Behaviour of domestic fowl in anticipation of positive and negative stimuli

P.H. Zimmerman [a,*], S.A.F. Buijs [a,1], J.E. Bolhuis [b,2], L.J. Keeling [a]

[a] Department of Animal Environment and Health, Swedish University of Agricultural Sciences
[b] Adaptation Physiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, The Netherlands

Underlying the study of animal welfare is the assumption that animals experience emotional states. Although there has been a bias towards studying negative emotions, research into positive emotions is necessary for an overall welfare assessment. The aim of the current study was to find behavioural expressions specific for anticipation of different events in domestic fowl, Gallus gallus domesticus. To this aim, we used a Pavlovian conditioning paradigm by which we induced anticipation of a positive, neutral and negative event. We investigated whether birds were able to discriminate between sound cues signalling these events with different valences and, if so, whether anticipation of different events is reflected in different behavioural responses. The birds showed a response of increased attention to all sound cues. In anticipation of the negative event, the birds showed more head movements and loco-motion than in anticipation of both the neutral and positive event, possibly reflecting the aversive nature of the negative event. In anticipation of the positive event, the birds showed more comfort behaviours, such as preening and wing flapping, which have been associated with a state of relaxation. Our study shows that laying hens are able to anticipate differentially a positive, neutral and negative event announced by different sound cues. It is also the first study to identify comfort behaviours as specifically associated with anticipation of a positive event in domestic fowl. Comfort behaviours may therefore be associated with a positive emotional state in domestic fowl.

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The assumption that animals experience emotional states (Dawkins 1990) has led to an increased number of studies aimed at the investigation of such states in animals to evaluate their welfare (e.g. Paul et al. 2005; Boissy et al. 2007; Düpjan et al. 2008). Although it has been pointed out that good welfare is not simply the absence of negative emotional states, but also the experience of positive affective states (Dawkins 1983; Fraser 1995; Duncan 1996; Seligman & Csikszentmihalyi 2000), there has been a bias towards the study of negative emotions in both animals and humans (Paul et al. 2005; Burgdorf & Panksepp 2006). An abundance of studies have identified behavioural and physiological indicators of poor welfare (e.g. see Broom 1986; Broom & Johnson 1993; Wiepkema & Koolhaas 1993). Studies providing indicators of positive affect and positive welfare in animals are, however, rather scarce. Boissy et al. (2007) therefore advocated research in the area of positive emotions with a view to being able to apply the knowledge gained for improving animal health and welfare.

Behavioural indicators of positive welfare could be identified by experimentally inducing a positive emotional state. One of the approaches to induce such a state in animals is the anticipation or expectation of a positive event or stimulus (Spruijt et al. 2001; Boissy et al. 2007). Positive anticipation can be defined as 'responses elicited by rewarding stimuli that lead to and facilitate consummatory behaviour' (Spruijt et al. 2001, page 160). The stimulus that signals the arrival of a positive event elicits a behavioural response, that is, positive anticipatory behaviour. The types of behaviour that are displayed during anticipation of a reward or positive event appear to be species specific. In rats, Rattus norvegicus (Van der Harst et al. 2003a, 2005), mink, Mustela vison (Vinke et al. 2004) and pigs, Sus scrofa (Dudink et al. 2006), anticipation of a positive event is characterized by increased activity and a rise in behavioural transition patterns. On the other hand, cats, Felis catus, decrease activity during anticipation of a food reward (Van den Bos et al. 2003). The current experiment was aimed at investigating whether anticipation of different types of stimuli is reflected in different anticipatory behaviour in the laying hen, Gallus gallus domesticus.

The conditions under which laying hens are kept remain a major animal welfare concern. It is one of the most intensive forms of animal production and the total egg-laying flock in Europe comprises

approximately 250 million birds (Blokhuis et al. 2007). Although there has been research to induce anticipatory behaviour in laying hens in a conditioning paradigm to study positive affect (Moe et al. 2009), it is still unknown whether domestic fowl exhibit different behaviours in anticipation of a positive event compared to a negative or neutral event. Behaviours that have been identified as potential indicators of positive affect in a variety of species are play behaviour, self-grooming and vocalizations (Boissy et al. 2007). Play behaviour (e.g. Bekoff & Byers 1992; Spinka et al. 2001) does not seem to be a good candidate for the study of positive affect in birds because it is mainly displayed by juvenile birds (Broom & Fraser 2007). Grooming in birds has been associated with a state of relaxation (Spruijt et al. 1992) or de-arousal (Delius 1967; Savory & Kostal 2006). Others have found, however, that self-grooming increases in response to a thwarting or conflict situation (Duncan & Wood-Gush 1972a, b) as does stereotyped pacing (Duncan & Wood-Gush 1972b). Also in rats, some studies reported an increase in self-grooming in situations that indicate poor welfare (Raab et al. 1986; Tornatzky & Miczek 1993; Hurst et al. 1997; D'Aquila et al. 2000), whereas others reported an increase in self-grooming under conditions that indicate positive welfare (e.g. Hurst et al. 1996; Baumans 2004). In rats, vocalizations have been identified that are thought to signal positive affect (e.g. Panksepp & Burgdorf 2003). Vocalizations in domestic fowl have been well studied but mainly in relation to a negative affective state, such as stress (Marx et al. 2001; Manteuffel et al. 2004) and frustration (for example, the gakel call, Zimmerman & Koene 1998; Zimmerman et al. 2000).

The aim of the current study was to find behavioural expressions specific for anticipation of different events. To this aim, we used a Pavlovian conditioning paradigm by which we induced anticipation of positive, neutral and negative events. We investigated whether birds were able to discriminate between cues signalling events with different valence and, if so, whether anticipation of different events is reflected in different behavioural responses.

METHODS

Subjects and Housing

Eighteen Lohman LSL laying hens of approximately 4 months of age were used in this study. The hens had previously been used in an experiment in which they had to go through a push-door to get access to a dust bath (Wichman & Keeling 2008) and so were already familiar with the facilities and equipment. The hens were housed in floor pens measuring 150×150 cm in groups of two to five birds per pen. The floor of the home pens was covered with wood shavings and each pen contained two perches (at a height of 10 and 20 cm) and one nestbox. Food (standard layer crumble) and water were available ad libitum, but only outside the experimental procedures. Lights were on between 0800 and 2000 hours. Hens could be individually recognized by coloured leg rings. The methods used in this study were approved by the Swedish Regional Ethical Board. After the experiment, all birds were euthanized by cervical dislocation after prior stunning, in accordance with national legislation.

Treatment Groups

Anticipatory behaviour was induced by subjecting the hens to a Pavlovian conditioning paradigm in which an initially neutral stimulus (conditioned stimulus, CS) was repeatedly paired with a positive (food: mealworms, which are known to be highly rewarding for hens; Bruce et al. 2003) or a supposedly negative (water spray) or neutral event (nothing) which served as unconditioned stimuli (US). As conditioned stimuli we used three different sound cues: 'music' (the first 15 s of the Gabriel Rios' song 'Broad

daylight'), 'beep' (a repeated 15 s beeping sound) and 'ring' (15 s ringing of an old-fashioned telephone). A 'muted' treatment (no CS and US) was used to control for the effect of sound per se in the other treatments. The sound cues were played from a computer at a sound pressure level of 65 dB. Each of the three sound cues (music, beep and ring) was used to signal each type of US. As a result, we had three cue groups of six hens that each had different combinations of CS and US (see Table 1). For half of the six hens in each cue group, the orientation of the experimental pen was rotated by 180° to control for hens' possible preference in orientation.

Experimental Apparatus

The experimental pen (see Fig. 1) was located in a room adjacent to the room in which the birds were housed. The experimental pen measured 200×125 cm and its walls were 60 cm high.

The experimental pen consisted of two compartments (a waiting compartment and a reward compartment) that were separated by a wire-mesh partition and connected by a push-door in the middle of the partition. The push-door could be locked and released by, respectively, increasing or decreasing a current going through an electromagnet attached to the door (see Olsson et al. 2002). The push-door only opened in the direction of the reward compartment. The walls of the experimental pen consisted of plywood, except for one of the walls of the waiting compartment, which was made of wire-mesh to allow video recordings from the side. A lamp, with a light at 1.5 m from the floor, was placed outside the pen adjacent to the partition. It could be operated by the experimenter with a foot-switch. This light, which shone into the reward compartment, was initially used to highlight the food bowl. Another light, at floor level next to where the experimenter was sitting, was also operated by the experimenter, but this light was not visible to the hens. It was switched on during tests to mark the onset of the CS. This light on the video made it possible to analyse the video files without sound, so the experimenter coding the videos would be 'blind' to the treatments. During tests, the experimenter sat behind a hardboard screen, out of sight of the hens.

A blue food bowl was present in the reward compartment. A few days before training started, this blue bowl, containing sunflower seeds, was placed in the hens' home pen to accustom the hens to feeding from the bowl. All hens readily ate the seeds from the bowl.

Training Procedure

The training consisted of several phases and each bird was required to reach criterion before moving on to the next phase. We started with training to the positive stimulus, then the neutral and finally the negative one.

In Phase 1 (Day 1), the birds were encouraged to push the door. All birds from one pen were put in the waiting compartment of the experimental pen. A trail of sunflower seeds led through the push-door, which was closed but unlocked, to the blue bowl containing sunflower seeds in the reward compartment. This was done to encourage the hens to go through the push-door and explore the reward compartment. After all hens of a pen had entered the reward

Table 1
Overview of the groups and combinations of conditioned stimulus and unconditioned stimulus (US)

	Conditioned stimulus		
	'Positive'	'Negative'	'Neutral'
Cue Group 1 (*N*=6)	US='ring'	US='beep'	US='music'
Cue Group 2 (*N*=6)	US='music'	US='ring'	US='beep'
Cue Group 3 (*N*=6)	US='beep'	US='music'	US='ring'
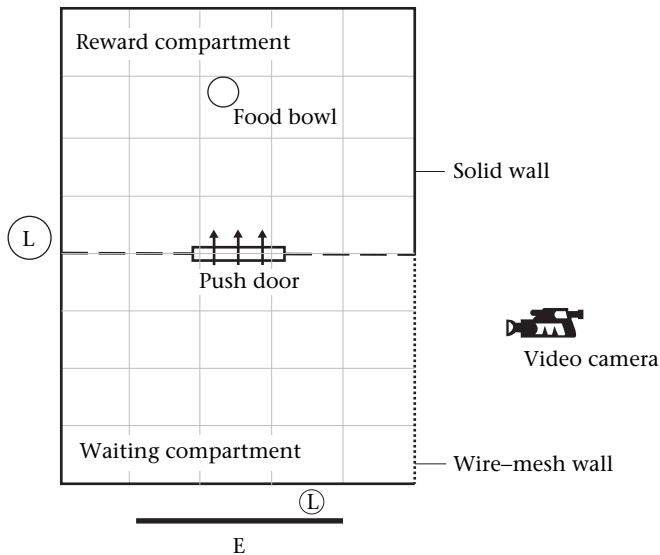
Figure 1. Schematic drawing of the experimental pen. 'E' indicates the position of the experimenter who sat behind a barrier; L indicates the position of a lamp.

compartment, and had eaten the sunflower seeds, they were returned to their home pen.

In Phase 2 (Days 2–4), individual birds were shaped to use the unlocked push-door. For this purpose, birds were mildly food deprived for, on average, 2.5 h (maximum: 5 h). A shaping session consisted of three consecutive trials: a bird was carried to the experimental pen and put in the waiting compartment. The push-door was kept unlocked. As soon as the bird approached the push-door from its current position in the waiting compartment, its specific positive CS (according to its cue group) was given and the light highlighting the blue food bowl, containing two mealworms, was switched on. After the bird had gone through the push-door to the reward compartment and had eaten the mealworms, the light and sound were switched off and the bird was gently guided back through the push-door, held open by the experimenter, to the waiting compartment. If the bird did not go back through the push-door to the waiting compartment voluntarily, she was picked up by the experimenter and put back into the waiting compartment via the push-door. As soon as the bird was in the waiting compartment again, the push-door was closed behind her and another two mealworms were put in the bowl. If a bird approached the push-door when the CS and light signal were switched on, but she did not go to the reward compartment within 10 min, she was gently pushed through the push-door. A bird progressed to the next phase of training when she approached (and stepped through) the push-door immediately after she returned to the waiting compartment and the door closed behind her. All birds reached this phase after 3 days of training.

In Phase 3 (Days 4–7), the CS and light signal were made contingent on the behaviour of the bird. For this purpose, the push-door was kept locked and a bird was allowed to try to push through the door twice. The third time the bird approached the door, the CS and light signal were given and the push-door was simultaneously unlocked to allow the bird to push through. This procedure was executed three times for all birds, after which the CS and light signal were given at random times when the bird was not near the door. In this phase, a training session lasted 8 min per day. When a bird went through the push-door immediately after the CS and light signal had been given in five consecutive trials, the training session was ended and the bird returned to her home pen. All birds reached the criterion by day 8 of training.

In Phase 4 (Days 8–10), the CS and light signal were made noncontingent on the behaviour of the birds. For this purpose, a delay conditioning procedure (Lieberman 2000) was used. The interval between the start of the CS and the light signal was gradually increased to 15 s. At the end of the 15 s, the CS stopped and the light signalling the push-door was now open came on. Birds reached criterion when they went through the push-door within 5 s after the lights had been switched on.

In Phase 5 (Days 11–13), all birds were introduced to their neutral CS. The procedure was the same as in the positive training trials, except that in the sound-neutral trials nothing happened (the door did not open, the hen was not sprayed with water (see Phase 6)) after the light had been switched on. In Phase 6 (Day 14), all birds were also introduced to their negative CS. The procedure was the same as in the positive trials, except that after the end of the sound cue, when the light was switched on, the birds were squirted with water once on the back using a water pistol. The birds learnt this association very quickly, judged by the escape behaviour they exhibited after a couple of negative trials. The push-door did not open in either the sound-neutral or the negative trials. In Phase 7 (Days 15–22), birds were given positive, neutral and negative trials in a randomized order. The total number of trials varied throughout the days, but the number of positive trials was at least as high as the sum of the number of neutral and negative trials, to try to ensure that the hens would not develop a negative association with the experimental conditions. The birds were considered to have completed training when, despite the mix of negative, neutral and positive cues, all 18 birds met the criterion for the positive trials. That is to say, they went through the push-door within 5 s after the positive CS had ended in nine of 10 trials.

Test Procedure

For testing, a bird was collected from her home pen and put in the experimental pen. Birds were not deprived of food. The order of testing was balanced between sound cue groups and, within groups, between home pens. Each hen received one test session per day, on 4 consecutive days. At the start of each session, the hen was allowed to habituate to the experimental pen for 1 min. A session consisted of eight trials which were composed of five trials with a positive (POS) treatment, one negative (NEG) treatment, one sound-neutral (SN) treatment and one muted-neutral (MN) trial. A session always started with a POS trial and in the rest of the session a POS trial was equally often preceded by a POS, NEG, SN or MN trial. This order was changed every test day and was the same for all birds.

After the start of each trial, the baseline behaviour of a bird was video recorded during a 15 s waiting period. Then the appropriate CS was given for 15 s in the anticipation period, during which behaviour was also video recorded. In a POS trial, after the end of the 15 s CS, the light was switched on signalling that the push-door was unlocked, the bird pushed through the door and ate the mealworms. Then the light signal was switched off and the bird was pushed back gently to the waiting compartment by the experimenter, who held the push-door open. In a NEG trial, after the end of the 15 s CS, the bird got a squirt of water on the back. In an SN trial, after the end of the 15 s CS, nothing happened. In an MN trial, no sound cue was given. Between the end of a trial and the start of a new waiting period, there was an intertrial interval of 10, 20 or 30 s. This intertrial interval was balanced between hens and was used to prevent them from anticipating the start of the next trial. This intertrial interval started when the experimenter had returned behind the screen after putting new mealworms in the bowl in the reward compartment. When the next trial was NEG, SN or MN, the experimenter pretended to put mealworms in the bowl (see Fig. 2).
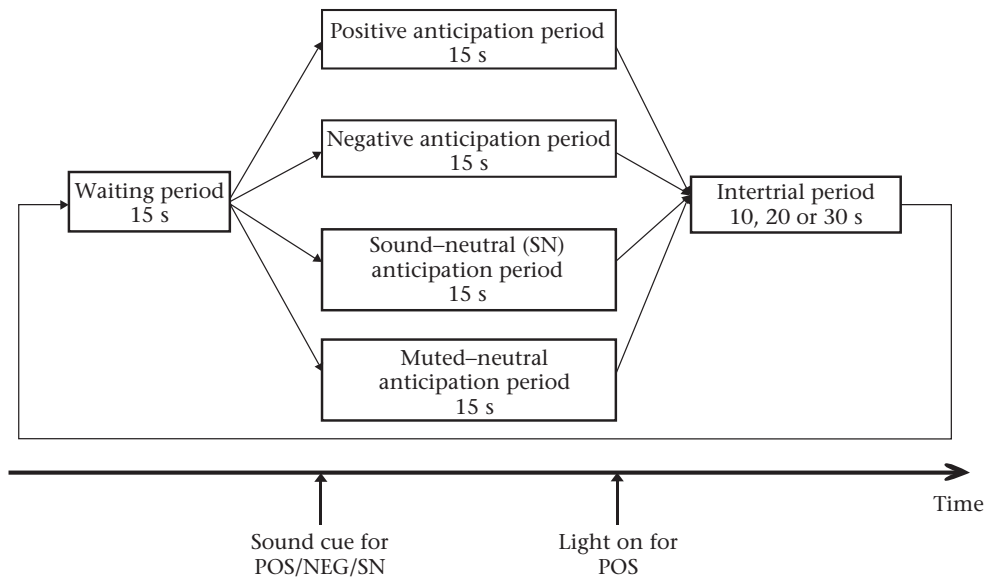
Figure 2. Schedule of testing in one trial.

Behaviour Analysis

The frequencies of the behaviours listed in Table 2 were scored from video recordings. Tail wagging was not observed during the trials. Pecking the cage occurred very infrequently and was therefore omitted from further analyses. The behaviours wing flapping, preening foot and feather ruffling were observed only during POS treatment trials, and therefore could not be subjected to separate statistical analyses for treatment effects. Similarly, scratching body occurred only in the SN and POS treatments and preening other only in the NEG and POS treatments. These behaviours (wing flapping, preening foot, preening other and feather ruffling) were therefore combined with preening neck/chest, yawning and scratching body and analysed as one heterogeneous category labelled 'comfort behaviour' (see also Table 2) which is a generic and commonly used term to refer to activities that may be involved in feather/body maintenance in domestic fowl (e.g. Black & Hughes 1974; Nicol 1989; Tanaka & Hurnik 1991; Sherwin & Kelland 1998; Carmichael et al. 1999; Appleby et al. 2002; Albentosa & Cooper 2004; Shimmura et al. 2007) and other avian species (Ainley 1974; Van Rhijn 1977; Delius 1988; Kreger et al. 2005). Preening neck/chest and yawning were also analysed separately for treatment effects. Only one type of vocalization, the gakel call (Zimmerman & Koene 1998; Zimmerman et al. 2000), was recorded during the video analysis.

Statistical Analysis

The data were analysed using general linear models in the SAS system (version 9.1.3, SAS Institute, Cary, NC, U.S.A.). Skewed distributions of frequencies were square-root transformed to achieve homogeneity of variances. Preliminary analysis showed no effects of day or orientation of the experimental pen. Therefore, day and orientation were omitted from the final models, and data from the 4 days were averaged per bird and treatment.

First, the effect of period (waiting versus anticipation period) on behaviour was analysed for each of the treatments. The aim of the muted (MN) treatment was to control for spontaneous changes in behaviour in the anticipation period as compared with the waiting period. No effect of period was found for any of the behaviours recorded in the MN treatment, indicating that changes in behaviour in the other three 'sound' treatments could not be attributed to the experimental procedure per se. Once this was demonstrated, the MN treatment was not included in further comparisons between treatments. Subsequently, the effect of the sound treatments (neutral, SN; positive, POS; or negative, NEG) on behaviour during the anticipation period was analysed using a model which included effects of cue group (i.e. the three groups with different sound–treatment combinations) and its interaction with treatment. Effects of cue group were tested against the effect of bird within cue group. Finally, to discern possible carryover effects, behaviour in the waiting and anticipation period of POS trials was analysed with a model that included preceding treatment (POS, NEG, SN or MN), cue group and bird within cue group as factors. Significant effects were further analysed using post hoc tests with Bonferroni adjustments for multiple comparisons. Data are presented as raw means ± SEM, both in the text and in Table 3.

Table 2
Ethogram

Name	Description
Foraging behaviour	Pecking at ground or moving foot backwards over ground
Pecking cage	
Pecking mesh	Pecking at wire-mesh dividing the rooms
Pecking wall or roof	Pecking at walls or roof of test pen
Head flicking	Short, vigorous shaking of the head
Head movement	Any movement of the head not (part of) a peck, preen, ruffle, head flick or yawn
Stepping	Lifting foot and putting it down again
Standing alert	Shifting front of body upwards while neck is stretched up
Comfort behaviour	
Preening neck/chest	Moving beak along feathers of neck and chest
Preening foot	Moving beak along its foot
Preening other	Moving beak along feathers, excluding neck and chest
Wing flapping	Lifting wings and flapping them
Feather ruffling	Stretching neck, raising ruff and ruffling feathers and body
Scratching body	Moving foot along feathers
Yawning	Opening mouth widely without vocalizing
Tail wagging	Lowering tail and moving it rapidly from side to side in the horizontal plane
Gakel call	An elongated vocalization followed by a few short notes

Table 3
Changes in behaviour in the anticipation period as compared with the waiting period for the muted-neutral (MN), negative (NEG), sound-neutral (SN) and positive (POS) treatments

Behaviour (frequency)	MN	NEG	SN	POS
Foraging behaviour	NS	↓***	↓***	↓***
Head flicking	NS	↑***	↑***	↑***
Head movement	NS	↑***	↑***	↑***
Stepping	NS	↑***	↑**	NS
Standing alert	NS	NS	NS	↑*
Comfort behaviour	NS	↓*	↓†	↑†
Gakel call	NS	NS	↓*	↓*

NS = no significant period effect; ↑ = increase and ↓ = decrease in anticipation period as compared with waiting period.
†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

RESULTS

Effects of Period (Waiting versus Anticipation)

Effects of period are summarized in Table 3.

In all sound treatments, we found an increase in the number of head movements ($F_{1,17} = 58.0, 25.8, 38.2$, for NEG, SN and POS, respectively, $P < 0.001$) and head flicks ($F_{1,17} = 19.3, 23.8, 16.9$, for NEG, SN and POS, respectively, $P < 0.001$) in the anticipation compared to the waiting period. Frequency of foraging behaviour was lower in the anticipation than in the waiting period for all sound treatments ($F_{1,17} = 30.5, 21.0, 38.7$ for NEG, SN and POS, respectively, $P < 0.001$).

In the SN and NEG treatments, we found an increased number of steps ($F_{1,17} = 15.6, P < 0.01$ and $F_{1,17} = 58.5, P < 0.001$, respectively) in the anticipation compared to the waiting period, but not in the POS treatment. Standing alert increased only in the POS treatment ($F_{1,17} = 4.8, P < 0.05$) from the anticipation to the waiting period. Comfort behaviour tended to increase in the anticipation period of the POS treatment as compared to the waiting period ($F_{1,17} = 3.5$, $P = 0.08$), whereas it decreased more than three-fold in the NEG

treatment ($F_{1,17} = 5.7, P < 0.05$). Furthermore, comfort behaviour tended to decrease in the SN treatment ($F_{1,17} = 3.8, P = 0.07$) in the anticipation period compared to the waiting period. We found a decrease in the number of gakel calls in the anticipation period compared to the waiting period of the SN ($F_{1,17} = 6.7, P < 0.05$) and POS treatment ($F_{1,17} = 6.4, P < 0.05$), but not in the NEG treatment.
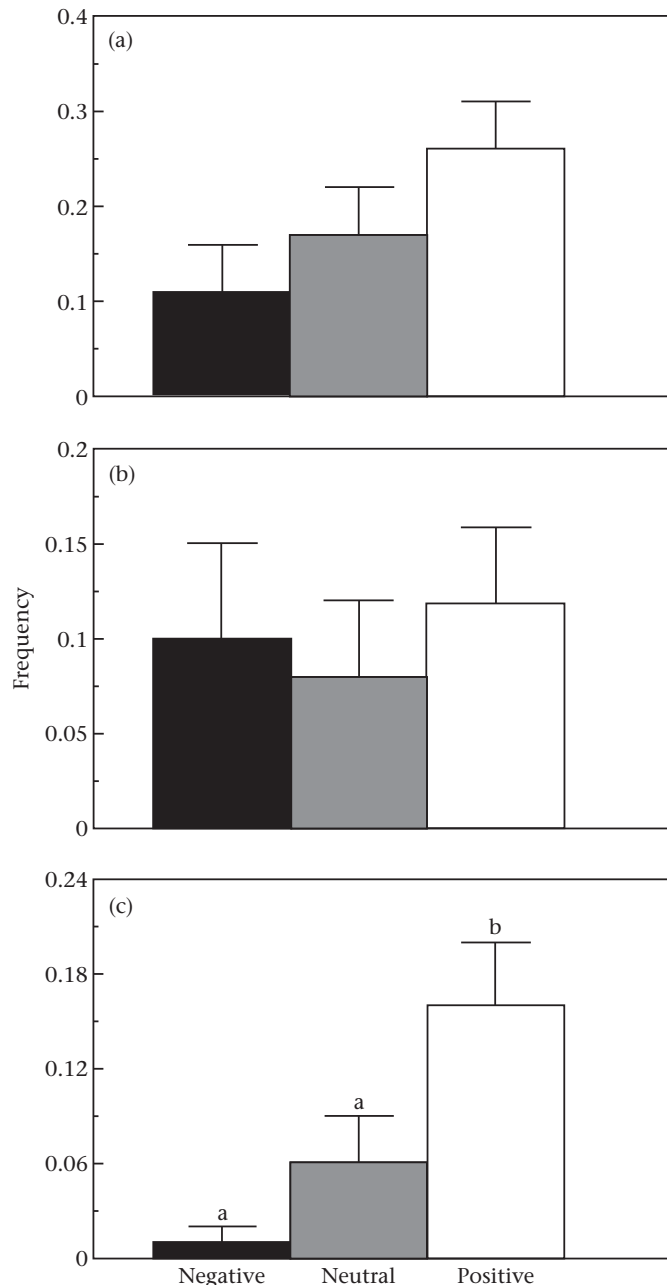
Effects of Treatment

Effects of treatment (NEG, SN or POS) on behaviour during anticipation periods are given in Figs 3 and 4. In anticipation of a NEG event, birds showed less foraging behaviour (treatment effect: $F_{2,30} = 5.5$, $P < 0.01$) and more head movements ($F_{2,30} = 9.5$, $P < 0.001$) than in anticipation of a POS or SN event (see Fig. 3). Head flicking was not affected by treatment ($P = 0.78$). There was an effect of treatment ($F_{2,30} = 39.4, P < 0.001$) on the number of steps. The number of steps was higher in the NEG treatment than in the SN and POS treatment, and higher in the SN than in the POS treatment. We also found an effect of the interaction treatment*cue group on the number of head movements ($F_{4,30} = 13.4, P < 0.001$), head flicks ($F_{4,30} = 18.6, P < 0.001$) and steps ($F_{4,30} = 4.6, P < 0.01$). Post hoc analysis revealed that in the NEG treatment, 'music' as a cue led to more head movements, steps and head flicking than 'beep' as a cue.

Frequency of standing alert and gakel calls during the anticipation period were not significantly affected by treatment ($P = 0.13$ and 0.82, respectively; Fig. 4). There was an effect of cue group on the frequency of standing alert ($F_{2,15} = 4.0, P < 0.05$). Birds from cue group 2 (NEG = 'ring', SN = 'beep', POS = 'music') displayed more standing alert than birds from the other two cue groups.

Comfort behaviour was shown more in anticipation of a POS event than in anticipation of a NEG or SN event ($F_{2,30} = 9.5, P < 0.001$; Fig. 4). Frequencies of the different comfort behaviours separately are given in Table 4. Birds displayed more preening neck/chest in the POS treatment than in the NEG treatment, with levels in the SN treatment in between ($F_{2,30} = 4.2, P < 0.05$). Yawning showed the same pattern but was not significantly affected by treatment ($F_{2,30} = 1.9, P = 0.17$).
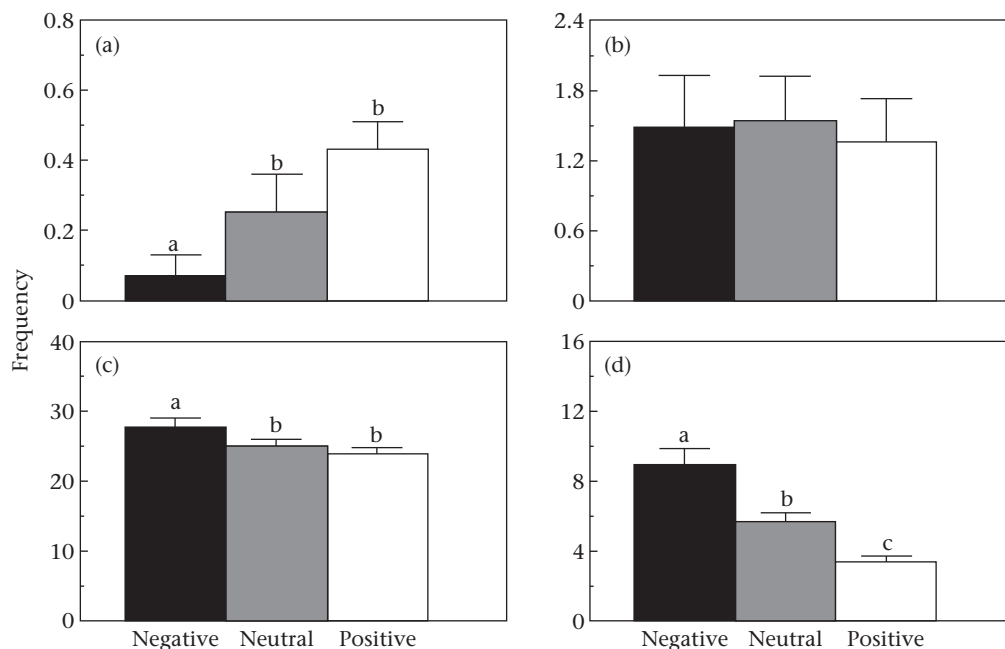
Figure 3. Mean frequency (per 15 s) + SEM of (a) foraging behaviour, (b) head flicking, (c) head movements and (d) stepping of birds during the anticipation of a negative (black), neutral (grey) or positive (white) event. Different letters (a, b, c) indicate significant differences ($P < 0.05$). Note different scales on the Y axes.

Figure 4. Frequency (per 15 s) + SEM of (a) standing alert, (b) gakel calls and (c) comfort behaviour of birds during the anticipation of a negative (black), neutral (grey) or positive (white) event. Different letters (a, b) indicate significant differences ($P < 0.05$). Note different scales on the Y axes.

Table 4
Frequencies (per 15 s) of comfort behaviours in the anticipation period of the negative (NEG), sound-neutral (SN) and positive (POS) treatments

Behaviour	NEG	SN	POS	P
Total comfort behaviour	0.042±0.023[a]	0.083±0.035[a]	0.247±0.069[b]	0.0006
Total preening	0.028±0.019[a]	0.028±0.019[a]	0.114±0.045[b]	0.013
Preening neck/chest	0.014±0.014[a]	0.028±0.019[ab]	0.072±0.033[b]	0.024
Preening foot	0	0	0.022±0.012	
Preening other	0.014±0.014	0	0.019±0.009	
Wing flapping	0	0	0.003±0.003	
Feather ruffling	0	0	0.025±0.007	
Scratching body	0	0.014±0.014	0.031±0.025	
Yawning	0.014±0.014	0.042±0.030	0.075±0.031	0.17

For some behaviours, no P value is given because the behaviour did not occur in all three types of treatment. Different letters (a, b) indicate significant differences between treatments ($P < 0.05$).

(24.0 ± 0.9) or MN (22.9 ± 1.2) compared to SN (22.7 ± 1.3). The number of steps was higher when the preceding treatment had been NEG (4.0 ± 0.5) compared to SN (2.8 ± 0.4).

DISCUSSION

The present study shows that laying hens differentially anticipate a positive, negative and neutral event. Anticipation of the positive event was specifically associated with an increase in comfort behaviour, although we also discuss possible additional associations with standing alert and increased foraging behaviour. Anticipation of a negative event was associated with an increase in activity.

Overall Effect of Sound Cues

The behavioural reaction to the CS, irrespective of the associated US, was generally one of increased attention. In all three sound treatments (POS, SN and NEG), we found an increase in the number of head movements, head flicks and steps, and a decrease in foraging behaviour compared to the muted-neutral control. The increase in head movements can be seen as an indicator of increased attention, as it is known that by swinging their heads from side to side birds scan the environment with different, specialized parts of their eyes (Dawkins 2002). Furthermore, by increasing their head movements the birds might have been trying to localize the source of the sound, as we did not use any particular visual cues in this experiment. The decrease in foraging behaviour might be explained by the birds paying more attention to the environment in all three sound treatments.

Effect of Negative Treatment Cues

In the NEG treatment, 'music' elicited more steps and head flicks than 'beep'. This was possibly the result of the birds being more alert because of the 'music' cue being continuous and more variable than the intermittent 'beep'. Similarly, birds in cue group 2, in which 'music' announced a POS event, showed more standing alert than birds from the other two cue groups. Although balanced in our study, in future, the nature of the sound cue should be taken into account when using sounds as CS in conditioning experiments with domestic fowl. Alternatively, cues other than sound should be used.

Overall, when the NEG treatment was cued, birds showed more head movements than when the SN and POS treatments were cued. Head movements could be an expression of the anticipation of a negative event in general (e.g. increased vigilance), or an expression of the particular type of negative event used in the current study, that is, a squirt of water on the back. Furthermore, the higher

Carryover Effect

In the waiting period of the POS treatment, we found an effect of preceding treatment on the number of head movements ($F_{3,51} = 4.4$, $P < 0.01$) and steps ($F_{3,51} = 6.9$, $P < 0.001$). The number of head movements was higher when the preceding treatment had been POS (18.5 ± 0.8) compared with SN (15.7 ± 0.8). The number of steps was higher when the preceding treatment had been NEG (4.2 ± 0.5) compared with SN (2.1 ± 0.3). The carryover effects in the anticipation period of the POS treatment were almost identical to those found in the waiting period. We found an effect of preceding treatment on the number of head movements ($F_{3,51} = 5.3$, $P < 0.01$) and steps ($F_{3,51} = 3.4$, $P < 0.05$). More head movements were shown when the preceding treatment had been POS (25.1 ± 0.8), NEG

number of steps seen in the NEG treatment compared with the SN and POS treatments might be interpreted as a stimulus-specific response. Stepping occurs in normal walking, but also in pacing behaviour, which is considered to be a frustration-related behaviour (Duncan & Wood-Gush 1972b). Whether birds were walking or pacing in the current study, the increased number of steps probably reflected the intention to escape a potentially aversive situation. We certainly saw that in the NEG treatment the hens moved away from the squirt of water. This is in line with the findings of Hansen & Jeppesen (2006) who suggested that the withdrawal behaviour of their mink was a specific response in anticipation of being trapped and taken out of their cage.

Effect of Positive Treatment Cues

In the POS treatment, we found no increase in the number of steps between the waiting and the anticipation period. Furthermore, the lower number of steps in the POS treatment compared with the SN and the NEG treatment is in accordance with the lower locomotory activity seen during anticipation of a food reward in cats (Van den Bos et al. 2003), but in contrast with anticipation experiments using rats (Van der Harst et al. 2003a, b, 2005), mink (Vinke et al. 2004; Hansen & Jeppesen 2006) and silver foxes, *Vulpes vulpes* (Moe et al. 2006). Van den Bos et al. (2003) explained the difference in anticipation behaviour between rats and cats as a difference between normal foraging behaviour of these species: rats search for food, while cats adopt a 'sit-and-wait' strategy when close to prey. This difference in foraging behaviour cannot explain the decreased activity in anticipation of a food reward in our birds, as their foraging behaviour resembles that of the rat (i.e. generally increased locomotion). A possible explanation might be the shorter anticipation period in our experiment. In the experiments mentioned above, the anticipation period between CS and US was between 1 and 10 min. Moe et al. (2006) found an increase in stereotypies during anticipation, which could indicate frustration. As the current experiment was aimed at finding a behavioural indicator of a positive emotional state, we deliberately chose to use a short anticipation period to avoid frustration. Indeed, we did not find higher levels of gakel calls or pacing behaviour, which are considered to be expressions of frustration (Duncan & Wood-Gush 1972b; Zimmerman & Koene 1998; Manteuffel et al. 2004), in the POS treatment compared to the SN and NEG treatments, implying that we were successful in minimizing frustration. This is supported by the fact that we found a decrease in the number of gakel calls in anticipation of a positive event as compared to the preceding waiting period. Finally, in the POS treatment, the number of steps did not differ between the waiting and the subsequent anticipation period, while the number of steps increased in the SN and NEG treatments, that is, locomotion showed an increase in the SN and NEG treatments, rather than showing a decrease in the POS treatment. Therefore, it is our conclusion that locomotion/general activity does not seem to be a good indicator of positive anticipation, at least in the laying hen, and that it does not seem to reflect a species-specific foraging strategy as suggested by Van den Bos et al. (2003).

Foraging behaviour also does not seem to be a good indicator of positive anticipation in domestic fowl. Foraging behaviour actually decreased in the anticipation period compared with the preceding waiting period for all treatments in this study, but less so in the POS treatment. The decrease in foraging behaviour in the POS treatment was probably smaller than in the NEG and SN treatments because the birds anticipated a food reward (mealworms) in the POS treatment. This is in accordance with findings in silver foxes (Moe et al. 2006) and rats (Van der Harst et al. 2003a) describing a stimulus-specific response to positive or negative events. In future studies, comparing anticipation behaviour in the hen to positive stimuli

other than food should confirm whether foraging behaviour is a specific response to a food reward.

Apart from a response of general increased vigilance (i.e. head movements and head flicks) which was seen in all treatments, there was an increase in standing alert in anticipation of a positive event compared to the waiting period. However, the total amount of standing alert was not significantly greater in anticipation of a positive event than in anticipation of the other events, making it unreliable as an indicator of a positive affective state.

Finally, we found an effect of treatment on the level of comfort behaviour. In the POS treatment, there was a tendency for birds to show more comfort behaviour (e.g. preening, wing flapping, feather ruffling, body scratching) in the anticipation period than in the waiting period. If we compare the anticipation period between treatments, in the POS treatment the hens displayed profoundly more comfort behaviour than in the NEG and SN treatments. Furthermore, birds in the anticipation period of the NEG treatment showed a decrease in the level of comfort behaviour compared to the waiting period (see Table 3). Comfort behaviour in birds has been linked to a state of relaxation (Delius 1988; Spruijt et al. 1992). For example, Delius (1988) found that preening is accompanied by slow waves in the electroencephalogram, which indicates that grooming is associated with de-arousal. These findings, together with those presented here, suggest that comfort behaviour in birds may reflect a positive emotional state. It is likely that the comfort behaviour observed in anticipation of a positive event in the present study is not a stimulus-specific response, because, unlike foraging behaviour, comfort behaviour is usually not contingent on a food reward being present.

The suggestion that comfort behaviour might reflect positive welfare is in accordance with findings of Nicol et al. (2009) that hens are more likely to choose an environment in which more preening, self-scratching and associated comfort behaviours are observed. Also in line with this, Bolhuis et al. (2005, 2006) found that pigs housed in enriched environments displayed more comfort behaviours than pigs housed in barren pens. Similarly, rats housed in groups with the least amount of social pressure, and those with good health, showed an increase in self-grooming (Hurst et al. 1996; Baumans 2004). It is also in keeping with the view that positive affective states are likely to be associated with performance of behaviours that enhance survival in the long term such as play. Therefore, a proximate reward system must be involved to promote their performance at appropriate times.

However, some types of preening have also been associated with frustration and a situation of uncertainty or conflict, that is, displacement preening (Duncan & Wood-Gush 1972a). Displacement preening has been described as shorter in duration than normal preening and relatively more directed at parts of the body that can be easily reached, for example the neck and chest (Duncan & Wood-Gush 1972a). Given the short duration of the trials, we could not distinguish between normal and displacement preening; therefore, we cannot fully exclude the possibility that some of the grooming behaviours observed, such as preening neck/chest, might have been displacement preening reflecting frustration. However, in the anticipation period of the POS treatment fewer gakel calls were recorded. This indicates a decreased level of frustration (Zimmerman et al. 2000).

A final possibility is that rather than eliciting a positive emotional state of anticipation, by our POS cue, we instead elicited a positive emotional state of 'relief' of not receiving the NEG cue or recovery from arousal. This would also explain the higher level of comfort behaviour, which as we stated earlier has been associated with relaxation (Delius 1988; Spruijt et al. 1992). However, more comfort behaviour was shown in anticipation of a positive event than in anticipation of a neutral event, suggesting that comfort behaviour did not just reflect 'relief' of not receiving the negative cue.

Effect of Preceding Treatment

When exploring the effect of preceding treatment on behaviour in the POS treatment, we found that the level of arousal, as reflected by head movements and steps, was higher in the subsequent waiting and anticipation periods when the preceding treatment was NEG or POS compared to the MN and SN treatments. This indicates that the temporary (short duration) emotional state of birds might affect their anticipatory response. This has been described as within-trial contrast (Zentall & Singer 2007). In our experiment, the valence of the positive event might have been perceived differently depending on whether it had been preceded by a positive/negative event compared to one of the neutral events. These effects of preceding treatment on anticipatory behaviour and the higher level of comfort behaviour both seem to be associated with positive anticipation. This merits further research into the relation between negative/positive anticipation and pessimistic/optimistic responses as, for example, tested in a cognitive bias paradigm (Harding et al. 2004; Bateson & Matheson 2007; Burman et al. 2008).

In conclusion, our study shows that laying hens are able to anticipate differentially a positive, neutral and negative event announced by different sound cues. It is also the first study to identify comfort behaviour specifically associated with the anticipation of a positive reward in the domestic fowl. Comfort behaviour may therefore reflect a positive emotional state in the domestic fowl. Based on this one experiment, however, it cannot be excluded that the increase in comfort behaviour was situation specific rather than a general indicator of a positive affect. Future studies in which a range of different positive and negative stimuli are used should further elucidate this.

Acknowledgments

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