparison, pigs have been found to have one of the largest olfactory receptor gene repertoires within the farm animal kingdom, with 1,113 functional olfactory receptor genes (Nguyen et al., 2012; Paudel et al., 2015). This is superseded by the mouse, with ~1,200 functional olfactory genes (Rouquier and Giorgi, 2007; Zhang et al., 2007) and the African elephant with 1,948 intact genes (Niimura et al., 2014), whereas the estimate for humans is about 339 functional genes and 297 pseudogenes (Malnic et al., 2004). Odors and the information contained in them may thus be important to cattle, although this is currently not considered in practical on-farm settings.

One of the areas where olfaction may play a crucial role is cattle management (reviewed in Archunan et al., 2014). Cattle have a substantial number of functional olfactory genes encoding olfactory receptors (**OR** genes), ranging from 900 to 1,100 depending on the study, compared with approximately 880 to 1,000 in dogs and fewer than 400 in humans (Quignon et al., 2003; Lee et al., 2013; Niimura et al., 2014). This shows that cattle have a well-developed basis for detecting a large array of odors. It also implies that cattle may use their sense of olfaction in many aspects of their daily life. As a farm animal with a keen sense of smell, the surrounding olfactory environment could (and likely does) affect their behavior and welfare, highlighting the importance of considering olfaction in future adaptations of commercial cattle housing and management. Odors may be used as calming agents or as attractive components of their environment that could improve welfare and optimize the functionality of housing systems for dairy cattle (Nawroth and Rørvang, 2022; Ginane and Rørvang, 2023). However, before implementing olfactory initiatives in a practical setting, more research is needed to uncover the olfactory abilities of cattle as well as their interest in and reactions to odors. Knowing which odors cows can detect and discriminate between, but also whether some odors elicit avoidant or explorative behaviors could be valuable indicators of how cattle perceive them. Such information will be pivotal in determining which odors to use and which to avoid.

Based on the OR genes described previously, it is reasonable to assume that cattle can detect a variety of fatty, sour, hay-like, sweet, rancid, and spicy odors (Lee et al., 2013). The limited body of research available on the topic shows that cattle can discriminate between complex odors such as coffee and orange juice (Rørvang et al., 2017). Cattle have also been suggested to recognize both the emotional states of conspecifics and humans through olfactory cues (Baldwin, 1977; Destrez et al., 2021). Additionally, using their vomeronasal organ, cattle can detect pheromones that indicate the reproductive or stress state of their conspecifics (Terlouw et al., 1998). Recent studies on the bovine appeasing pheromone (**BAP**) have uncovered beneficial effects on milk yield, as well as health and welfare parameters, in both dairy and beef cattle. One study observed a significant increase in milk yield during an environmental transition from indoor to outdoor housing of dairy cows treated with synthetic BAP (Osella et al., 2018), and in beef calves use of synthetic BAP during weaning and transport reduced stress indicators, including lower cortisol levels, lower haptoglobin levels, and improved feed efficiency and growth (Colombo et al., 2020; Cooke et al., 2020). Dairy calf welfare (measured as increase in heart rate variability, live weight gain, and resting time after weaning) also improved in groups treated with a synthetic BAP analog (Garcia-Alvarez et al., 2025). Studies on natural odors of nonsocial origin are, however, surprisingly scarce, and there is thus a need for more studies to map cattle olfactory abilities, as well as behavioral reactions and emotional responses to odors.

Measuring ear positions is a relatively new but promising and noninvasive method to assess affect and emotional valence in farm animals. Previous studies have made various attempts to categorize different ear positions and movements during emotional treatments or routine activities, for example by mapping ear positions according to the arousal-valence model (de Oliveira and Keeling, 2018). A relaxed ear position has been suggested to indicate positive low-arousal states in both dairy cows (Proctor and Carder, 2014) and sheep (Reefmann et al., 2009), whereas negative states have been characterized by an increase in ear movements and, in sheep, a higher proportion of forward and asymmetric ear positions (Reefmann et al., 2009; Boissy et al., 2011) possibly mediated by attention (Vögeli et al., 2015). Backward ear positions have, however, been observed both when cows were experiencing pain (Gleerup et al., 2015) and when gently stroked, which has been suggested to induce positive affective states of low arousal (Schmied et al., 2008; Proctor and Carder, 2014). The relationship between specific ear positions and affective states in cattle is still underexplored, and the patterns are still not fully understood, but they appear to follow similar patterns as for sheep (Lambert and Carder, 2019). If confirmed, ear positions during olfactory exploration could provide further insights into the emotional response of cattle exposed to specific odors.

The aim of this study was to investigate whether cows can detect and discriminate 4 odors of natural, nonsocial origin and if any of the specific odors evoked more interest than others. The 4 odors selected for the study (peppermint, lavender, cedarwood, and orange) are within the spectrum of what cattle can detect based on the genetic potential (Lee et al., 2013), and thus we hypothesized that cows would be able to detect these odors. The study further aimed to assess if age, parity, or breed affected this. In addition, the study aimed to investigate olfactory-exploration behavior of dairy cattle and ear positions to elucidate if certain behaviors and ear positions were more prevalent in response to certain odors.

MATERIALS AND METHODS

Ethical Considerations

The experiment was carried out at the research facilities for dairy cattle at the Swedish Livestock Research Centre at the Swedish University of Agricultural Sciences, Uppsala, Sweden. All procedures were conducted in accordance with the research center's ethical permit for the use of animals in education, issued and approved by the Swedish Board of Agriculture's Uppsala Ethics Committee on Animal Research (ethics approval number Dnr. 5.8.18–12184/2023), in compliance with EC Directive 86/609/EEC on animal studies. The procedure further met the ARRIVE guidelines (Kilkenny et al., 2010) and the ethical guidelines proposed by the Ethical Committee of the International Society of Applied Ethology (ISAE; Tahamtani et al., 2023).

Animals and Experimental Conditions

The experiment was carried out over a period of 9 d in April 2024. The building in which the experiment was done was the cows' home environment. In this barn, normal daily activities were carried out and no changes were made to the normal work schedule during the experiment to keep a familiar work flow for the personnel and animals involved. The air inside the barn was electronically monitored and ventilated via the building's ventilation system (system based on temperature adjustments; UBA ventilation, Malmö, Sweden, adjusted by DeLaval, Tumba, Sweden). The average temperature in the barn was 14°C (mean ± SD = 14.5°C ± 3.5°C), and the average airflow was 0.2 m^3/s (mean ± SD = 0.21 ± 0.06 m/s). Humidity was not possible to monitor inside the barn.

Inside the barn, cows and heifers were divided into 5 subsections of voluntary milking system (**VMS**) groups. One of these subgroups was chosen as the experimental group (Figure 1). The group had access to 30 cubicles with bedded packs. The cubicles were situated in the middle of the group, and on either side, the cows had access to 14 feed bins (CRFI, BioControl, Ås, Norway) where they were provided a roughage mix consisting of grass-clover and corn silage ad libitum. Fresh roughage was delivered 4 times per day (Distribution wagon FS1600, DeLaval International AB, Tumba, Sweden). The cows also had access to 2 transponder-activated concentrate feeders where they were provided individual rations of commercially pelleted concentrates (Komplett Norm 180, Konkret Mega 28; Lantmännen Lantbruk & Maskin, Malmö, Sweden). The slatted aisles between the cubicles and the feed bins were floored with concrete slats, from where cows had access to 2 automatic water troughs (175 × 40 × 20 cm, water flow: 10–12 L/min), and one mechanical brush in the slatted aisle (Figure 1). On the feed bin row, 5 headlocks were located in the middle of the metal fixture (Figures 1 and 2). This area was used as the experimental area.

Before the experiment, 2 cows were enrolled in a pilot test to train the experimenter in the testing regime and to test the experimental design. During the pilot test, it was determined at what distance the odor boxes would be presented to allow the cows to sniff but also to move away and avoid sniffing while locked in the headlocks. Headlocks were used to ensure the cows were all presented with the odors at the same distance and in a controlled manner. The 2 pilot cows were excluded from the experimental trials, and the experimental group thus consisted of 28 cows, 16 Swedish Holstein (**SH**) and 12 Swedish Red (**SR**). The sample size was based on previous studies using the same test paradigm on large mammals (e.g., Hothersall et al., 2010; Rørvang et al., 2017, 2022). At the time of testing, the cows were on average 51.6 mo of age (range: 39.7–74.9 mo, average per breed SH: 55 mo, SR: 44 mo), and none of the cows were pregnant during testing. One cow was in their fifth parity, 7 were in their fourth parity, 8 in their third parity, and 12 in their second parity (for more information about all cows see Supplemental Table S1; see Notes). The age range, parity spread, and breeds were based on the availability at the farm. The health status of the cows was monitored via the farm's health surveillance system (Delpro Farm Manager 10.2, DeLaval, Tumba, Sweden) and no health issues were detected before, during, or after the experiment. The overall activity of the cows was monitored by a real-time location system (DeLaval Plus Behavior Analysis, DeLaval International AB, Tumba, Sweden) automatically collecting the position data of the cows. Tags in the cows' left ear sent ultra-wideband (**UWB**) signals to receivers that were installed in the ceiling throughout the barn. The UWB sensors worked with a 2.2-s fixed rate and automatically aggregated positional data at a 24-h level for time spent in predefined areas including walking alley, cubicle, feed table, and milking area (Figure 1). Positioning data were downloaded directly from the local DelPro server at the Swedish Livestock Research Centre from 3 d before to 3 d after testing. No marked changes in activity pattern were found between the days before testing, test day, and the days after testing apart from a small increase in time spent at the feed table on the first day post-testing (Table 1).

Table 1. Overview of the positioning data from the barn system[1]

Day	Feeding	Walking	Cubicle	Milking
Pretest 3	14.7	23.2	52.0	8.3
Pretest 2	14.2	25.9	49.2	8.2
Pretest 1	13.7	27.0	47.6	9.6
Test day	14.1	26.8	49.1	8.1
Post-test 1	18.1	24.7	46.9	8.4
Post-test 2	13.9	27.0	48.5	8.2
Post-test 3	11.5	22.5	43.0	6.4

[1]The positions of the cows were tracked by ear-mounted tags, and the data are presented as percentage of time (per 24 h) spent in the feeding, walking, cubicle, and milking areas of the barn. In addition to these areas, the categories "farm-specific area" and "out of reach" were also included but not reported in the table. Pretest x = x number of days before being tested, test day = the day the cow was tested, and post-test x = x days after the cow was tested.

Before testing, the cows were divided into pairs by the experimenter based on balancing of breed and parity (in prioritized order) across the treatments of the experiment. Pair testing was chosen over individual testing to avoid habituation to social isolation, which can be stressful for the cows and time consuming. All cows within the group were familiar with one another and had been in the current group for on average 11 d (range: 2–12 d) before testing. All cows were also familiar with the experimenter. All animals were used to the headlocks situated in their home environment. Before the testing, all animals were tested on a habituation criterion: the cow was moved to the headlock and stayed in the headlock without showing any fear-related behavior such as freezing, backing, flight reactions (e.g., head tossing), or excessive vocalizations (e.g., constant vocalizing for the 5 min), for 5 min. During this period, the experimenter would squat down in the stable aisle 1 m away from the headlocks (i.e., in front of the cows). Animals who did not

Table 2. Ethogram of the recorded behaviors[1]

Behavior	Description
Sniffing	The cow's muzzle being close to (i.e., less than the length of a cow muzzle, approximately 12 cm) or in direct contact with the odor box.[2]
Licking/biting	Muzzle of the cow is in direct contact with the odor bucket, with open mouth and teeth touching the bucket at least once, or muzzle of the cow is less than the length of a muzzle away from the odor bucket, with tongue protruding and touching the odor bucket at least once. See Supplemental Video S1 (see Notes).
Flehmen[2]	The cow curls the upper lip backward and inhales simultaneously in both mouth and nose. Head may be elevated and neck may be extended.
Head movement	The cow moves its head upward in a sudden movement that may include hitting the headlock. No steps are taken. See Supplemental Video S1.
Backing	The cow lifts its head and takes at least one step backward.
Snorting[3]	Short powerful exhalation(s) from the nostrils.

[1]All behaviors were recorded as durations in seconds using continuous behavior sampling during all 1-min odor exposures.

[2]Adapted from Rørvang et al. (2022).

[3]Adapted from Christensen et al. (2005).

fulfill the habituation criterion were excluded; however, no cows were excluded because all animals successfully fulfilled the habituation criterion.

The Habituation-Dishabituation Test

In the experiment, a habituation-dishabituation test paradigm was used based on previous methods reported in Rørvang et al. (2017) and Rørvang et al. (2022). Because this test paradigm relies on the animals' curiosity of, and motivation to, explore odors (Yang and Crawley, 2009) it is crucial to the functionality of the test that animals are motivated to explore. To avoid having unmotivated animals as a result of sniffing an odorless or neutral control, an odorless control was excluded in this experimental design as has been suggested previously (Rørvang et al., 2017, 2022). Cows' interest in the odors was assessed by summing the total sniffing duration of an odor as was previously done for rodents (Coronas-Samano et al., 2016), horses (Rørvang et al., 2022), and pigs (Rørvang et al., 2023). The definition of sniffing is specified in the ethogram, Table 2.

Odor Boxes

The choice of experimental odors was based on a similar study conducted on horses (Rørvang et al., 2022) to enable comparison of both species' reactions to the same odors. The 4 odors were essential oils approved for human use: orange oil (*Citrus aurantium*), peppermint oil (*Mentha arvensis*), cedarwood oil (*Cedrus atlantica*), and lavender oil (*Lavandula angustifolia*), all obtained from Fischer Pure Nature (Fredensborg, Denmark). For more information about the odors, contents, and origin, see Supplemental Table S2 (see Notes). Using commercially available natural odors ensures that the substances are safe, accessible and easy to standardize (using a standard single-use 3.5-mL pipette to apply standardized amounts of drops). The 4 experimental odors were further assumed to be novel to the cows because none of these substances were found in their feed, hay, bedding, or as ingredients in any products used on the farm (e.g., lotion or soap) and were of 100% natural and nonsocial origin. The odors were all complex odors, meaning that they were composed of many different odorant components. During the tests, the odor samples were presented to the cows in plastic containers, from here on referred to as odor boxes. Fresh odor boxes were prepared in the morning before each test, and odors (50% of original amount) were re-applied every hour during testing to keep the odor intensity at a comparable level for all test animals. Odor boxes (including re-application of odor and cleaning of equipment) were prepared in a separate, closed-off room approximately 100 m away from the cows, and shut off from the cattle barn airflow. Six drops of odor (~0.20 mL, using the standard plastic single-use pipette) were applied to a filter paper (unbleached, light brown filter paper, model 7.607, Harald Nyborg, Viborg, Denmark) and placed in the plastic container (Figure 3). The filter paper absorbed and dispersed the odor and ensured that all odor boxes were visually similar. The container was a plastic (polypropylene, BPA free) 28 × 19 × 14 cm box (4.5 L), with matching lid (Orplast, Marynarki Polskiej 73a, 80–557 Gdánsk, Poland; Figure 3).

Odor boxes were prepared at the same ambient temperature as in the barn in which the tests were done (see "Animals and Experimental Conditions" section), and all odor boxes had a matching plastic lid to keep the odor contained between presentations. The odor box also contained a ballast weight (concrete pave stone: 20.5 × 13.7 × 5 cm, weighing 270 g; Benders AB, Kvänum, Sweden) to prevent the cows from tossing the box (Figure 3). The odor box was covered by a grid made from duct tape (50 mm wide; Bruksbo, Hörnefors, Sweden). The grid pre-

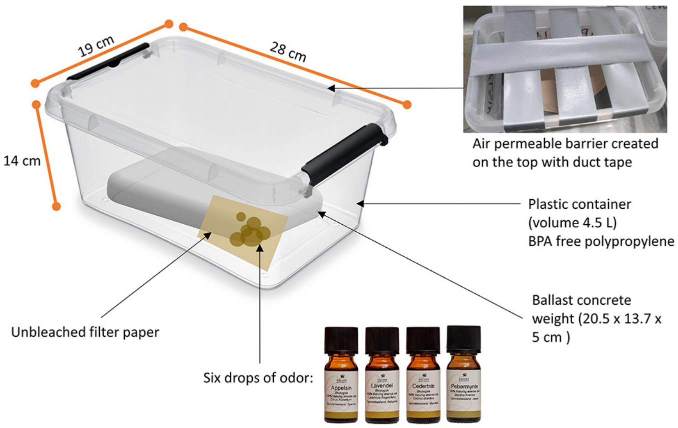

Figure 3. Odor samples were presented in a plastic (polypropylene) container (4.5 L capacity) by adding 6 drops of odor (oil-based essential oil) using a standard pipette, to an unbleached filter paper (brown square). The odor box was kept sturdy by adding a 20.5 × 13.7 × 5 cm ballast weight of concrete to the box (gray pave stone). The total weight of an entire odor box was 3.3 kg. The top of the odor box was fitted with a duct tape grid preventing the cows from eating or licking the odors (to avoid mixing taste and smell) while still allowing odors to disperse from the container.

Table 3. Odor presentation order with first, second, third, and fourth odor given as O = orange, P = peppermint, C = cedarwood, and L = lavender[1]

First	Second	Third	Fourth	N
O1 O2 O3	P1 P2 P3	L1 L2 LE	CI C2 C3	1
		C1 C2 C3	L1 L2 L3	2
	C1 C2 C3	L1 L2 L3	P1 P2 P3	1
		P1 P2 P3	L1 L2 L3	1
	L1 L2 L3	P1 P2 P3	C1 C2 C3	1
		C1 C2 C3	P1 P2 P3	1
P1 P2 P3	O1 O2 O3	C1 C2 C3	L1 L2 L3	1
		L1 L2 L3	C1 C2 C3	1
	C1 C2 C3	O1 O2 O3	L1 L2 L3	2
		L1 L2 L3	O1 O2 O3	1
	L1 L2 L3	O1 O2 O3	C1 C2 C3	1
		C1 C2 C3	O1 O2 O3	2
L1 L2 L3	P1 P2 P3	O1 O2 O3	C1 C2 C3	1
		C1 C2 C3	O1 O2 O3	1
	O1 O2 O3	P1 P2 P3	C1 C2 C3	1
		C1 C2 C3	P1 P2 P3	1
	C1 C2 C3	O1 O2 O3	P1 P2 P3	1
		P1 P2 P3	O1 O2 O3	1
C1 C2 C3	P1 P2 P3	O1 O2 O3	L1 L2 L3	1
		L1 L2 L3	O1 O2 O3	1
	O1 O2 O3	P1 P2 P3	L1 L2 L3	1
		L1 L2 L3	P1 P2 P3	1
	L1 L2 L3	O1 O2 O3	P1 P2 P3	2
		P1 P2 P3	O1 O2 O3	1

[1]Each odor was presented three times in a row, represented as 1–3 in the table. Each presentation order sample size is represented as n in the last column (N). For information on the treatment distribution across the individual cows, refer to Supplemental Table S1 (see Notes).

vented the cows from eating or licking the odor sample during testing (Figure 3), and the 6 openings allowed the cows to sniff the odors using both nostrils. The flat grid ensured that the box could be closed with the plastic lid in between odor presentations to limit odor contamination of the surrounding air. A specific odor box, including a matching plastic lid and ballast weight, were used for each of the 4 experimental odors to prevent any cross-contamination. At the end of a test day, the filter paper and tape grid was removed and discarded, and all other equipment (plastic container, lid and ballast weight) were cleaned with warm water and odorless soap in the preparation room. Once clean, the materials were left to dry for at least 12 h before the next test day. During all odor preparations the person handling the equipment wore latex gloves to avoid odor contamination.

Test Procedures

Before testing, a balanced odor presentation order was determined (Table 3), to ensure all possible odor order combinations were tested. Each cow was assigned to a distinct odor order randomly before the experimenter arrived at the barn (using a random number generator for each odor combination). The cows were tested in pairs but with individual odor orders, hence the experimental unit was the individual cow.

When inside the barn, the experimenter located the cow pair to be tested, and manually moved them to the headlocks (Figures 2 and 4). The staff at the farm (2 people who were familiar with the cows) assisted the experimenter in moving the cows by use of negative reinforcement (applying pressure by moving toward the cow or placing a hand on the cow's hind or side and then releasing the pressure (reducing distance or removing the hand) when the desired movement was elicited), which was standard practice for moving cows at the farm (e.g., when moving cows to be milked). Once in the headlocks the cows were rewarded with feed to ensure reinforcement of being in the headlock. The other cows in the group (not being tested) were free to move during testing and were kept at least 2.5 m (corresponding to the length of a cow) away from the odor sample.

In the test situation, all odor boxes were moved from the preparation room to the experimental area, and the 2 odor boxes to be tested had the lids removed. For each odor presentation, the 2 open odor boxes were placed in front of each cows' individual headlock, 40 cm from the fixture (Figure 4), in the same manner as during habituation. Each odor was presented 3 times in a row for a duration of 1 min each, with an inter-trial interval of 2 min. After 3 presentations and the final removal of one odor, the cow again had a 2 min interval without odor (Wesson et al., 2008) before being presented with the next odor. During all intervals, the experimenter removed the odor boxes from the ex-

Table 4. Ethogram of ear positions observed during sniffing and direct contact with odor box, adapted from de Oliveira and Keeling (2018)[1]

Ear position	Description
Axial	Ears straight out to the side, perpendicular to the head-rump axis.
Forward	Ears are directed forward, with the tip of the ear at an angle of more than 30% from the perpendicular.
Backward	Ears are directed backward, with the tip of the ear at an angle of more than 30% from the perpendicular.
Asymmetric left	Ears are oriented in opposite directions; left ear is backward and right ear is axial or forward.
Asymmetric right	Ears are oriented in opposite directions; right ear is backward and left ear is axial or forward.

[1]Ear positions were recorded as durations in seconds using continuous behavior sampling during all 1-min odor exposures.

perimental area and covered them with the lids to limit intertrial contamination. After placing an odor box, the experimenter moved one meter away from the headlock, still positioned in the experimental area (in the aisle 1 m directly in front of the cows being tested) in a squat position as during habituation. The same odor boxes with corresponding grid were used for both cows in the pair. The same experimenter prepared the odor boxes, and performed the tests for all cows throughout the experiment. The experimenter was not naive to the odors or the odor presentation order (because the person could smell the odors used, and needed to keep track of the odor orders and equipment), or blind to the cows' age, or parity. One full test of 12 odor presentations (3 presentations × 4 odors) lasted 35 min, and all cows were immediately released from the headlocks after the test concluded. To limit potential odor contamination in the barn, a maximum of 10 cows (i.e., 5 pairs) were tested on the same test day.

Observations

Two stopwatches (model KH-061, art: 31–2468; Clas Ohlson, UK) were used during the experiment; one to time the duration of each odor presentation trial (1 min) and the other to time the intertrials interval (2 min). Two GoPro Hero 7 cameras (GoPro Inc., San Mateo, CA) recorded the behavior of each cow during the full test. The main response variable from the test was sniffing duration. Sniffing behavior was recorded using continuous behavior sampling (Bateson and Martin, 2021) during all odor presentations using the video footage recorded during the test. Habituation to an odor was defined as a significant decrease in sniffing duration per presentation, measured over the 3 consecutive presentations of the same odor. Dishabituation was defined by reinstatement of sniffing when a new odor box was presented. This was done by an experienced observer not naive to the cows' age and parity, but blind to the specific odor and odor presentation order being tested. Licking and biting when in contact with the odor box (i.e., while sniffing) as well as flehmen, head movement, backing and snorting during the odor presentation (Table 2) were recorded as durations separately but alongside the recording of sniffing behavior. This was done by the same observer using behavior sampling (Bateson and Martin, 2021). Olfactory-exploration behavior has in humans been linked to olfactory interest (Han et al., 2022), and thus duration of sniffing and occurrence of licking and biting was used as an indicator of cows' interest in the odors. Behaviors backing and snorting were used as indicators of avoidance toward the odors. Ear positions were recorded during sniffing as well as direct contact with the odor box (i.e., licking/biting; Table 4).

Statistical Analyses

All statistical analyses were performed in the online software R, version 4.2.2 (R Core Team, 2022), using the interface RStudio version 2022.12.0 + 353 (RStudio Team, 2023). We used 5% as the significance level, and 10% as the level for tendencies.

Sniffing Behavior. The data consisted of 12 repeated measures for each cow; 3 presentations per odor, of a total of 4 odors (Table 3). The total data set thus comprised 336 odor presentations for analyses. Before modeling, parity and age were investigated in a correlation analysis and were found to be significantly correlated ($P < 0.001$, r = 0.98). Hence, only age was used in the analyses. Sniffing duration data were right-skewed due to a large number of zeros and were thus analyzed using methods appropriate for this type of data. As all cows were tested in pairs but on individual odor orders, and the experimental unit was the individual cow, but potential effects of being tested in a pair were accounted for by adding the cow pair as a random effect in the model.

A gamma generalized linear mixed-effect model with log-link was fitted to the data using R-package lme4 (Bates et al., 2015) to investigate sniffing duration. Sniffing duration data had a small constant added before modeling (0.01). The full model included fixed effects of presentation (categorical variable with 3 levels: first, second, third), odor (categorical variable with 4 levels: orange, peppermint, lavender, cedarwood), the interaction between presentation and odor, breed (categorical variable with 2 levels: SH or SR, see definitions on the

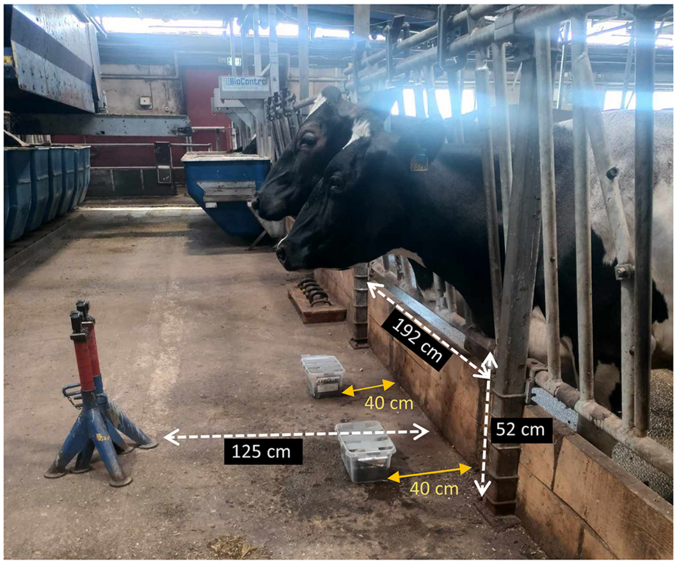

Figure 4. Illustration of the experimental setup during testing. The 2 cows to be tested were positioned into each of their headlocks. The cameras were placed on a tripod (red/blue tripod) 125 cm away from the headlock, each camera filming one of the 2 cows. The odor box was placed 40 cm away from the base of the headlock (illustrated by the yellow arrows) to allow the cows easy access while still offering them the opportunity to move away from the odor.

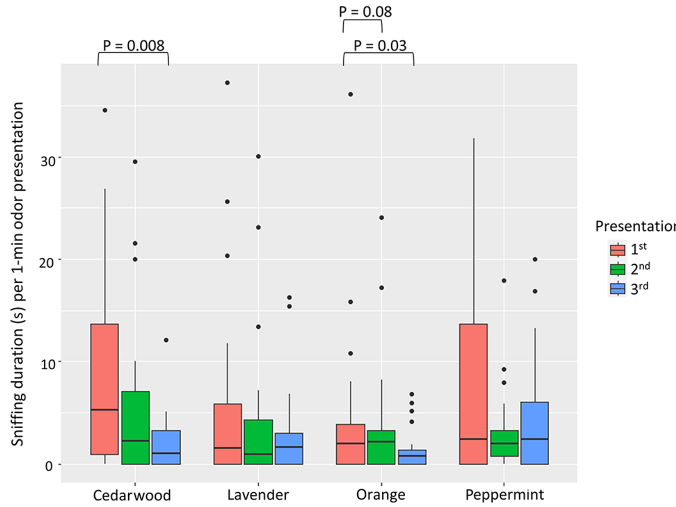

Figure 5. Sniffing duration (seconds) per 1-min odor presentation (raw data without transformation), with first presentations in red, second in green, and third in blue. The sniffing durations (y-axis) are shown for each odor (on the x-axis) separately: cedarwood, lavender, orange, and peppermint. The boxes represent the 25% and 75% quartiles, the horizontal lines in the boxes are the medians, and the error bars illustrate the maximum and minimum within the sample, with outliers shown as black dots. The classification of outliers in the plot was based on the interquartile range rule.

breeds in the "Animals and Experimental Conditions" section), and age (numerical variable: mean ± SD: 51.6 ± 17 mo of age), and random effect of cow ID (1–28) to account for repeated measures (i.e., 12 presentations) on each cow and of cow pair (categorical variable with 14 levels, pairs: A–N) to account for possible effects of social transmission. Stepwise reduction was applied in the model fitting stages (with $P > 0.1$ as the threshold), and the reduced model was checked against the full model in a likelihood-ratio test. During these steps breed was excluded from the final model. The final model thus included the fixed effect of presentation, odor, and their interaction, age, and the random effect of cow ID and cow pair. Residuals were evaluated visually in quantile-quantile (**QQ**)-plots, overdispersion was checked and actual versus predicted values were visually assessed. To investigate if habituation occurred over the 3 repeated presentations of the same odor (2 comparisons per odor per cow: n = 224; Table 1), contrasts were performed using the contrasting function in package emmeans (Lenth, 2021) within each odor. To investigate if dishabituation occurred between the third presentation of one odor and the first presentation of a new odor (3 comparisons per cow: n = 84; Table 3), pairwise comparisons of third and first presentations were compared using the same contrasting function. To analyze whether specific odors evoked more sniffing behavior, contrasts were used on the same model (above) to compare the total sniffing duration of each odor.

Other Odor Exploration Behavior. Other odor exploratory behavior was recorded over all odor presentation, hence data also consisted of 12 repeated measures for each experimental unit; 3 presentations per odor, of a total of 4 odors (Table 3) resulting in 336 odor presentations for analyses. The behavior categories flehmen and snorting (Table 2) were not observed and were thus not analyzed. The durations of the other behavior categories (licking/biting, head movement, and backing) were low and included a large number of zeros. For the durations of licking and biting, the same model as for sniffing was fitted to the data. A small constant (0.01) was added before modeling. The full model included fixed effects of presentation, odor, the interaction between presentation and odor, breed, and age, and random effect of cow ID to account for repeated measures on each cow and of cow pair to account for possible effects of social transmission. Stepwise reduction was applied as previously described, and the reduced model was checked against the full model in a likelihood-ratio test. During these steps breed was excluded from the final model. The final model thus included the fixed effect of presentation, odor, and their interaction, age, and the random effect of cow ID and cow pair. Residuals were evaluated visually in QQ-plots, overdispersion was checked, and actual versus predicted values were visually assessed.

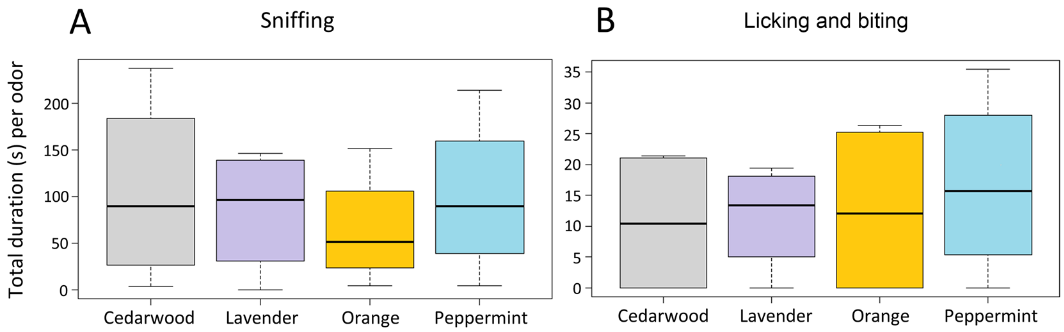

Figure 6. Total duration (seconds) of (A) sniffing and (B) licking and biting behavior per odor. Odors are specified on the *x*-axis and shown in colors: gray = cedarwood, purple = lavender, orange = orange, light blue = peppermint. The bars represent the 25% and 75% quartiles, the horizontal midline represents the median, and the error bars the range within the sample.

Data for head movement and backing contained even more zeros and short durations, and hence these data were converged to a binomial response variable before modeling (0 = not expressing the behavior, 1 = expressing the behavior). Two binomial generalized linear mixed effects models with logit-link were fitted to the data (i.e., one model for head movement and backing, respectively) using package lme4 (Bates et al., 2015). The models both included the fixed effects of odor, breed, and age, and random effect of cow ID to account for repeated measures (i.e., 4 first presentations) on each cow and of cow pair. The models were checked visually using scaled Pearson residuals and deviance residuals. Stepwise reduction was applied as above, and the reduced model was checked against the full model in a likelihood-ratio test, and during these steps breed was excluded from both models. Models were checked using the same methods as previously described.

For the analysis of ear positions only first presentations were used and hence data included 112 first presentations of odors (n = 4 each for all 28 cows). Data for ear positions also contained a large number of zeros, and all durations were low, hence data for each ear position were also converged to a binomial response variable before modeling (0 = not showing ear position, 1 = showing ear position). Five binomial generalized linear mixed effects models with logit-link were fitted to the data (i.e., one model for each ear position variable: axial, forward, backward, asymmetric left, and asymmetric right, separately) using package lme4 (Bates et al., 2015) in the same manner as for head movements and backing. The models all included the same fixed effects as for head movements and backing and additionally the fixed effect of side (left/right), meaning the side to which the cow was placed in the pair. The models were checked visually using scaled Pearson residuals and deviance residuals.

RESULTS

Overall, 24 cows (21% of total 112 first presentations of any odor) showed no interest in the test situation (i.e., did not approach the odor box) when presented with cedarwood for the first time (n = 6), lavender for the first time (n = 10), orange for the first time (n = 4), and peppermint for the first time (n = 4). The number of cows not investigating the odor boxes during second and third presentations (of the same odor) increased, in line with what is expected during habituation, but generally the majority of cows explored the odor box upon the first presentations of their odors.

The sniffing durations for cows presented with each odor (cedarwood, lavender, orange, and peppermint) over all presentations (first, second, third) are presented in Figure 5, and illustrate a relatively large individual variation indicated by the error bars (i.e., range in the sample) and outliers (black dots). The analyses showed that within each odor, only first-to-third presentation of cedar wood and first-to-second and first-to-third presentation of orange differed significantly (all P-values <0.05; Figure 5).

The model fitting further showed that there was a significant effect of age on sniffing duration with younger cows sniffing longer than older cows (estimate ± SE = -0.03 ± 0.009, t-value = -2.9, $P = 0.004$), and that specific odor did not significantly affect sniffing (all $P > 0.1$; Figure 6A).

Considering dishabituation, the comparison between third and subsequent first presentations of a new odor showed that the cows only displayed a significant increase in sniffing when orange odor (as habituation stimulus) was replaced for cedarwood, lavender, and peppermint, respectively (as dishabituation stimulus; Table 5).

With respect to the other behavior categories, cows expressed most licking and biting (numerically) with peppermint (Figure 6B), but this was only a tendency found between lavender and peppermint (GLMM [post hoc pairwise comparisons]: estimate ± SE = −0.7 ± 0.3, z-ratio = −2.5; P = 0.08), and age did not significantly affect the expression of the behavior either (GLMM Wald z-test, age: estimate ± SE = −0.01 ± 0.03, z-value = −0.3; P = 0.7).

The data on ear positions showed a large individual variation among the cows (Figure 7). For axial ear positions, no significant effect of odors (Figure 7) or breed was found (GLMM: all P-values >0.1), but a tendency for younger cows to express axial ear positions for longer was found (GLMM Wald z-test: z-value = −1.8; P = 0.07). Specific odor did not significantly affect forward, asymmetric left, or right, ear positions (Figure 7), and neither did age or breed (GLMM Wald z-tests: all P-values >0.1). For backward ear positions, orange numerically elicited shorter total durations than all other odors (Figure 7) but no significant difference was found comparing the odors for this ear position. Although age did not affect the expression of backward ear position, there was a tendency for SH cows to express more of this ear position compared with SR cows (GLMM Wald z-test: SH vs. SR: z-value = −2.0; P = 0.05). The side to which the cow was placed in the pair when tested significantly affected the asymmetric right and left ear positions. The probability of expressing asymmetric left was significantly higher if the cow was placed to the right (GLMM Wald z-test: left vs. right: z-value = 2.3; P = 0.02), and the probability of expressing asymmetric right was significantly higher if the cow was placed to the left (GLMM model Wald z-test: left vs. right: z-value = −2.5; P = 0.01).

DISCUSSION

This study investigated whether cows were able to detect and discriminate 4 odors of natural, nonsocial origin. The study further investigated whether any of the odors evoked more interest than others and if age, parity, or breed affected this. As a secondary aim, the study explored olfactory-exploration behavior of dairy cattle (behavior other than sniffing) and ear positions during odor exploration to elucidate if certain behaviors and ear positions were restricted to certain odors. Although the results showed a numerical decrease in sniffing time over repeated presentations of the same odor, only first-to-third presentation of cedar wood and first-to-second and first-to-third presentation of orange differed significantly. Hence, it remains unclear if cows were able to detect all odors. The results comparing the dishabituation trials were also unclear with respect to whether cows could discriminate between all odors. Cows numerically sniffed orange the least and cedarwood the most, but this was not reflected in the results because specific odors did not significantly differ in total sniffing duration. Breed did not influence sniffing but a tendency for age to affect sniffing was found, with younger cows sniffing all odors longer than older cows. Cows expressed more licking and biting behavior when exploring peppermint but this difference was only a tendency. Head movements and backing behavior did not differ significantly among the 4 odors and age and breed did not affect the expression either. Expression of axial ear positions was not affected by odor or breed but a tendency for younger cows to express axial ear positions for longer was found. Odor, age, and breed did not affect forward or asymmetric ear positions, but the side to which the cow was placed in the test pair significantly affected asymmetric left and right ear positions. For backward ear positions, breed had a significant effect on the expression, with SH cows having higher probability to express this ear position during odor exploration.

Cows' Ability to Detect and Discriminate Odors and Future Adaptations of the Testing Regimen

To investigate whether cows were able to detect the odors, a habituation-dishabituation test was conducted, using odors that cows, based on their genetic capability (Lee et al., 2013), should be able to detect and discriminate between. Although the results showed a numerical decrease in sniffing time over repeated presentations of the same odor, only first-to-third presentation of cedarwood and first-to-second and first-to-third presentation of orange differed significantly. According to the definition of habituation, this implies that not all cows significantly habituated across repeated presentations, and it is thus unclear if all cows could detect all 4 odors apart from cedarwood and orange. This could imply that there may be a discrepancy between cattle's genetic capability (Lee et al., 2013) and actual ability. The lack of a significant decrease in sniffing time does not align with previous studies (Rørvang et al., 2022, 2023). It is possible that the design of the current test could have affected these results. Cows were tested in pairs with relatively short distance between the 2. Within the cow pair to be tested, the odors presented were never the same for both cows at the same time, posing a risk of a cow being able to sniff an odor before being tested on this odor. Additionally, because the odor boxes were reused during testing it presented a risk of contamination with individual body odors (contained in saliva and nasal fluid). Although all cows were familiar with one another, and thus also to the body odors of their companions, it is possible that body odors of other cows could have influenced the results. On a test day, the odor sample (filter paper) and the grid was changed during breaks between tests (cow pairs) and if the equipment got wet from nasal mucus or saliva, hence this contributed to lowering the risk of contamination.

Table 5. Overview of the results of the analysis of dishabituation trials from the third presentation of an odor to first presentation of a new odor[1]

Dishabituation trial	Estimate ± SE	z-ratio	P-value	n
Cedarwood third to lavender first	−1.08 ± 0.45	−2.42	0.24	12
Cedarwood third to orange first	−0.71 ± 0.45	−1.58	1.00	12
Cedarwood third to peppermint first	−1.20 ± 0.5	−2.68	0.12	12
Lavender third to cedarwood first	−1.09 ± 0.45	−2.45	0.23	12
Lavender third to orange first	−0.46 ± 0.45	−1.02	1.00	12
Lavender third to peppermint first	−0.95 ± 0.45	−2.13	0.54	12
Orange third to cedarwood first	−1.77 ± 0.45	−3.97	0.0011**	12
Orange third to lavender first	−1.51 ± 0.45	−3.38	0.012*	12
Orange third to peppermint first	−1.63 ± 0.45	−3.65	0.0042**	12
Peppermint third to cedarwood first	−0.52 ± 0.45	−1.17	1.00	12
Peppermint third to lavender first	−0.27 ± 0.45	−0.60	1.00	12
Peppermint third to orange first	0.11 ± 0.45	0.25	1.00	12

[1]The specific dishabituation trials are given along with estimate ± SE, z-ratio, P-value, and sample size (n). Level of significance is given along with the P-values.

$P > 0.1$ = nonsignificant, $P \leq 0.09$ = tendencies; *$P \leq 0.05$; **$P \leq 0.01$.

In addition, all cows had been present during several habituation sessions, and were thus not particularly interested in the tests when their herd mates were tested. It is, however, not possible to rule out that the not-tested animals could have been able to detect an odor while not being tested because it is currently unknown what detection thresholds cows have for the odors. The distance from the odor sample (>5 m away) and the oil-based odors nevertheless limit this risk. Collectively, these aspects could have contributed to the slightly lower sniffing durations in this study and hence the overall results. The sniffing durations reported in this study are overall lower than those reported for horses using the same 4 odors (Rørvang et al., 2022) and for pigs (Rørvang et al., 2023), but comparable to sniffing durations reported on dairy cows using different odors: orange juice and coffee (Rørvang et al., 2017). Hence, pair testing with short distance between the test subjects could have resulted in lower sniffing durations. However, the finding that time spent sniffing each odor did not increase across presentations and decreased between first and both second and third is an indication that habituation to the different odors might have occurred.

A significant increase in the time spent sniffing the dishabituation odors was observed only during certain dishabituation trials. Therefore, it cannot be concluded that all individuals were able to distinguish between the 4 odors. In previous studies using the same test paradigm, researchers found that dairy cows (Rørvang et al., 2017), horses (Hothersall et al., 2010; Rørvang et al., 2022; Jardat et al., 2023), and pigs (Mendl et al., 2002; Rørvang et al., 2023) were able to distinguish between different odors. However, in this study the pair testing may (as mentioned previously) be the cause of why a significant reinstatement of sniffing was not seen between the third and subsequent first presentations. If cows could smell the odor before being tested, their first presentation of the odor in the test is not equal to a first exposure, which is likely to have resulted in lower sniffing duration. For social animals, social testing is preferred to limit stress from isolation, which was the main reason for the pair testing in this study. Social testing could, however, have been done with one experimental and one nonexperimental animal to ensure no pre-exposure of animals to their odors. In this case, such a test was not possible due to a limited number of experimental animals, but future studies could introduce nonexperimental companions to avoid social isolation and pre-exposure to odors. Another option is to habituate the experimental animals to temporal separation, which has been done in several species to allow for individual testing, for example, horses (Christensen et al., 2005) and calves (Michalski et al., 2023). The effect of the close proximity of the test animals was also visible in the results on asymmetric ear positions. The significant effect found of the side to which the cow was placed in the test pair on asymmetric left and right ear positions highlights that the cows affected one another. A cow placed on the right side in a test pair had significantly higher probability of expressing asymmetric left ear position (and vice versa for a cow placed to the right), which could be explained by a risk of having ears touching the head of the other test cow. It is therefore also important to consider the distance between test subject and companion animal, in relation to the behavior observed (other than sniffing), when testing animals in a social setting.

Another possible explanation for why a reinstatement of sniffing did not occur in dishabituation trials is that the cows sniffed the new odor less because they were unmotivated, either as a result of pre-exposure or loss of interest after being exposed to several other odors. The risk of unmotivated animals has been raised previously in other studies in dairy cows (Rørvang et al., 2017) and humans (Cometto-Muñiz and Cain, 1995) and is something to be aware of when using repeated testing in the habituation-dishabituation paradigm. A possible adaptation of the test to prevent this could be to alternate or mix the odors more to create more curiosity. This could be done for instance by foregoing the 3 repetitions of each odor and instead adopting a scheme of mixing odor presentations. We thus encourage future studies to try different versions of the test, mixing various dishabituation trials to investigate whether this may circumvent the risk of having unmotivated animals. Future studies could also focus on optimizing the test design, for instance by ensuring that cows are tested in an isolated space, such as a methane chamber or similar, to limit odor contamination, while still minimizing the risk of cows being stressed from social isolation.

Factors Affecting Cows' Interest in Odors

The time animals spend sniffing an odor sample can be linked to their interest in the odor (Yang and Crawley, 2009). The cows in this study did not sniff any of the odors significantly more than others, although they sniffed cedarwood numerically more than the other odors. However, the odor intensity could have affected the level of interest and hence the sniffing duration of the cows. The current study used undiluted essential oils applied in their pure form and in the same quantity to a filter paper. The odors were presented in the same manner, at approximately the same temperature, and with controlled airflow inside the barn. It is, however, possible (and likely) that each oil had different intensity or potency depending on the properties of the specific oil, which could have influenced the results. Full chemical analysis of the essential oils was beyond the scope of this study, but from the data sheets belonging to the oils (see details in Supplemental Table S2), it is clear that they differ in chemical composition, and properties emphasizing that intensity may also likely differ. Additionally, it remains unknown if the olfactory sensitivity of cattle could have made their perception of these odors overwhelming or aversive, causing lower overall sniffing durations. Studies of detection thresholds of cattle for various odors are thus encouraged.

Another important aspect to the specifics of the odors when assessing cows' interest is the novelty aspect of the odors presented. All 4 odors were hypothesized to be within a spectrum of what cattle can detect based on their genetic potential (Lee et al., 2013), yet novel to the cows to ensure that none of the individuals had a pre-established association with the odors. Presenting cows with a novel stimulus might, however, cause aversive reactions due to novelty in itself (Herskin et al., 2003; Forkman et al., 2007). Occurrence of behavior indicative of avoidance (i.e., backing, snorting, and head movements) was nevertheless uncommon in this study. Cows expressing behavior indicative of avoidance in a first presentation of an odor did not continue to do so in subsequent presentations of that same odor and it is thus possible that this behavior was caused by novelty and not aversiveness. This aspect may have influenced the results, at least for the first presentations of the odors, and we therefore encourage future studies to observe odor exploration behavior over multiple presentations.

Cows in the current study sniffed cedarwood the most, which is a contrast to the finding in horses (Rørvang et al., 2022), where horses sniffed cedarwood the least. Another contrast between horses and cows worth highlighting is the difference between horses sniffing peppermint significantly longer than the 3 other odors (Rørvang et al., 2022), whereas cows did not express such an interest. The interest in odors may thus differ between these 2 species. These results, however, should be interpreted alongside the results on the expression of licking and biting behavior. Peppermint evoked the most licking and biting in dairy cows as was also the case for horses (Rørvang et al., 2022). This result could indicate that cows perceived peppermint as edible, but it may also indicate that cows had a pre-established association with the odor. Checking the content of the feeds and feed supplements used at the experimental farm, however, revealed no mint components, which makes the latter notion unlikely. Peppermint, and other types of mints, have in other species been found to increase activity, for example in captive mice (Umezu et al., 2001), dogs (Graham et al., 2005), and zoo-kept lions (Powell and Powell, 1995). In humans, variants of mints (eucalyptus) have also been rated as among the more pleasant odorants, like strawberry (Ferdenzi et al., 2013). Last, peppermint odor has a marked trigeminal component, which may be lacking, or at least weaker, in the other 3 odors. Such differences can also have affected the results, and could have contributed to the cows expressing more licking and biting of peppermint odor. These findings, along with the fact that any type of mint was novel to these cows, suggests that peppermint may evoke an innate reaction or interest in cows resulting in them expressing more licking and biting behavior. This highlights that sniffing duration in itself may not suffice as indicator of preference or interest, and thus warrants further investigations into indicators of olfactory preference.

Factors Affecting Cows' Odor Exploration Behavior

The current study found a tendency for younger cows to generally sniff odors longer. This result was also found in the aforementioned horse study, but for the horses

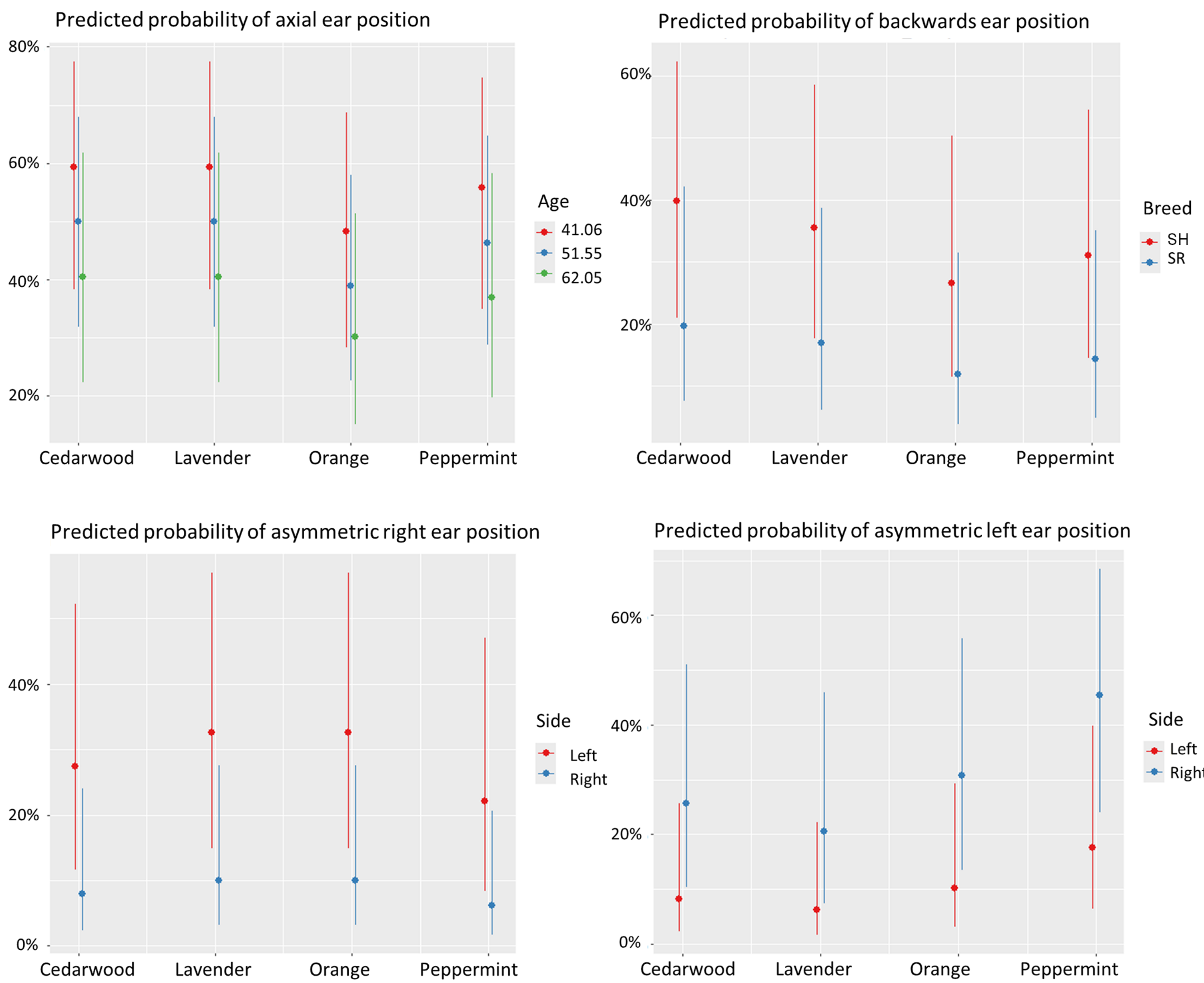

Figure 7. Predicted probabilities (%) of significant fixed effects on ear positions (first presentation of each odor only): Axial, backward, asymmetric right, and asymmetric left, respectively. The probabilities are shown within each odor separately (cedarwood, lavender, orange, and peppermint) on the *x*-axis. The bars represent the range within each odor, and the fixed effect with levels are given to the right in each plot. For axial ear position, the significant effect is of age, with older cows displaying significantly lower probabilities of expressing the ear position than younger cows. For backward ear position the fixed effect of breed significantly affected the expression of the ear position, with SH cows having higher probabilities for expressing the ear position than SR cows. For asymmetric right ear position, the fixed effect of side (left or right) significantly affected the probability of cows expressing the ear position, with the cows placed to the left having higher probability of expressing this ear position. For asymmetric left ear position, the fixed effect of side (left or right) significantly affected the probability of cows expressing the ear position, with the cows placed to the right having higher probability of expressing this ear position.

the age-effect was only significant within cedarwood, with younger horses sniffing this particular odor for longer than older horses. This could be due to changes in the olfactory system as the animals age (as reported in humans; Han et al., 2022) or changes in interest in odors with age (Rørvang et al., 2022). It is also possible that younger individuals were more curious about the odors, thus spending more time sniffing them (Schulz et al., 2007; Christensen et al., 2021). Although age was one factor significantly influencing the time cows spent sniffing the odors, age did not affect expression of licking and biting, backing, or head movement. The ear positions were also not affected by age, but a tendency for younger cows to express axial ear positions for longer was found. A previous study including ear positions as a measure of affect found that axial ear position was the predominant ear position of cows queuing for the automatic milking system (de Oliveira and Keeling, 2018).

When described with the arousal-valence model, axial ear position has been suggested to be indicative of a low arousal and neutral to slightly positive emotional state (Proctor and Carder, 2014; de Oliveira and Keeling, 2018). Curiosity, at least in the human literature, is however often described as a product of high arousal (see e.g., McNary, 2023), hence the link between axial ear position and curiosity is not clear and warrants further investigation. In addition, breed affected ear positions, with a tendency for SH cows expressing more backward ear positions than SR cows. Backward ear positions have been associated with unpleasant and negative emotions in sheep and goats (Boissy et al., 2011; Briefer et al., 2015), but also with positive states in sheep and cattle (Verbeek et al., 1994; Reefmann et al., 2009; Proctor and Carder, 2014). Hence, it remains unclear if backward ear positions during odor exploration correspond to a positively or negatively valenced state.

Collectively, age and breed are 2 important factors to take into consideration when exploring cattle olfactory-exploration behavior. In this study, we included both and additionally considered previous experience with the odors. Additional factors worth including are sex because females outperform males in olfactory sensitivity and acuity (Sorokowski et al., 2019) and respond more rapidly to olfactory stimuli (Kass et al., 2017), internal state because hunger or satiety can alter sensitivity (Hanci and Altun, 2016), and chemical pollution because various chemical compounds can affect or even damage the olfactory system (Ajmani et al., 2016). In the current study, these factors were either not relevant (we tested only females), or not possible to control for within the practicalities of this experiment. Future studies could add both males and females, control for chemical pollution, and consider the animals' level of satiety to further control for these factors.

In conclusion, this study adds important information to the basic knowledge and understanding of cattle olfaction. The results show a tendency for habituation (i.e., a decrease in sniffing behavior) to the repeated presentation with all 4 odor stimuli, and in contrast, only a significant dishabituation effect (i.e., an increase in sniffing) with cedarwood, lavender, and peppermint odor, respectively, when the cows had been habituated with orange odor. Testing cows in pairs likely affected the results and thus further studies on refining methods for olfactory testing in cattle are needed. Future research could enhance odor discrimination tests by controlling for social factors and by mixing various dishabituation trials to investigate if this may circumvent the risk of having unmotivated animals. The results can aid the understanding of the behavioral reactions of cattle to different odors, and in the future, it may be possible to relate these to the physiology and health of cattle.

NOTES

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Nonstandard abbreviations used: BAP = bovine appeasing pheromone; OR = olfactory receptor; QQ = quantile-quantile; SH = Swedish Holstein cattle; SR = Swedish Red cattle; UWB = ultra-wideband; VMS = voluntary milking system.

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