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Acute stress enhances sensitivity to a highly attractive food reward without affecting judgement bias in laying hens

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ABSTRACT

Affective states can be evaluated by assessing shifts in the animal's expectation of a positive and negative outcome in response to ambiguous cues, also known as judgement bias (JB). The aim of this study was to use a JB methodology, using a go/go type of task where animals are required to make an active choice, to assess the effects of acute stress on affective states in hens. Thirty ISA-Brown hens were trained in a two-choice (left–right) test in an arena to associate a high-value (H) reward (four mealworms) with a 100% black and a low-value (L) reward (one mealworm) with 5% black (visually white) cues. Twenty hens that learnt the tasks were randomly allocated to either a control (C) or stress (S; 5 min social isolation in a novel environment) group. During testing, hens were presented with H and L (rewarded) and three novel ambiguous (un-rewarded) cues: 75%, 50% and 25% black. Order of cue presentation was balanced between treatments to either having ambiguous cues always preceded by L cues (L-Ambiguous) or by H cues (H-Ambiguous). Latency to approach a reward and active choice made (i.e. reaching side associated with either H or L reward) were recorded. Data are log-transformed least square mean (LSmean) latencies(s) ± SEM with back-transformed LSmean in parentheses. Latency data showed that S-hens were faster to approach a reward cue than C-hens (S 0.8 (2.3) ± 0.04 vs C 0.9 (2.6) ± 0.04 (s), $P < 0.05$). Hens were faster to approach H and 75% than 25% and L cues (H = 0.7 (2.0) ± 0.04 and 75% = 0.7 (2.0) ± 0.07 (s) vs 25% = 1.1 (2.9) ± 0.07 and L = 1.1 (3.1) ± 0.04 (s), $P < 0.05$) with intermediate responses to 50% cues (50% = 0.8 (2.3) ± 0.07 (s)). S-hens were faster to approach ambiguous cues preceded by an H reward compared to C-hens (S H-Ambiguous = 0.7 (1.9) ± 0.06, S L-Ambiguous = 1.0 (2.7) ± 0.06, C H-Ambiguous = 0.9 (2.4) ± 0.06 and C L-Ambiguous = 1.0 (2.7) ± 0.06 (s), $P < 0.05$). Active choice was not affected by treatment. These results show that acute stress enhances sensitivity to a previously rewarding outcome without affecting judgement bias in laying hens. Hens are sensitive to events occurring immediately before the test and the order in which cues are presented and these issues should be considered in future studies.

1. Introduction

Public concerns about animal welfare are largely based on the assumption that animals can perceive and subjectively experience their environment as being positive (pleasurable) or negative (suffering) (Burman et al., 2008;

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Mendl et al., 2009). However, little is known about how hens perceive their environment and how this influences their affective state. Therefore, it is important to determine how the animals "feel" about different environments/situations in terms of their subjective experiences (i.e. positive or negative affective states) in order to understand fully the welfare implications of different production systems (Dawkins, 1990; Fraser et al., 1997). Because subjective experiences cannot be directly measured, different proxy measures and tests have been developed to assess affective states in animals including judgement bias tests (Harding et al., 2004), appraisal theory studies (Desire et al., 2002, 2004) and assessment of behavioural responses during the anticipatory/appetitive (Spruijt et al., 2001; Moe et al., 2009; Zimmerman et al., 2011) and post-consummatory (Burman et al., 2011) stages of a food reward cycle. The present study will focus on the first approach, judgement bias.

Several studies have shown that various aversive stimuli can negatively influence an animal's subjective experience of its environment and modify its affective state. For example, restraint in isolation (Doyle et al., 2010), unpredictable environments (Doyle et al., 2011) and shearing (Sanger et al., 2011) in sheep, disbudding in cattle (Neave et al., 2013) and unpredictable environments in rats (Harding et al., 2004) all result in shifts in the expectation of a positive (i.e. optimistic) or negative (i.e. pessimistic) outcome, as measured by their responses to an ambiguous cue. This shift in the animal's expectation of a positive or negative stimulus, also known as judgement bias (JB), is thought to be determined by the animal's affective state or mood (Harding et al., 2004; Mendl et al., 2009). This concept is based on previous studies in humans showing that anxious or depressed people tend to expect more negative outcomes than control people (MacLeod and Byrne, 1996) and was first applied in animals by Harding et al. (2004).

The most commonly used judgement bias test in livestock animals is a go/no go type of test where the animals are trained to respond to a cue, or to approach a location associated with a positive outcome (go), and to avoid responding to a cue/location associated with a negative outcome (no go). The latencies to respond to ambiguous cues/locations are then interpreted as indicating an optimistic response if the latency to respond is similar to that of the positive cue. However, if the animal does not respond or the latency to respond is similar to that of the negative cue/location then the response is considered as being pessimistic. This type of test has been used to assess judgement bias in livestock including sheep (Doyle et al., 2010; Sanger et al., 2011; Destrez et al., 2012), pigs (Douglas et al., 2012), cattle (Neave et al., 2013) and poultry (Salmeto et al., 2011; Wichman et al., 2012) and most such tests are sensitive enough to detect differences attributed to the affective state of the animals. However, it has been argued that this type of task, where the only outcome measured is the latency to approach or not approach within a time limit, has limitations in its interpretation as negative affective states may be associated with a general reduction in activity and feeding motivation reflecting a response bias rather than a judgement bias (Brilot et al., 2010). Furthermore, it is difficult to differentiate an omission from a no/go response (Enkel et al., 2010) and it has been suggested that go/go tasks where animals need to make an active choice are preferable to go/no go tasks (that rely on latencies alone) since it is easier to determine when an animal has learned the tasks (Murphy et al., 2013). For these reasons, go/go tests have been used to assess judgement bias in rats (Enkel et al., 2010), pigs (Murphy et al., 2012) and starlings (Matheson et al., 2008; Brilot et al., 2010). In a go/go task, the animals are required to make an active choice between two possible options, one associated with a high value (positive) reward and the other associated with either a low value reward or a negative stimulus. In this type of test, the animal's latencies to reach reward as well as the active choice they make can be assessed, thus minimising the potential confounding factors mentioned above. A change in the active choice as well as a change in latency are both indicators of a change in affective states. Therefore, the aim of the present study was to use a modified judgement bias methodology using a go/go task, previously developed for starlings (Brilot et al., 2010), to assess affective states in laying hens.

Short-term social isolation has been used to induce negative affective states in poultry with well characterised behavioural and physiological responses (Sufka et al., 2006; Salmeto et al., 2011). For example, 5 min social isolation in 5–6 day old chickens has been shown to induce increased distress vocalisation rates, increased plasma corticosterone levels (Sufka et al., 2006) and pessimistic-like responses, measured as increased latencies to reach ambiguous cues of morphed images of a chick silhouette (attractive) with an owl silhouette (aversive; morphing ratios of 75% chicken:25% owl, 50% chicken:50% owl and 25% chicken:75% owl), during a judgement bias test using a go/no go type of task (Salmeto et al., 2011). These responses seem to have good repeatability and normal behaviours can be restored using anxiolytic and antidepressant drugs suggesting the responses share a common mechanism to human affective disorders (Sufka et al., 2006; Hymel and Sufka, 2012). For these reasons, 5 min social isolation was used to induce a negative affective state in hens in this study. We hypothesised that birds exposed to the stress of social isolation would experience a negative affective state and display a more pessimistic response than non-stressed birds during a two-choice judgement bias test.

2. Materials and methods

2.1. Animals and treatments

Thirty ISA Brown laying hens were kept in a barn from 18 weeks of age until the end of the experiment. The hens were kept in a floor system with straw litter. The birds had free access to water from nipple drinkers, commercial layer pellets provided in hanging poultry feeders, nest boxes and perches. Hens were given 9 weeks to acclimatise to the barn environment before training in the judgement bias test commenced. To increase motivation to perform the tasks, food was removed 2 h before training and testing. The hens were moved to a holding pen (2.5 m × 2.5 m) 10 min before training and testing to facilitate handling the animals. Water, but no food, was freely available inside the