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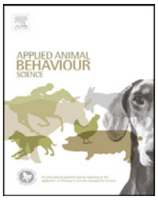

Cognitive bias and anticipatory behaviour of laying hens housed in basic and enriched pens

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ABSTRACT

The performances of adult laying hens, housed in either a basic or an enriched pen, were investigated in a cognitive bias and an anticipation test. Both tests were designed to measure the assumed emotional state of the birds. The behaviour of birds in each test was compared to see whether both tests provided similar evidence of changed emotional state. In addition, measures of fear and negative social interactions within the home pen were taken, and their association with the measures of emotional state were examined. No significant differences between treatments were found, which suggests that the basic and enriched pens did not induce large enough differences in the birds' emotional state to have a significant impact on their behaviour in the tests. On the other hand, the number of social interactions exhibited in the home pen correlated with some aspects of behaviour in the anticipation tests and the amount of training needed to reach testing criteria in the cognitive bias tests correlated with latencies to approach the near rewarded probe in the same test. This suggests that when animals are not put under more severe treatment differences, other individual differences have a greater impact on how they perform in the tests.

1. Introduction

An important component of animal welfare is the affective state of the animal (Fraser et al., 1997) whether it is positive or negative (Boissy et al., 2007). Affective states are subjective experiences and thus impossible to measure, especially in non-human animals, but they can be investigated with indirect methods and traditionally, methods such as preference and motivational testing have been used to estimate feelings in animals (Duncan, 2006). New methods are being developed to measure aspects of emotion thought likely to reflect subjective states and three prime approaches are: appraisal theory (Désiré et al., 2004), cognitive bias (Harding et al., 2004) and the use of anticipatory behaviour (Spruijt et al., 2001). In the current study we will compare two of these; the cognitive bias and the anticipatory behaviour approach.

The cognitive bias task for animals is derived from a similar language task for humans (Mendl and Paul, 2004) and usually it consists of a task where an animal is trained to respond to a positive or negative cue and then tested with ambiguous cues. This type of test is based on the finding that depressed and anxious people in general interpret ambiguous stimuli more pessimistically compared to control persons (Mathews et al., 1995). When using this approach with animals, Harding et al. (2004) found that rats kept under unpredictable housing conditions were slower to respond to ambiguous tones, which was interpreted as these animals being more pessimistic. Bateson and Matheson (2007) found that starlings, which had recently been deprived of the environmental enrichment in their

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home pens, less often flipped open the lids of food pots of an ambiguous colour than did control birds. Results such as these support the hypothesis that non-human animals in a negative mood also respond more pessimistically to ambiguous cues than individuals in a relatively more positive mood. For a review of research in the area of emotional judgement biases see Mendl et al. (2009).

The other approach used in this paper is based on observations of anticipatory behaviour which has been studied by Spruijt et al. (2001). They propose that anticipatory behaviour indicates the state of the animal's reward system and that it can be used to understand and measure the coping capacity of animals. This method therefore also potentially has implications for animal welfare research (Broom, 1996). Through the use of Pavlovian conditioning it is possible to observe an animal's behaviour when it is expecting a reward. Observations of anticipation in laying hens have been carried out by Moe et al., 2009 who described hens as showing attentive movements and stretching their neck and Zimmerman et al. (2011) found that birds expecting a positive event performed more comfort behaviours and were standing alert more. The extent of anticipatory behaviour is modulated by recent experiences leading to an increased or decreased sensitivity to the reward. Jones et al. (1990) found an increased sensitivity to reward in socially deprived rats and van der Haarst et al. (2003) found a decreased sensitivity to rewards in rats housed under enriched conditions. On the other hand, it is known that animals in a depressed like state fail to show anticipation at all when expecting a reward (von Frijtag et al., 2000).

Poultry present a very interesting model for the study of these cognitive responses as they are at the forefront of discussions about animal welfare. Laying hens are kept under different housing conditions such as aviaries and furnished cages. When evaluating these systems different measures give different indications of which systems might provide the best welfare. Thus it would be of particular relevance if there were possibilities to get an estimate of how the birds experience their situation.

The main goal of the current study was to investigate how the results of the cognitive bias and anticipation tests are related to each other. To study this we used laying hens kept in either an enriched or in a basic housing environment. As well as comparing the short term response of the animals, we also had the aim of making long-term comparisons to determine if the birds' response to the tests changed with the amount of time they had been kept in the same environment. In estimates of animal welfare it is well accepted to use several different measures to compare how these relate to each other. In laying hens, for example, preferences for different environments have been compared with physiological measures and behavioural activity and suggestions for indicators of positive and negative valence have been made (Nicol et al., 2011). Therefore, in addition to the main tests and treatments, other measures which could be relevant for how the birds experienced their situation were gathered and compared with the behaviour in the cognitive bias and anticipation tests. These measures were the number of negative social interactions performed and received in the home pen for each individual bird, the state of its plumage and if it had any wounds. Finally an estimate of the birds' fear level, as measured with a tonic immobility and novel object test, was carried out (Forkman et al., 2007).

2. Materials and methods

2.1. Animals, housing and treatments

The animals were adult laying hens (Lohmann white, 67 weeks old, previously kept in cages in commercial egg production). Initially there were 47 hens housed in 10 floor pens in groups of either four (3 groups) or five (7 groups). Cannibalistic pecking led to 13 culled birds, resulting in between two and five birds per pen. The birds were individually marked with leg rings of different colours. The pens were 1.5 m × 1.7 m and contained wood shavings, a nest box with straw (30 cm × 90 cm), perches 20 and 40 cm high and ad lib. access to food (standard commercial layer diet), mussel shells and water. All birds were kept under these conditions during the first eight week long training period. Three days before the first test period the birds' home environment was changed to either of two different treatments, the enriched (5 pens, 18 birds) and the basic environment (5 pens, 20 birds). In the enriched pens, in addition to the previous resources, there was one additional perch (100 cm high), a box (42 cm × 30 cm) with peat and the floor was covered with a generous amount of wood shavings and new litter was added several times per week and hay once a week. Either apples or sunflower seeds were put in the litter every day. In the basic environment the 40 cm high perch was removed, so that only the 20 cm high perch remained, and the amount of wood shavings on the floor and in the nest box was kept to a minimum.

Lights were on from 07:00 to 17:00 h, except during the first two weeks after the hens had arrived, when they were on from 07:00 to 13:00 h. In addition the room had windows which let in some daylight. The study was carried out during the summer at the experimental farm Rørrendegård at the University of Copenhagen, Denmark.

2.1.1. Ethical note

Since the study did not involve any invasive procedures the national Danish legislation did not require an ethical approval. The birds were checked at least once daily and any injured birds were treated with anti-pecking spray, removed to a single pen or culled, depending on the severity of the wound.

2.2. Experimental setup

The birds were tested in two periods with a cross over design (Fig. 1). Training of the birds in preparation for the first anticipation and cognitive bias tests began a few days after the birds' arrival at the experimental farm. Each test period was one week long and the birds were 76 weeks old when the first test period was started and 85 weeks old in the second test period. Birds that did not succeed in the training were not tested. In the first test period 15 birds (8 enriched and 7 basic) were tested in the cognitive bias test and 16 birds (7 enriched and 9 basic) in the anticipation

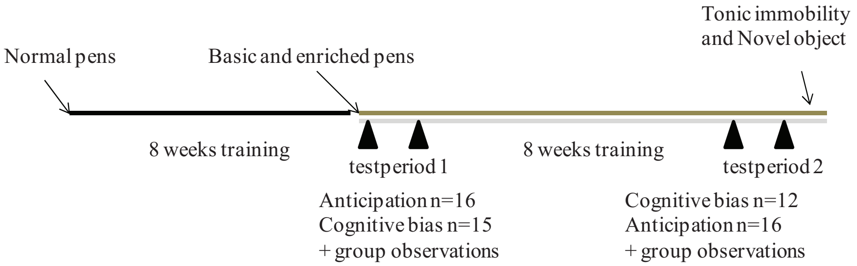

Fig. 1. Outline of the experimental set up.

test. In the second test period 12 birds (6 enriched and 6 basic) went through the cognitive bias test and 16 birds (10 enriched and 6 basic) the anticipation test. Twenty-four birds participated in both of the tests. Tonic immobility and novel object tests were carried out on each individual at the end of the study to not interfere with the cognitive bias and anticipation tests.

2.3. Cognitive bias training

The cognitive bias test was based on spatial cues and followed the methods used on rats described by Burman et al. (2008). The test arena was 2.2 m × 2.35 m and in the middle along one short side there was a start box with a manually operated sliding door. The birds should learn that the position of a food bowl (either to the right or left at the furthest end of the arena with a distance of 1.2 m between the two locations) indicated whether it contained corn or not. Half of the birds were trained that the reward location was to the right side and the other half that it was to the left side and this was balanced between treatments (basic/enriched) and pens. Training started by familiarising the birds to eating grains of corn in their home pens after which they were habituated in pairs to the start box and the test arena, where corn was scattered over the floor. After this initial habituation all training was carried out with one individual at a time. In the beginning of the individual training, the food bowl was always placed at the designated rewarded location. The reward was a small amount of corn. When birds reached the criterion of walking up to the bowl and eating the corn within 2 min, three times in a row, they were introduced to the rewarded/unrewarded training.

Each session of this training consisted of eight trials where the bowl was placed four times in the rewarding position (i.e. contained food) and four times in the unrewarding position (i.e. was empty). The same treatment was applied a maximum of two times in a row and then the bowl was placed in the other location. The order of rewarded and unrewarded trials was otherwise randomised and differed between training sessions. The time from the opening of the start box until the bird's feet were less than 10 cm from the bowl was measured. The maximum time allowed was 1 min from leaving the start box. If the bird had approached the bowl and ate within 1 min, it was picked up and returned to the start box (if the bowl was in the rewarded position) and training was continued. If it had not approached the bowl it was encouraged to feed from the bowl by throwing corn near the bowl. If the bird still did not eat, the session was ended and the bird returned to the home pen.

During the training (and also during later testing) to the rewarded/unrewarded locations, a half circular piece of cardboard covered the half of the bowl closest to the start box to prevent the birds from being able to see whether the bowl contained food or not from a distance. The criterion for when a bird was finished with its training and ready to be tested was that the latency to reach the food bowl from the start box should be at least 5 s longer in at least three out of the four times the food bowl was located in the unrewarded position, compared to the four occasions when it was located in the rewarded position, in the eight trials in that session. However, after six training sessions there was no indication that any of the birds had reached the learning criterion with birds quickly approaching the bowl irrespective of its location. Therefore a more intensive, individual-based training schedule was used where the number of unrewarded trials was increased. To reach the learning criterion the birds were trained in total 56.8 ± 1.5 (mean ± se) times to the rewarded bowl and 93.5 ± 7.6 times to the unrewarded bowl across at least 12 training sessions. Despite this, ten birds, six from the basic and four from the enriched environment, did not approach and feed reliably from the rewarded bowl and did not proceed to the cognitive bias test.

2.4. Cognitive bias testing

Each bird was tested three times with one day in between test days. The test sessions were recorded on video and the latency from the time the bird could leave the start box until it pecked at the bowl was measured. If the bird did not leave the start box within 60 s it was given a latency score of 60 s. One test series consisted of five cues for reward (bowl in rewarded position) and five cues for non-reward (bowl in unrewarded position) and three probe tests. The three probes (ambiguous cues) were placed at points 25%, 50% and 75% between the rewarded and unrewarded cues. A series started with four rewarded (R) and unrewarded (U) cues (order varied), then one probe (P), followed by three rewarded and unrewarded cues. The second probe was followed by three more unrewarded and rewarded cues in varied order before the last probe. A rewarded or unrewarded cue was never given twice in a row during the test series. An example of a test series was U, R, U, R, P1, U, R, U, P2, R, U, R, P3. The order of where in the series the three probe locations were tested was balanced between treatments. When a test series was started it was controlled that the bird differentiated between rewarded and unrewarded bowls during the first four cues, meaning that the latency to approach the unrewarded food bowl should be at least 5 s longer in at least one pair of a rewarded and unrewarded choice. If this was not the case, the bird was exposed to one more rewarded/unrewarded choice and if it still did not show any difference between these, the testing was interrupted and the bird given further training before it was tried in a test again.

2.5. *Anticipation training*

The anticipation test was carried out in a separate arena (1.5 m × 1.7 m). In the middle of the front wall of the arena there was a tube leading to a bowl, so that corn could be dropped into the bowl from outside. Two different light signals, one white and one blue light chain (string of light) were placed beside each other along the wall next to the tube and were used to announce either the arrival of the reward (corn), or that nothing would happen. The colour of the light was counter-balanced. Initially the birds were only trained with the signal predicting food. The light chain was lighted manually for 1 s and within a few seconds after that, two pieces of corn were presented in the food bowl. This was repeated 12 times during one training session irrespective of whether the birds ate the corn or not. The time interval between the signals varied randomly between intervals of 20, 30 or 40 s within the same session. When the birds had learnt to eat the corn from the bowl, they were trained with both signals. Only one signal was used in each training session. After five sessions with the positive signal and four sessions with the neutral signal, a delay was introduced between the positive signal and the food reward. Four training sessions with a delayed reward were carried out and the delay was gradually increased, by 5 s per training session, so that during the last training a 20 s delay was used. Training sessions with the positive signal and delayed reward were paired with training sessions with the neutral signal so that one session of each type was carried out on the same day.

2.6. *Anticipation testing*

Each bird went through six anticipatory test sessions. Three sessions with rewarded and three sessions with unrewarded signals. Within each session, the light signal was given three times. Thus, in total each bird was exposed to nine positive and nine neutral signals. One rewarded and one unrewarded session per individual was carried out on the same day, with one day in between test days. The first signal was given 25 s after the bird had been released in the arena and then the light signals were repeated with 1 min intervals between each signal. The delay between the positive signal and reward was set to 25 s since Moe et al. (2009) had found an increased rate of anticipatory behaviours in chickens during 22 s delay between signal and reward.

The test sessions were video recorded and the recordings used for the behavioural observations. Behavioural observations were carried out during the 25 s after each signal and, in the case of the positive signal, finished immediately before the reward was given. Previous studies of anticipatory behaviour in chickens (Moe et al., 2009; Zimmerman et al., 2011) suggested that attentiveness towards the location for the expected reward and head movements were indications of anticipation. Therefore in this study the behaviours observed were head movements and time spent with the feet within 30 cm of the bowl, with the front of the head directed towards the bowl, i.e. being in a position where they could see if corn appeared in the bowl, versus time away from the bowl (feet further away than 30 cm or with the head directed away from the bowl). Since they continually moved their heads during the observations only major head movements were scored. These were the number of times the bird raised its head up over the base of the neck and the number of times it lowered its head below the base of the neck. Whether the bird put its head down into the food bowl or down to the ground was also noted. The number of steps taken was also scored. Comfort behaviours such as preening and wing flapping were observed, but occurred so seldom that the results are not presented.

2.7. *Group observations*

Observations of negative social interactions between birds were carried out in the home pens. The behaviours scored were severe feather pecks, aggressive pecks and avoidance and the identities of the individuals performing and receiving the behaviour were noted. The observations were carried out continuously for 10 min per pen and occasion. All pens were observed on five different occasions during the first test period (total 50 min) and this was repeated during the second test period.

2.8. *Tonic immobility test*

After the second test period all birds underwent a tonic immobility (TI) test. The bird to be tested was taken to another part of the same room, within hearing distance of the other birds, but out of sight. It was put on its back in a cradle and gently held down for 5 s by the experimenter placing one hand on its chest and one hand over its head. The hands were removed and then the time was recorded until the bird righted itself, with a maximum time of 10 min. If it remained shorter than 10 s a new attempt was made up to a maximum of three attempts.

2.9. *Novel object test*

A novel object test was carried out in the home pen after the second test period was finished. All hens were removed from the home pen and returned singly for the test. Birds were first habituated to eating grains of corn from a small metal container placed in the middle of the pen. They were judged to have achieved this when they ate from the container within 10 s of being placed in the pen. Most hens ate from the container on the first or second try and when they had reached the criterion they were taken out again whilst the other birds from the home pen were trained. When the bird was placed in the pen the next time, a novel object (a red and yellow coloured plastic ball 12 cm diameter) had been placed 20 cm behind the container with the corn. The time was taken for the bird to feed and maximum time was set to 60 s.

2.10. Body weight, plumage condition and wounds

The birds were weighed before each test period. In addition, a scoring of any skin wounds as well as plumage condition was carried out. Wounds were given a score between 0 and 3 with 0: no wounds, 1: minor (less than 1 cm^2) older wounds, 2: medium sized (between 1 and 4 cm^2) old and fresh minor wounds and 3: medium sized fresh and large (>4 cm^2) fresh and older wounds. The plumage was given a score between 0 and 3 with 0: no obvious naked parts, 1: small naked part less than 5 cm × 5 cm, 2: more than 5 cm × 5 cm naked, 3: more than three quarters of the birds body is naked.

2.11. Statistical analyses

To account for the fact that the we had set the maximum time of the latency to peck at the bowls in the cognitive bias test to 60 s and thus censored the data for many of the birds, the score for each animal was based on the median latency (of the three occasions) to peck at each of the three probes and median latency (of the 15 occasions) to approach the rewarded and unrewarded bowls. The difference between treatments and test period was then analysed using Fisher's Exact test on the number of animals which pecked at the bowl with a median time within 20 s after being allowed to leave the start box.

Anticipation results were analysed using Mann–Whitney *U* tests on the mean frequencies of the different behaviours and time spent close to the bowl from the three positive sessions, based on the mean of the three repeated signals within one session. Analyses were carried out both of the effect of treatment and of test period. Comparisons between the birds' responses to the positive and neutral signal were tested using a Wilcoxon matched-pair signed-rank test. A Spearman correlation test was used to test for correlations between the TI test, novel object test and the number of negative interactions in the home pen with the results in the cognitive bias and the anticipation tests. In addition, the amount of training to reach the learning criterion in the cognitive bias test was correlated with the latencies to approach the probes during testing.

A Mann–Whitney *U* test was used to compare results in the TI and novel object tests between birds that did not reach the learning criteria and the birds that did reach criterion and proceeded to the cognitive bias test. A Mann–Whitney *U* test was also used to compare effects of treatment on body weight, wounds, plumage condition and the number of negative interactions performed and received. A Wilcoxon matched-pair signed-rank test was used to compare the effect of test period on the same parameters.

The reduced number of birds in the study reduced the power of the statistical analyses and therefore results $P < 0.1$ are reported.

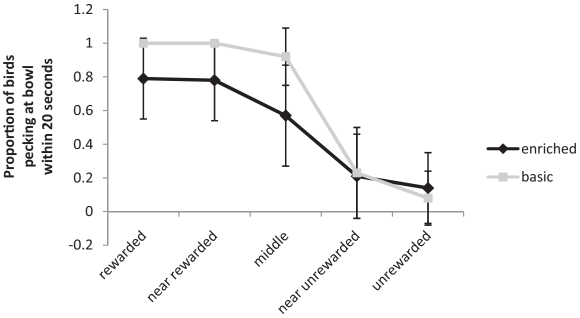

Fig. 2. Proportion of birds (±CI 95%) from the enriched and basic treatments which pecked at the bowl within 20 s in the cognitive bias test.

All analyses were carried out with PASW Statistics 18.

3. Results

3.1. Cognitive bias

Twenty-four out of 27 birds pecked at the bowl within a median time of 20 s when it was placed at the rewarded location, whereas when it was positioned at the unrewarded location only three birds did so. No significant differences were found between treatments in the number of birds which pecked at the bowl when the bowl was placed at the *rewarded* (Fisher's exact test; $P = 0.22$, $N = 27$), *near rewarded* ($P = 0.22$), *near unrewarded* ($P = 1.00$) and *unrewarded* ($P = 1.00$) locations. However, more birds from the basic treatment, though not significantly, pecked at the *middle* probe within 20 s compared to birds from the enriched treatment ($P = 0.08$, Fig. 2).

There was no difference between the first and second test periods in the number of birds that pecked at the bowls within 20 s for any of the five locations (all $P > 0.10$).

3.2. Anticipation

Overall the birds were spending most of their time close to the food bowl (22.4 ± 0.8 s, mean ± se) when in the anticipation arena. There was no difference between birds housed in the enriched or basic environment in their behaviour (Mann–Whitney *U* test; *time close to the bowl*, $P = 0.24$, $N = 32$; *head up*, $P = 0.58$; *head down in bowl*, $P = 0.47$; *head down other*, $P = 0.31$; *steps*, $P = 0.66$; *total number of movements*, $P = 0.60$) during the period after the positive signal. There was however an effect of test period for the behaviour after the positive signal, with birds being more active (more steps taken) in the first test period (first period 2.5 ± 0.5, second period 1.3 ± 0.3, $P = 0.04$, $N = 31$). Birds also spent more time close to the bowl, though not significantly, in the second test period (first period 21.5 ± 1.0, second period 23.5 ± 0.9, $P = 0.06$), but there was no difference in any other behaviour (i.e. all $P > 0.10$).

Birds spent significantly more time by the bowl (Wilcoxon test; $P = 0.003$, $N = 31$), put their *head up* ($P = 0.03$) and *head down in bowl* ($P = 0.000$) more often after the rewarded signal than they did after the control signal. There were no significant differences for *number of steps*

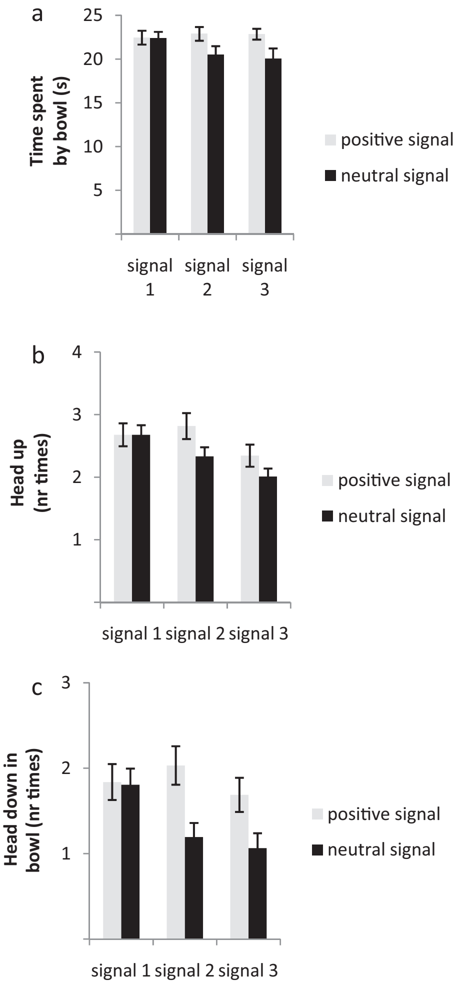

Fig. 3. Mean ± se of the time spent close to the bowl after the three signals during the same session as for the positive and neutral signals. For (a) time spent by the bowl, (b) head up, (c) head down in bowl.

(P = 0.17), *head down* (P = 0.18) or *total number of movements* (P = 0.60). Descriptive information of the amount of *time spent by the bowl, number of times head up* and *number of times head in bowl* after each of the three signals given within the same session indicates that the birds behaved similarly after the first signal and that differences between the positive and neutral signal occurred after the second and third signal (Fig. 3a–c).

3.3. Relationship between different tested parameters

The behaviour of the birds in the cognitive bias and the anticipation tests were correlated with the rate of negative interactions performed and received in the home pen, latencies in the novel object test and the amount of training needed not to approach the unrewarded bowl in the cognitive bias test. The negative interactions observed in the home pen during the first test period correlated with some of the behaviours during the anticipation test and the trend was that birds performing more negative interactions than they received, performed more *head up* and *head down in bowl* movements and tended to spend more *time by the bowl* in the anticipation test (Table 1). Longer latencies in the novel object test tended to correlate with longer latencies to approach the *near rewarded* probe in the cognitive bias test and shorter *time by the bowl* in the anticipation tests. There was an effect of the different amounts of training on the birds' performance in the cognitive bias test in that the number of times a bird needed to be trained *not* to go to the unrewarded bowl and time to the *near rewarded* probe were correlated, i.e. if the bird required more training they had a shorter latency to go to the probe. There was also a similar tendency for the *middle* probe but not for the *near unrewarded* probe (Table 1).

3.4. Influence of fear level on trainability for the cognitive bias test

The mean time of TI for all birds was 198 ± 30 s and there were two birds that remained in TI for the maximum time of 10 min. In the novel object test the mean latency to feed was 26 ± 5 s and 12 birds did not eat within 60 s. There was no difference in the fearfulness of birds, as assessed by their time in TI, between those birds that did and those that did not (due to not feeding reliably during training) proceed to carry out the cognitive bias test. However, the birds that did not proceed had a significantly longer latency in the novel object test (Mann–Whitney test; P = 0.05). In fact, out of the seven birds which never carried out the cognitive bias test, six of these did not feed from the metal container within 60 s in the novel object test. Although, they ate just as quickly from the container when the novel object was not present (4.5 ± 0.5 s for birds which carried out the cognitive bias test compared to 4.9 ± 0.7 s for birds which did not, one bird is not included here since it did not eat corn at all). There was a significant positive correlation between the TI and novel object tests (R_s = 0.57, P = 0.001, N = 32).

3.5. Comparisons of the physical state of the birds and their rate of negative interactions in the home pen between treatments and between test periods

There were no significant differences between treatments in *body weight*, *wounds* and *plumage condition* for either of the two test periods. There were, however, significantly more *negative interactions received* in the basic environment than in the enriched environment during the first test period (Mann–Whitney; P = 0.026), but there was no significant difference between treatments in the second test period (P = 0.93). There was no difference in body weight between the two test periods, but a significant decrease in the score of wounds (Wilcoxon signed rank test; first 0.74 ± 0.14, second 0.19 ± 0.09; P = 0.006) and improved plumage condition (first 1.97 ± 0.20, second 1.5 ± 0.23; P = 0.005) from the first to second test period. There was also a significant decrease in number of performed negative interactions by birds from the first to second test period (first 5.4 ± 1.2; second 2.8 ± 0.8; P = 0.02).

Table 1

Correlations between median latency to peck at bowl at the three ambiguous probes in the cognitive bias test, frequency of the different behaviours in the anticipation test, social situation in home pen (number of negative interactions performed minus number of negative interactions received,) TI, novel object test and training required to learn to avoid walking up to unrewarded bowl in the cognitive bias test. Spearman rank correlation coefficients (R_s) and significance values (P) are shown. P values <0.10 are presented.

	Negative interactions in home pen first test period		Tonic immobility (s)		Novel object (s)		Training required for the cognitive bias test	
Cognitive bias	R_s	P	R_s	P	R_s	P	R_s	P
Near rewarded	ns	ns	ns	ns	0.39	0.054	−0.72	0.001
Middle	ns	ns	ns	ns	ns	ns	−0.33	0.09
Near unrewarded	ns	ns	ns	ns	ns	ns	ns	ns
Anticipation								
Time close to bowl	−0.32	0.07	ns		−0.39	0.03	*	*
Steps	ns	ns	ns	ns	ns	ns	*	*
Head up	−0.37	0.04	ns	ns	ns	ns	*	*
Head down bowl	−0.50	0.004	ns	ns	ns	ns	*	*
Head down other	ns	ns	ns	ns	ns	ns	*	*
Total movements	−0.39	0.03	ns	ns	ns	ns	*	*

The asterisks indicate that no analysis of correlation between those parameters were carried out.

4. Discussion

Individual factors such as fear level, relationship to their conspecifics and motivation to feed were correlated to the birds' behaviour in the anticipation and cognitive bias tests. The home environment, on the other hand, did not give the expected results in the tests, since the only indication of a difference between treatments were the tendency for birds from the basic environment to approach the middle probe in the cognitive bias test more readily than the birds from the enriched environment. This suggests that individual differences were more influential than the supposed induction of emotional states on a group level in this experimental setting. From these results it is therefore difficult to draw any conclusion as to whether the anticipation and cognitive bias tests indicate the same emotional state.

The tendency for enriched birds to be slower to approach the middle probe in the cognitive bias test was contrary to the hypothesis since it was expected that birds housed in the enriched environment would have a better welfare, be more optimistic and therefore approach the bowls at the ambiguous cues faster than birds housed in the basic environment (Burman et al., 2008 (although see Parker, 2008; Burman et al., 2011 for studies with results opposite to predicted)). One explanation for the results in our study could be that the extra food, provided as part of the enrichment in the home pens, made the birds from this treatment less motivated to work for the food reward in the test. This would be in accordance with observations made of anticipatory behaviour where it has been found that animals lacking essential stimuli react more to different stimuli, both rewarding and aversive, than animals kept under more stimulating circumstances (Spruijt et al., 2001). An alternative explanation is that the differences between the basic and the enriched environment were too small (see e.g. Doyle et al., 2011). In the original study showing cognitive bias in rats, the rats had been exposed to a considerable level of disturbance (Harding et al., 2004). All our birds on the other hand had access to a perch, nest, scratching possibilities and at least limited dust bathing, which are known to be important for their welfare (Weeks and Nicol, 2006), and it is not clear how much better the enriched environment was compared to the control, even if the quantity and quality of the resource varied between the treatments.

In most other studies of cognitive bias a transition from good to worse, i.e. a reduction in environmental conditions from enriched to non-enriched has given the predicted results that the animals with the assumed worse welfare has responded more negatively to the ambiguous cues (Burman et al., 2008; Harding et al., 2004). In line with this Bateson and Matheson (2007) found no difference in judgement bias for starlings which experienced an improvement in their housing, whereas those that experienced a decline of their environment did show a difference. These authors suggested that judgement bias was more indicative of a negative affective state (Bateson and Matheson, 2007). Although, there are now a few studies which have shown indications of increased optimism in the cognitive bias test (Doyle et al., 2010; Burman et al., 2009). Thus a third possible explanation for our results is the birds' previous experience of being kept in cages. For the birds whose environment was converted to a poorer one before the first test, the basic environment might still have been experienced as relatively good compared to the cages they were used to before they entered the study.

In combination, such findings indicate that interpretations of results in cognitive bias tests might not be as straightforward as was originally supposed, and that many parameters need to be considered. In particular we suggest that these are parameters related to the individual; their characteristic such as fearfulness and health status, as well as parameters related to their unique role in the dynamics in the group e.g. social interactions. These will influence the individual's affective state and interact in complex ways with the treatment to which all individuals and groups are exposed. More work is clearly needed to investigate the relative nature of these biases, their interactions and even how the aspects of the treatment interact with aspects of the test, like the animal's willingness to work for the reward. For example, given the results in our study one might speculate that the biologically relevant food enrichment improved the mood of the birds whilst at the same time making them less motivated for the food rewards. The amount of training required before the animals could be tested in the cognitive bias test had two main drawbacks.

The first one was that training could have had an influence on the birds' emotional state, such as for example being a positive stimulation for the animals that lessens the effect of the housing conditions. The second point is that it also tends to reduce the number of animals that is practically possible to test. In our study, that, in combination with the loss of birds, led to a fairly low sample size. This paired with individual variation reduced the possibility of detecting more subtle differences between treatments (if such existed).

Moe et al. (2009, p. 174) described anticipatory behaviour in laying hens as a "duration of standing still or walking with slow steps, with legs, body and neck stretched upwards and eyes open, head sometimes moving up and down and kept at an angle, sometimes but not always directed at the light source and/or reward bowl". In our anticipation test, the birds were moved to a separate arena and after entering they spent most of the time close to the bowl and were attentive towards the pipe which delivered the food reward. Thus according to Moe's description they would all probably have been scored as showing anticipatory behaviour during their stay in the arena. This suggests that placing the birds in the test arena itself induced anticipation, which would be supported by the findings in our study comparing the response of birds to the positive and control signals. Although, the birds responded differently in the positive and control sessions, i.e. birds stayed closer to the bowl when there were positive (rewarded) signals but tended to spend more time away from the bowl after repeated control (non-rewarded) signals, this difference was not evident until after the second and third signal.

In conclusion, individual factors like the birds' motivation to feed in the tests and social interactions in the home pen influenced behaviour in the anticipation and cognitive bias test more than the different treatments. This suggests that individual differences should be taken into consideration and that using animals as their own control could help in detecting more subtle treatment effects.

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