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The potential of a light spot, heat area, and novel object to attract laying hens and induce piling behaviour

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Piling behaviour of laying hens often results in smothering or death due to suffocation. Mechanisms leading to piling are not yet understood though various potential factors have been suggested. In this experimental study, we predicted that the presence of a light spot, a novel object (metal foil), or a heat area within animal pens would increase animal numbers around the stimulus leading to piling behaviour. We presented the cues in a 4 × 2 Latin-square design in eight identical experimental pens including each 55 Lohmann Selected Leghorn hens. The cues were presented in two test areas per pen, at two bouts per day in the morning, consecutively for 5 days, over four periods (age: 20, 22, 24, 26 weeks). Each pen received a cue and control condition simultaneously (test areas without cue presentation) once. For a bout, each cue was presented for 35 min except for the light spot where the duration was 10 min. Birds' responses to the cues during bout and non-bout times were video recorded and analysed for the first bout of each period. To assess the cues' attractiveness, the number of hens during bout times was counted at predefined times within the test and control areas. To assess the cues' effects on piling, we described piling behaviour (pile number, duration, animal numbers, trigger) in control and test areas during bout times. Furthermore, we described piling behaviour during bout times and non-bout times on the first day of the first period and fourth period. The best model explaining the number of hens included the interactions of treatment and bout time, and treatment and area. Over the bout's time course, more hens were attracted to the light spot compared to the control condition, and more to test areas compared to control areas. In the novel object condition, more hens were drawn to the test areas compared to the control areas. Hens were not attracted to the heat area. Piling in bout times was observed twice when hens pecked at the novel object. During non-bout times, piling behaviour occurred frequently at midday and in the late morning compared to the afternoon, mostly in corners and mainly preceded by the mutual attraction of hens. Overall, hens were attracted to light spots and less so to the novel object though neither reliably induced piling behaviour. The occurrence of piling behaviour in non-bout times shows that more work is needed to understand mechanisms eliciting piling behaviour.

Implications

Piling, the dense aggregation of laying hens, often results in death due to suffocation. Despite being an animal welfare and economic problem for producers of loose-housed laying hens, mechanisms of piling behaviour are not understood yet. In this study, we tested the potential of a light spot, a novel object (metal foil), and a heat area to attract laying hens and induce piling behaviour. We found that light spots and the novel object attracted laying hens, though neither reliably resulted in piling behaviour. Overall, more work is needed to understand mechanisms eliciting piling behaviour.

Introduction

Piling behaviour, the dense clustering of laying hens in the litter area, is the behavioural phenomenon preceding smothering, i.e. death due to suffocation. Piling behaviour and smothering are frequently observed in loose-housed layer flocks raising animal welfare and economic concerns (Barrett et al., 2014; Gray et al., 2020; Winter et al., 2021). Despite the relevance of the subject, studies have only recently begun investigating mechanisms eliciting piling behaviour with little definitive results. For example, in an explorative study on 13 Swiss commercial layer farms, piling beha-

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viour was mostly preceded by hens joining other hens' behaviour (e.g. pecking at a barn wall), group movements of birds, and the attraction of hens to various cues or events (e.g. uneven distribution of natural or artificial lighted areas) (Winter et al., 2021). Furthermore, limited studies involving two North American layer flocks (Campbell et al., 2016) and one British layer flock (Herbert et al., 2021) proposed variations in local temperature within the barn to be a factor. Though these explorative findings suggest that various cues can elicit piling behaviour, no controlled effort has tested if the attraction of hens to specific cues leads to piling behaviour. However, identifying cues that elicit piling behaviour is an essential step in developing effective measures that prevent piling behaviour and smothering from occurring. To fill this gap, the overarching research question of this study was whether the presentation of specific environmental cues could attract hens and elicit the behaviour. The objective of this study was thus to evaluate the potential to induce piles of three environmental cues previously shown to associate with piling behaviour. We predicted that: 1) environmental cues would attract laying hens, and 2) an increase in the number of hens elicited by these cues would result in piling behaviour. Cues included were 1) a light spot, 2) a heat area, and 3) a novel object (light-reflecting metal tape). The basis for the cues was that unequal light (Huber and Fölsch, 1985; Winter et al., 2021) and temperature variations (Campbell et al., 2016; Herbert et al., 2021) are common in commercial layer barns (e.g. due to sunlight spots). In addition, we observed that hens pecking at various environmental cues could result in piling behaviour (Winter et al., 2021), whereby a strong attraction of hens to novel objects consisting of metal foil was previously reported (Murphy, 1977). Choosing an experimental approach to test our predictions allowed us to control for confounds such as flock colour, age, size, and housing design (Campbell et al., 2016; Winter et al., 2021).

Material and methods

Animals and housing

The experiment involved 440 Lohmann Selected Leghorn hens, a standard hybrid showing high piling incidences in Swiss commercial layer flocks (Winter et al., 2021). The hens were reared from 1 day of age as chicks (hatchery: Rüegg Gallipor AG, Märstetten, Switzerland) in one pen of an on-site rearing barn (pen floor area: 19.2 m^2, total accessible area: 41 m^2, animal density: 10.7 animals/m^2 for the total accessible area). The pen was equipped with a rearing aviary system (Natura 187 Aufzuchtvoliere, Inauen AG, Appenzell, Switzerland), including nipple drinkers, an automatic feeder-line, a manure belt, and round plastic-coated metal perches. The hens had access to a wood-shaving covered litter area throughout rearing and, additionally from 6 weeks of age (43 days) to a covered outdoor area (winter garden) with perches. Chicks were fed *ad libitum* with a standard chick-feed (Hühnerküken Alleinfutter, FORS Futter, Kunz Kunath AG, Burgdorf, Swizerland) for the first 10 weeks followed by a standard commercial diet (Junghennen Alleinfutter H&N/L, FORS Futter, Kunz Kunath AG, Burgdorf, Swizerland) until 20 w. The light regime followed a standard schedule as recommended by the breeder, including 9 h of daylight from the second to the 16th week of age. During the rearing period, the hens participated in an experimental study assessing the use of ramps in chicks. The hens used in the present study originated from the control group where no treatment was applied. At 17 weeks of age, the hens were transferred to an experimental barn and evenly distributed to eight experimental pens (height: 2.0 m, width: 2.0 m, length: 8.13 m) resulting in 55 hens per pen (7 birds/m^2). Pen dimensions followed the minimal requirements according to the Swiss Welfare Ordinance which allow for 7 birds/m^2 on a grid surface plus litter area for flocks smaller than 150 birds. Nearly, all commercial layer flocks (>150 birds per flock) are kept under stocking densities differing to the experimental dimensions with 12.5 birds/m^2 on a grid surface plus 3.5 birds/m^2 in the litter area. Birds within each pen were visually separated from each other by grey plastic sheets (height: 1.5 m, thickness: 2 mm, SIMONA ® PP-DWU Alpha Plus 55606 Kirn, Germany) attached to the pen structure. Each pen (Fig. 1) was equipped with one nest box (height: 55, length: 110 cm), five perches on different heights (length: 155 cm, 14 cm/hen) mounted above an elevated grid area with a faecal pit (width: 1.02 m, length: 2.6 m), two automatic feeding chains (length: 2 m, 8 cm/hen), nipple drinkers (seven nipples), and a litter area with evenly distributed wood chips (12.3 m^2). The animal density was 3.6 hens/m^2, including the litter space and elevated grid area. The resources were provided in the middle of the pen to create two experimental sections at either end of the pen (length: 2.0 m, width: 2.0 m). The sections were visually separated from the middle section using grey pen-wall elements (width: 1.0 m, height: 1.5 m) and accessible to the hens without interruption. From 17 to 18 weeks of age, hens were provided with a 10 h light period (0700–1700 h) that increased to a 14 h light period (0200–1600 h) at 20 weeks of age. The light was dimmable, artificial, flicker-free LED light (SILOX-DIM LED Tube 22 W, iLOX GmbH, Vechta, Germany) and created a homogenous light distribution in the barn. Natural daylight was not provided as it could not be controlled in a standardised manner across pens and was previously associated with the occurrence of piling behaviour (Huber and Fölsch, 1985; Winter et al., 2021). A two-step dimming phase was provided in the evening: the first step included switching the lights off in the experimental sections half an hour before lights off (1530 h), followed by a dimming phase starting 15 min before lights off in the middle section (1545–1600 h). The hens were fed a layer starter diet (Legehennen Starter, FORS Futter, Kunz Kunath AG, Burgdorf, Switzerland). The feed chain freshly delivered the food six times a day (2–3 h intervals) throughout the experimental period. The barn temperature was measured at four hen-level locations within the animal area of the barn during the experimental period (HOBO U12-013, Osetcomp, Bourne, US) and was on average 21.4 ± 3.0 °C. The barn climate was controlled using a fan-ventilation system and adjustable, window (light-proof) openings. Management procedures always took place between 0900 h and 1300 h by experienced farm staff. Animal welfare checks were performed daily as part of the management care routine. Injured animals were treated

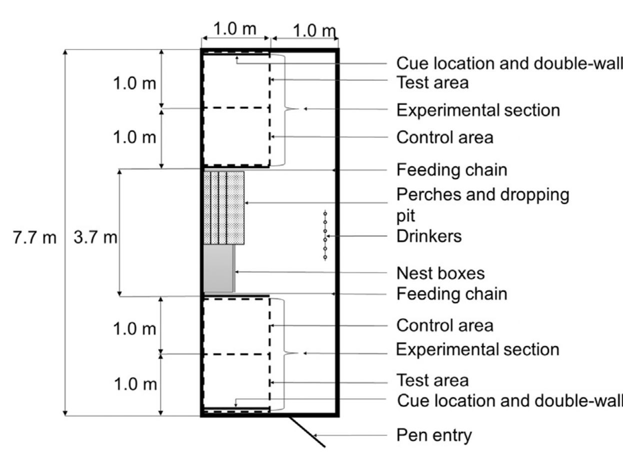

Fig. 1. Experimental pen design to test the potential of a light spot, a novel object and a heat area to attract laying hens. Dashed squares indicate the experimental and control areas that were accessible for the birds throughout the complete study period.

by staff trained in veterinary care and separated until recovery in an adjacent pen containing the same equipment as the experimental pens. Farm staff did not disperse piles on purpose.

Experimental setup

The experiment started at 20 weeks of age (May 2018) and lasted until 26 weeks of age (July 2018). A 2-week delay (i.e. from the initial transfer from the rearing barn) was chosen to provide enough time for the hens to habituate to their new environment. Also, the results of former studies indicated the selected age range as a period of frequent smothering (Bright and Johnson, 2011; Barrett et al., 2014). Furthermore, adjustments of the light period until 20 weeks of age as part of the standard light regime (see 2.1) could have influenced hen behaviour and, thus, did not allow an earlier start. The cues were presented in a four-treatment, complete crossover design (Fig. 2), in four experimental periods (i.e. 20, 22, 24, 26 weeks of age) involving five consecutive days per period with two bouts per day starting at randomised times in the morning (start of bout 1 between 0400 h and 0504 h am and bout 2 between 0615 h and 0719 h). Both experimental sections per pen were divided into a 1 m^2 test area and a 1 m^2 control area (Fig. 1); cues were presented in the test areas of the experimental sections only. The first bout started no earlier than 2 h after lights on to provide birds enough time to feed and nest in the earliest hours of the day. An automatically randomised time gap of approximately 2 h (0218 h ± 0012) was used between the start of the two bouts aiming to prevent birds from habituating to the bout start times. In addition, a 9-day washout between experimental periods aimed to reduce carryover effects between treatments. The treatments consisted of the following cues:

a) A control with no environmental manipulation
b) A heat area with an increased local temperature created by a heating plate (POLYESTER-HEIZFOLIE 230 V 100 W, thermo Flächenheizungs GmbH, Rohrbach, Germany, width 8.0 cm × length 38.0 cm). The heating plate was positioned in the test areas of both experimental sections at hen height behind the visual barriers of the pen wall. The temperature at the wall area, measured with a thermal camera (FLIR C2 Compact Thermal Camera, FLIR Systems, Inc., Wilsonville, USA) before the experiment, ranged between 20.7 °C and 48.6 °C with a mean of 38.1 °C, SD: ± 7.8 and was on average 19.5 °C higher than the mean barn temperature. The heating plate needed approximately 90 s to raise the temperature at the spot to 10 °C above environmental temperature and around 15 min to cool off to 10 °C above environmental temperature. The heat area was activated for 35 min per bout at the desired temperature not taking the cool off period into account.
c) A light spot (diameter: Ø10 cm, range: 4 000–5 000 Lux) created by a spotlight (Eurolite LED PST-3 W Spot, Steinigke Showtechnic GmbH, Waldbüttelbrunn, Germany) in the litter of the test area where the approximate distance of the light spot to the pen wall was 10 cm, providing at least 5 cm lighted area on the floor. The light spot was presented for 10 min per bout, a duration that was shorter than the other treatments to prevent smothering.
d) A novel object (reflective aluminium tape, 5 cm × 10 cm, 3 M, Neuss, Germany), placed at hen height in the centre of the pen wall of the test area. An automatically liftable double wall (same material as pen wall) was used to present the novel object during bouts (time to lift wall: 12 s, Belimo LF230, Belimo Holding AG, Hinwil, Switzerland). The novel object was presented for 35 min before the double wall was lowered.

All cues were operated remotely and automatically using a smart home control system (LOXONE ®, Loxone Switzerland, Seestrasse 16, 8266 Steckborn) to ensure that all bouts began simultaneously and reduced human influences on response to treatments. After each bout had finished, the feeding chain was automatically activated to distract hens from the test areas to reduce smothering risks. Hen behaviour in the experimental sections was constantly recorded throughout the whole experiment using two custom-built recording systems (10-channel MULTIEYE 3 GreenWatch Network Video recorder, Artec technologies AG, Diepholz, Germany) and IR-sensitive, high-resolution, wide-angled video cameras (n = 16, SNO-L6083RP, Samsung, South Korea) installed in the middle of both experimental sections per pen covering the complete test and control areas.

Video data analysis

Cue attraction

To assess the attraction of hens to the presented cues, the number of hens in the test and control areas was counted for the first bout of each experimental period at predefined time points after the bout started (control, heat area, novel object: 0, 5, 10, 17.5, 25, 30, 35 min; light spot: 0, 5, 10 min after bout start) resulting in 768 observation points for all periods across all pens. We chose the first bout of each experimental period to exclude potential habituation effects. A hen was counted when its comb was inside the test or control area using the open-source video player software KINOVEA (version 0.8.15, Charmant, kinovea.org).

Piling behaviour

To assess the cues' potential to induce piling, we analysed piling behaviour during the assessed bout times. To provide a general overview of piling behaviour in non-bout times, assessments were

Period	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8
20 w	L	H	N	C	N	H	C	L
Break	-----	-----	-----	-----	-----	-----	-----	-----
22 w	H	L	C	N	L	C	H	N
Break	-----	-----	-----	-----	-----	-----	-----	-----
24 w	N	C	L	H	C	L	N	H
Break	-----	-----	-----	-----	-----	-----	-----	-----
26 w	C	N	H	L	H	N	L	C

Fig. 2. Study design: Latin-square design with four treatments (L = Light spot, H = Heat area, N = Novel object, C = Control) distributed over eight pens and presented to laying hens in experimental sections. Each pen received each treatment once in two test areas per pen and served as self-control. Cues were presented for 5 days in each period (4 weeks of age, w), followed by a break (9 days) implemented to minimise carryover effects.

made for the entire light period of the first day of the first (20 weeks of age) and fourth (26 weeks of age) period. We defined piling behaviour as three or more mostly immobile (maximal movement duration < 5 s) hens standing in the closest possible proximity (overlapping of body outlines), with most heads (estimated over 50% of hens) facing in the same direction (Winter et al., 2021). A piling event started when all conditions were met and ended when one of the conditions was absent. We assessed the number of hens involved in the pile (i.e. pile size) at the visually estimated peak size by counting the number of hen combs and tail tips of hens that crawled below a pile. We defined the pile duration as the time between pile start and pile end. We described the location where piles started (i.e. test and control areas) and visual events preceding piling. Preceding events were defined as any visible events in the hens' environment observed at the same time and location where the birds' behaviour fulfilled the piling definition first. We checked videos for the occurrence of piles by 1-min scan sampling including pausing the videos every minute and checking bird behaviour for approximately 1–3 s. Therefore, we only considered piles longer than 1 min in the data analysis. Once a pile was identified, its exact start time, end time and pile size were assessed by a detailed analysis including playing the video forward and backward.

Reliability checks

The number of hens in the test and control corner during bout times was assessed by one observer. The intra-observer reliability was checked during each week of video assessment by repeatedly reassessing 21.6 per cent of the analysed data points per week and applying the R-package 'irr' (Gamer et al., 2019). High intra-observer reliability rates were achieved (week 1: $n = 142$, $\kappa = 0.994$, confidence level: $0.991 < \kappa < 0.99$, $P < 0.001$, mean difference: 0.014 ± 0.16 birds; week 2: $n = 24$, mean difference: 0 ± 0 birds, identical scoring).

Another observer assessed all piles after being trained by the observer who performed the primary observations. To assess inter-observer reliabilities between both observers after the training, the observers reassessed the number of piles, pile start and end time, the peak pile size, and the number of birds at the maximal pile size at a predefined time point (entire daylight portion of first four pens of the first period). Within this period, both observers agreed on 20 piles (>1 min) but did not agree on seven piles (7/27 = 25.92%). For the agreed piles, mean inter-observer differences for pile start times were low (mean ± SD: 6 ± 9 s), about half a minute for the visually estimated peak pile size times (35 ± 42 s) and end times (32 ± 51 s), and one hen for the number of birds at the time of maximal pile size (1 ± 1 bird). Reasons for no agreement included a low pile duration close to 1 min (3/7 piles), a high-level movement of hens surrounding the pile (1/7 piles), a short interruption of the pile (1/7 piles) or were of unknown origin (2/7 piles). To assess intra-observer reliabilities of pile detection of the trained observer, the observer assessed pile start- and end times at each week of video assessment and after finishing data analysis for seven randomly chosen piles. For the reassessed pile start- and end times, the observer showed very high intra-observer reliabilities during (mean differences ± SD, start time: 7 ± 9 s, end time: 3 ± 4 s) and after finishing (start time: 4 ± 4 s, end time: 9 ± 19 s) the data analysis.

Statistical analysis

We analysed the cues' effects on the response variable number of attracted hens by applying a generalised linear-mixed effect model (lme4 package, Bates et al., 2015) in R (version 4.0.2, R Core Team, 2020) using the user interface RStudio (Rstudio Team, 2020). Independent variables included treatment (i.e. control, heat area, novel object, and light spot), bout time (i.e. for the heat area and novel object: 0, 5, 10, 17.5, 25, 30, 35 min, for the light spot: 0, 5, 10 min, as a continuous factor), area within the experimental section (i.e. test or control area) and experimental section (pen entrance or opposite). In addition, all 2- and 3-way interactions of treatment, bout time and area within the experimental section were included as fixed effects. The random term in the models included pen (1–8) crossed with hen age (20, 22, 24, 26 weeks of age). We included a model performance optimiser (bobyqa) for all models, including a maximal number of 100 000 iterations. We based the model selection on Akaike Information Criterion (AICc) differences (ΔAICc) between models (corrected for small sample sizes), AICc weights, model likelihoods and evidence ratios (aiccmodavg package, Mazerolle, 2020). We considered a ΔAICc > 2 in model comparison as substantial (Burnham and Anderson, 2004) in order to select the most parsimonious model. We controlled model assumptions by visually checking residual QQ-plots (DHARMa package, Hartig, 2018) and following a statistical protocol (Zuur et al., 2010). We calculated predictive model values and 95% confidence intervals for the most supported model using the package 'effects' (Fox and Weisberg, 2018). We graphically displayed the predictive model values using ggplot2 (Wickham, 2016) and calculated descriptive values (mean, SD) of piling characteristics using dplyr (Wickham et al., 2020) and Microsoft Excel.

Results

Cue attraction

The best model explaining the effect of the cues on the hens included the interactions between treatment and bout time and treatment and area (Table 1, Δ AICc to second-best model: 0.64, evidence ratio compared with second-best model: 1.37, AICc weight = 0.42) and experimental section. During the course of the assessed bout times (light spot: three bout times; control, novel object, heat area: seven bout times/treatment), the number of hens attracted to the light spot was greater (mean ± SD of raw data: 2.0 ± 2.6) in comparison to the control group (mean ± SD of raw data: 0.5 ± 1.0). However, no difference was seen in comparison to the control group for the novel object (mean ± SD of raw data: 0.6 ± 1.0) and heat treatment (mean ± SD of raw data: 0.6 ± 1.0) (Fig. 3). In comparison to the control areas, the number of hens in the test areas was greater during the light spot (mean ± SD of raw data, control area: 0.8 ± 1.4, test area: 3.1 ± 3.0) and novel object (control area: 0.4 ± 0.8, test area: 0.7 ± 1.2) treatment, but no difference was seen in the heat treatment (control area: 0.5 ± 1.1, test area: 0.6 ± 1.0) (Fig. 4). More hens were attracted to the experimental sections located opposite to the entrance (0.9 ± 1.5) than to the entrance (0.4 ± 1.1).

Piling behaviour

We observed nine piling events during all treatment bout times (total observation time: 61.2 h) in the first (seven piles) and third (two piles) periods. Two piling events were preceded by an animal responding towards a presented cue. A hen focused and pecked at the novel object in both cases, resulting in increased animal densities, hens squeezing against each other in the nearest corner (distance: ~50 cm), and piling (Fig. 5). Five piles appeared unlikely to be related to the presented cues as they either occurred in the control area (3/5) or the control treatment condition (2/5). We could not exclude an influence of cues for two piles occurring in the test areas during the heat and novel object bouts. However, both piles were preceded by hens pecking in a corner close to (distance: 50 cm) but not at the presented cue, making involvement of cues

Table 1
Overview of the best models explaining the attraction of laying hens to the presented cues. Explanatory variables were treatment (light spot, novel object, heat, control condition), bout time, area (control or test), and all two- and three-fold interactions.

Fixed Effects	K	AICc	ΔAICc	AICcWt
Treatment * bout time + treatment * area + experimental section	16	1 753.91	0.00	0.42
Treatment * bout time + treatment * area + bout time * area + experimental section	17	1 754.54	0.64	0.30
Treatment * bout time * area + experimental section	20	1 754.78	0.87	0.27

Model selection included the number of model parameters (K), Akaike Information Criteria (corrected for small sample sizes), Akaike Information Criteria differences (ΔAICc > 2) and Akaike weights (AICcWt).

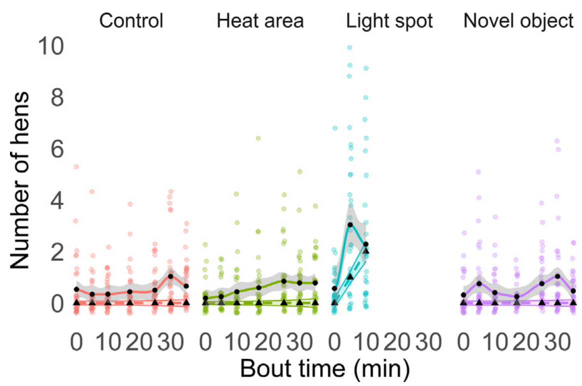

Fig. 3. Effect of the interaction of treatment (control, heat area, light spot, novel object) and bout time (light spot: 0, 5, 10 min; heat area, novel object, control: 0, 5, 10, 17.5, 25, 30, 35 min) on the number of attracted laying hens. Dashed lines connect the model estimated means (black triangles), seamed by the SEs of the estimated means. Solid lines connect raw data means (black dots), seamed (grey) by the SEs of the raw data means. Coloured dots show raw data points.

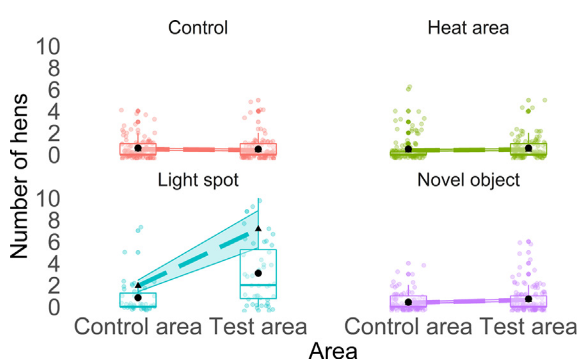

Fig. 4. Effect of the interaction of treatment (light spot, heat area, novel object, control) and area (control area and test area) on the number of attracted laying hens. Dashed lines indicate the model estimated means and SEs of the estimated means. Triangles highlight the model estimated means. Box plots and coloured dots reflect raw data. Black dots highlight raw data means.

in the pile formation less likely. Neither the duration nor the number of involved hens had noticeable differences for piles induced by the novel object cue and non-cue related piles during bout times (Table 2).

In non-bout times, during the first day of the first (20 weeks of age) and last (26 weeks of age) period, we observed 81 piles (total observation time: 864 h). Piles occurred in all pens and experimental sections, more often in experimental sections located opposite the pen entrance (52 piles) than those located at the pen entrance (29 piles), and more frequently in the test areas than in the control areas. Most piles (73/81, 90.1 %) were preceded by hens joining one or more hens (e.g. facing towards a corner: 51/73 piles, pecking at wall spots close to a corner: 18/73, resting in a corner: 3/73, scratching in a corner: 1/73). Six piles (6/81, 7.4%) were preceded by group movement of hens towards a pen wall and two piles (2/81, 1.2%) by agonistic interactions between hens. Piles lasted between one (inclusion criteria, minimum pile duration) and 8 min and involved three to 23 hens. The average pile size and duration did not differ between bout and non-bout times (Table 2).

Taking all piles at the first and fourth period together (88 piles: 7 piles occurring during bout times and 81 piles during non-bout times), piles mainly occurred around noon (10–12 h) and in the late morning (7–9 h) but were less frequent in the early morning (2–7 h) and the afternoon (13–16 h) (Fig. 6).

Discussion

In this study, we predicted that 1) specific environmental cues would attract laying hens into a test area and 2) the attraction to the test area would result in piling behaviour. Our results showed that hens were attracted to the light spots, occasionally to the novel object, and never to the heat area. Piling behaviour could not be explained by hens being attracted to the cues since only two piles were elicited by hens pecking at the novel object during the bout times. However, we frequently found hens piling in non-bout times which complicated explanations for the attraction of laying hens to environmental cues, piling behaviour during bout times, and factors eliciting piling behaviour in non-bout times.

Cue attraction

Hens reacted differently towards the three environmental cues. Whereas the number of hens increased prominently over the course of the light spot condition, no increase in the number of animals was observed in the novel object and the heat area condition compared to the control condition. Furthermore, the number of hens in the test area was higher in the light spot and novel object conditions but not in the heat area condition compared to the control area, a partial fulfilment of our predictions. Various reasons could explain the variable attractivity of presented cues, including their visibility and relevance to the birds.

In our experiment, there was a clear difference in the visibility of cues. Whereas light spots had a high luminosity and contrasted strongly with the litter area, the novel objects were of a shiny grey colour, less strongly deviating from the grey pen walls. Other research in operant task conditions found that hens are more likely to respond and show more responses to cues with a higher luminosity (Gover et al., 2009). Therefore, differences in visibility could be a key factor explaining why hens strongly responded to the light spots but only occasionally to the novel object in our experiment. Our observation confirms on-farm findings that hens are attracted to artificial (Huber and Fölsch, 1985; Winter et al., 2021) and natural light spots (Gibson et al., 1985; Winter et al., 2021). As observed in commercial settings (Winter et al., 2021), hens in the current study approached the light spot within a short time.

In contrast, treatment areas with increased temperature would probably have been the most difficult for the birds to detect. Hens do not visually perceive IR (heat) wavelengths (Prescott and Wathes, 1999) but discriminate temperature differences in their environment using thermoreceptors (Gentle, 1989) requiring that

Fig. 5. Pile formation of laying hens in the test area during a bout time of the novel object treatment condition. The pile formed 85 s after hens pecked at a novel object against the top-right corner of the experimental pen.

Table 2
Descriptive values for the number of piles, number of piles observed per hour, pile size (mean ± SD) and pile duration (min, mean ± SD) of laying hens during bout and non-bout times in test and control areas of the experimental pens.

Time	Area	Observation time	Pile number	Piles per hour	Pile size (mean ± SD)	Pile duration (min, mean ± SD)
Bout	Control	30.6 h	3	0.09	6.0 ± 2.0	1:35 ± 0:21
	Test*	30.6 h	4	0.13	10.7 ± 4.3	5:03 ± 2:01
	*Cue related		2	0.06	7.0 ± 0.0	2:27 ± 1:07
	Total	61.2 h	9	0.14	8.3 ± 3.6	3:19 ± 2:08
Non-bout	Control	432 h	33	0.07	7.0 ± 2.2	2:53 ± 1:36
	Test	432 h	48	0.11	10.3 ± 4.1	3:41 ± 1:56
	Total	864.0 h	81	0.09	8.9 ± 3.8	3:22 ± 1:50

Asterisk (*) indicates two piles occurring during bout time induced by the interaction of a hen with a presented cue (novel object).

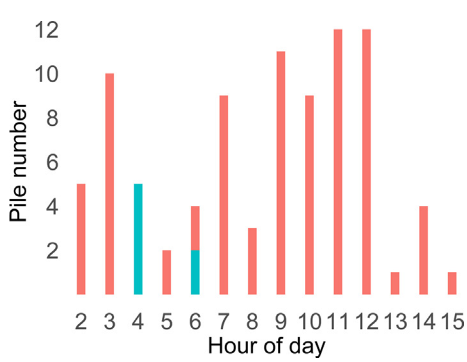

Fig. 6. The number of laying hen piles at bout times (green) and non-bout times (red) for the time of the day during the entire daylight period (0200–1600 h).

hens come in contact or within close proximity to the heat source. The provided bout time was probably too short for the hens to detect the area with increased temperature and may explain the lack of a treatment effect.

In addition to their visibility, the cues' biological relevance for the birds may also explain why hens were more attracted to the light spot and less to the novel object. Hens are highly motivated to seek light. In our study, hens often pecked and scratched at the lighted litter areas, showing that hens might have perceived light spots as a source of exploration. In other experimental studies, increasing the light intensity on the litter area stimulated comfort behaviours, including dustbathing behaviour in laying hens (Duncan et al., 1998). Light has also been suggested to play an essential part in behaviours such as feeding and interactions with conspecifics (Prescott et al., 2003). Novel objects can also be a source of exploration for hens. For example, an experimental study found that a large number of hens approached and pecked at a presented novel object (a shiny pencil) within half a minute (Rozempolska-Rucinska et al., 2017). It could be that the small size, grey colour, and potential difficulties in manipulating the novel object (it was attached to the wall) in the current effort were unattractive to the birds. In addition, hens may have been fearful of approaching the novel object. Experiments presenting various novel objects to white layer breeds showed that the birds approached novel cues with caution (Keer-Keer et al., 1996; Uitdehaag et al., 2008; Rozempolska-Rucinska et al., 2017), especially in the first 30 min of presentation (Murphy, 1977). Thus, the provided bout time (30 min) may have been too short to observe attraction to the novel object cue in this experiment.

Piling behaviour during bout times

Our prediction that an increase in the number of hens in the test areas due to the attraction of the cues would result in piling behaviour was not supported as the increased number of animals during light spot provision did not lead to piling behaviour. Though high animal densities after hens pecked at the novel object induced two piles, piling behaviour was not reliably induced by the novel object. However, we frequently observed piles during non-bout times and bout times that were not induced by the cues indicating that other mechanisms were active.

According to our definition, various conditions must be met to call an aggregation of hens piling behaviour (Winter et al., 2021). These conditions include when hens are in close body contact with other birds showing an overlapping of body outlines, motionless, and having the heads positioned in the same direction. Though animal densities in the test areas increased in the light spot condition, hens were not motionless and did not align in the same direction. For example, we observed an increased number of hens scratching and pecking at the lighted floor area, which is not compliant with our piling definition. In this sense, certain elements of piling behaviour were present and, with longer time or varying other factors, piling behaviour may have taken place, though piling behaviour still was not detected.

An explanation why we did not observe piling in the light spot condition is that pecking, and scratching elicited by the light spot may have led to greater distances between birds. For example, it was shown that hens increase bird distances when pecking (Keeling and Duncan, 1991) and adjust positions to increase individual distances when animal densities increase at an approached resource (Sirovnik et al., 2021). While light spots have been described to elicit piling behaviour and dense agglomerations of birds in commercial settings (Huber and Fölsch, 1985; Winter et al., 2021), larger numbers of birds and restricted space may explain piling behaviour instead of light itself (Gebhardt-Henrich and Stratmann, 2016). Finally, light spots may not have led to piling due to a short bout time. In commercial settings, where piling can be observed when light appears on the floor (Huber and Fölsch, 1985; Winter et al., 2021), light spots likely last longer than 10 min, potentially attracting a higher number of hens and therefore leading to piling. Future studies should consider increasing group sizes in pens and presenting the light spots in a more restricted space and longer duration to induce piling behaviour.

Piling behaviour in non-bout times and non-treatment location effects

We frequently observed piling behaviour in non-bout times (i.e. when the cues were off), more frequently around midday and late morning, and less in the early morning and afternoon. In addition, piling behaviour more often occurred in experimental sections located opposite to the pen entrance and was preceded by hens joining other hens (e.g. standing and pecking) in pen corners. Overall, only a small proportion of birds participated in piling at the same time.

The variance across daytimes is in line with other studies (Campbell et al., 2016; Winter et al., 2021). One explanation for the observed variation of piling behaviour across the day could be the diurnal variation of other hen behaviours. For example, we suggested that variation of piling behaviour across the day could be related to diurnal patterns of nesting, dustbathing, and feeding behaviour (Winter et al., 2021). Overall, finding similar daytime effects on piling behaviour occurrence despite the different housing conditions (i.e. experimental vs commercial setting) may confirm that this is a general pattern of piling behaviour.

Piling behaviour occurred more often in experimental sections located opposite to the pen entrance indicating that the pen design may affect piling frequencies. Staff passed the experimental areas at the pen entrance twice per day (once on their way to the middle sections and once on their way back) and experimental areas opposite the pen entrance once per day (to check bird health and look for floor eggs). These management times took place between 0900 h and 1300 h and lasted approximately half an hour. Though hens are known to accustom to the presence of human beings over time (Jones and Faure, 1981), it may be that hens preferred accessing areas that were less disturbed by farm staff, potentially resulting in higher animal densities and increased piling behaviour. Such may also be supported by our finding that hens more often approached cues in the experimental sections located opposite to the pen entrance than at the pen entrance.

Next to lower possibilities of hens leaving bird accumulations located closely to barn corners, specific corner qualities might have attracted hens as well. For example, it may be that the corner's shape, shadows, or other corner characteristics may have drawn hens into barn corners, though we attempted to minimise any differences as much as possible. Finally, we suggested in previous work that piles occur more frequently in barn corners due to anti-predation behaviour (Winter et al., 2021). Independent of the reason, the attraction of hens to corners may have increased hen densities and the likelihood of piling there independent of cue.

Piling behaviour was mostly preceded by hens joining other hen behaviours in pen corners or along the pen wall confirming previous findings that social factors play a role. Hens mostly piled after joining individual hens facing a corner, pecking at wall spots close to a corner, or resting in a corner. Similar patterns were observed on-farm, where hens were joining other hen behaviours (Campbell et al., 2016; Winter et al., 2021) close to walls and in corners preceding piling behaviour. Various social mechanisms could explain the observed behaviour of hens joining other hen behaviours. For example, in domestic fowl, social facilitation was frequently suggested as involved in the mutual attraction or synchronicity in bird behaviours (Hoppitt et al., 2007; Collins et al., 2011; Winter et al., 2021). However, given that hens joining other hen behaviours resulted in piling when birds were close to a pen wall or a corner, an interaction between a social stimulus and location seems relevant. The interaction between a social stimulus and location may also explain why the attraction to conspecifics' behaviours in pen corners led to piling, whereas the attraction to hens pecking and scratching at the light spot on the floor at the centre of the pen wall could not.

Even though the absolute numbers for pile frequencies as well as pile size were smaller in the experimental setting compared to commercial flocks, comparing the relative numbers shows a different outcome. When comparing piling frequencies and pile sizes of this experimental study to results of commercial farms, taking flock sizes into consideration suggests that more piling and more birds per flock were engaged in piling in this study. For example, the ratio in a commercial setting was 0.63% of birds per flock piling (Winter et al., 2021: given an average flock size of 4 935 birds per flock and an average pile size of 31 birds per pile). Comparing these numbers to those of the current effort (i.e. flock size of 55 birds and 9 on average engaging in a pile) would lead to a ratio of 16% of birds per flock/ pen engaging in piling. Thus, the likelihood for an individual hen to be involved in piling seems higher in experimental pens compared to commercial flocks. Multiple reasons, such as altered social dynamics (Estevez et al., 2007) and shorter distances to approach piles in experimental pens, may have contributed to an increased likelihood of hens to be involved in piling in smaller pens.

Conclusion

Taken together, the prediction that cues attract laying hens was true for light spots but not for the novel object and area with increased temperature. However, the attraction to light spots did not lead to piling behaviour. Instead, piling behaviour was often observed in non-bout times and appeared to be more likely around midday and when hens performed various behaviours in pen corners. Future studies may increase the visibility and explorative value of the novel object, expand bout times, present piling cues in an increased number of pen corners, and increase pen animal densities and group sizes to increase the likelihood for attracting hens and inducing piling behaviour. In addition, further work is needed to elaborate the diurnal variations of piling behaviour, for example, by varying daylight cycles, to decipher behavioural mechanisms involved in piling.

Ethics approval

The project was granted ethical approval by the veterinary office of the canton of Bern, Switzerland (BE97/17). In consideration of the 3Rs, all animals originated from a previously approved research trial (BE55/17) and were treated in compliance with the Swiss regulations regarding the treatment of animals involved in research.

Data and model availability statement

None of the data were deposited in an official repository. The data are owned by the University of Bern. Upon request, the authors will provide the reviewers' access to the data and model scripts.

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Author contributions

All co-authors substantially contributed to the conception of the work. Jakob Winter and Ariane Stratmann were responsible for the acquisition, analysis and interpretation of data for the work. Michael Toscano was responsible for acquisition of funding. All co-authors drafted and critically revised the work for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work.

Declaration of interest

None.

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