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ORIGINAL ARTICLE

Influence of a maternal odorant on copying strategies in chicks facing isolation and novelty during a standardized test

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Abstract

A synthetic analogue of a mother-hen odour named MHUSA (Mother Hen Uropygial Secretion Analogue) reduces stress-related behaviour in the chicken. We hypothesize that MHUSA may have an attractant effect on chicks. In order to test this, 30 chicks were individually exposed to MHUSA, placebo or neutral when self isolated in a straight shuttle box. The location of the chicks within the test chamber was recorded with 360 consecutive scan-sampled images. During the first three minutes immediately after introduction to the test area chicks spent more time in the neutral zone ($p<0.05$). However, taking the results from the total observation period, chicks spent more time in the MHUSA and placebo zones combined than in the neutral zone ($p=0.07$). They were more often observed in the MHUSA zone compared to the placebo zone ($p<0.05$). These results suggest that during the first three minutes in the shuttle, individuals were adapting to their new environment. After this period, chicks directed themselves towards specific local stimuli, as they tried to reach their group or something that resembled it. After a stressor was introduced, we observed a return to the same situation as during the first three minutes of the test, with chicks returning to the neutral zone, suggesting that the chick had its confidence in the environment. Three main conclusions may be drawn. Firstly, MHUSA has an attractant effect on naïve chicks. Secondly, it appears to play a role in the reaction of chicks faced with a stressful event, and finally, the reaction to MHUSA seems innate and does not require previous experience.

INTRODUCTION

Chickens (*Gallus gallus*) are social animals that aggregate to create a social structure (Bradshaw, 1992). Runway and treadmill tests are often used to assess social behaviour or the motivation of birds to be near to conspecifics (Vallortigara *et al.*, 1990). These methods require the chick to go through a corridor and are usually used to measure bird's attempt to remain close to siblings (Mills & Faure, 1991). For example, following a stressful event, the readiness of a bird to traverse a runway in order to reach conspecifics has been used to estimate the disinhibition of induced fear (Nicol & Scott, 1991). Chickens are generally attracted to familiar stimuli (Clarke & Jones, 2000), with chicks tending to approach live conspecifics placed at either end of a two choice runway (Carmichael *et al.*, 1998).

When a hen is in the presence of chicks, it produces specific odour chemicals, regardless of whether or not the chicks are its own (Richard-Yris *et al.,* 1983). When chicks are isolated from a hen, they tend to show specific patterns of behaviour (Roden & Wechsler, 1997).

Recent studies have demonstrated the efficacy of a synthetic analogue (Pageat, 2002) of a mother-hen odour secretion (Mother Hen Uropygial Secretion Analogue, MHUSA) in lowering stress, as indicated by blood parameters, in industrially raised broiler chickens (Madec *et al.,* 2006; Madec *et al.,* 2008). Whilst these blood parameters are commonly referenced as indicators of stress in birds (Mumma *et al*., 2006), they may in fact be less sensitive than behavioural indicators (Feltenstein *et al*., 2003). In addition, it is often difficult to take blood samples from chicks, and the procedure itself may have a detrimental impact on their welfare. Thus, the aim of the present trial was to test the attractiveness of MHUSA in chicks facing isolation and novelty.

Chicks are naïve during the first five to seven days after hatching (Nicol, 2004) and there is an optimal period for imprinting in chicks. Collias (2000) showed that the ability to imprint decreases as early as the first day after hatching. In processing plants, chicks are typically delivered at one day of age, after artificial incubation and hatching. They have therefore had no contact with adult birds after hatching. Handling, transportation and novelty are known sources of stress (Grandin, 1997), and Warnick *et al*. (2006) suggest that isolation stress in chicks resembles panic. So, it appeared ideal to perform the odour preference test on previously minimally stressed chicks at three or four days of age, after adaptation to their rearing environment. We chose to present individual chicks with a simple task using a linear corridor, rather than a v-shaped runway, to test the attractiveness of MHUSA, as this provided a strong motivation and facilitated behavioural observations (Vallortigara *et al.,* 1990). As shown by Hazard *et al.* (2008), it was anticipated that studying the response to isolation and placing the chick alone in a novel environment might provide greater understanding of responses to restraint, since both these stressors are components of the restraint test.

MATERIAL AND METHODS

Subjects and housing conditions

Thirty chicks, from the ROSS PM3 strain were used for this trial. The chicks had been vaccinated for Marek's, Gumborow and Newcastle disease at the hatchery, on the day prior to their arrival on site. Chicks stayed in a dedicated barn for three days after arrival. They had free access to conventional food and water, and the rearing conditions were consistent with commercial temperature and hygrometry standards. Because it has been shown that circadian rhythm may influence reaction to stress (Dubovicky *et al*., 2007), the procedure was performed under full artificial uniform light. Methods of breeding and experimentation were in accordance with the 95/29/CE European convention.

Treatments and apparatus

A shuttle box, measuring 200 × 50 × 25 cm (length × width × height) was built for the trial. This test apparatus was situated in a room from the barn, but with similar ambient temperature. The shuttle box was marked into three equal sections. The ends were labelled A and B, and the central part was labelled N (neutral). Within the shuttle box the soil base was covered with fresh straw, the same as in the barn. As shown in Figure 1, a 20cm wide drawer allowed the chick to be gently introduced into the middle of the shuttle (zone N).

Two different treatment blocks were placed at opposing ends of the shuttle box, allowing a comparison of response to the two treatments. The treatment blocks consisted of slow-release macromolecular gelatin composed of water (>90%), non ionic surfactant (4%) and a gelling gum (3%) (Nicols S.A., 59980 Bertry, France). Control blocks were composed of this matrix alone, whilst MHUSA contained 2% of the active principle. Control and MHUSA blocks were visually indistinguishable and both weighed 150g. MHUSA and control blocks were placed at opposite ends of the shuttle box,

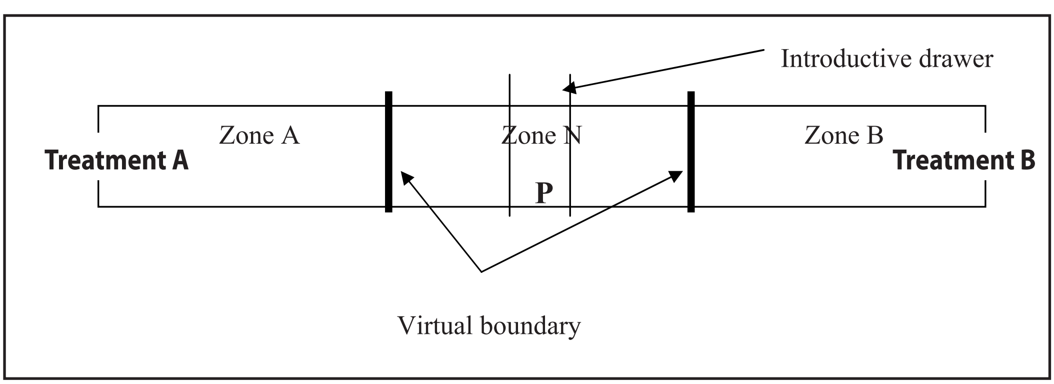

Figure 1: Overhead view of the testing apparatus: the shuttle.

Table 1. Attractiveness of the MHUSA treatment on naive chicks experiencing isolation and novelty – MHUSA vs placebo

T_{int}[1]	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{9p}[2]	S_{10}	S_{11}	S_{tot}[3]
MHUSA	2	5	7	8	11	9	8	12	10	13	13	11	109
Neutral	22	19	19	15	11	9	15	14	12	10	10	12	168
Placebo	6	6	4	7	8	12	7	4	8	7	7	7	83
Significance[4]	ns	ns	ns	ns	ns	ns	ns	p<0.05	ns	p=0.1	p=0.1	ns	*p*<0.05

[1]Tint: time (minutes) post introduction of the chick in the shuttle
[2]S_{9p}: 5sec after the stress
[3]S_{tot}: cumulated 360 observations

Table 2. Moves of the naive chicks experiencing isolation and novelty – N vs (MHUSA+placebo)

T_{int}[1]	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{9p}[2]	S_{10}	S_{11}	S_{tot}[3]
Neutral	22	19	19	15	11	9	15	14	12	10	10	12	168
COM[4]	8	11	11	15	19	21	15	16	18	20	20	18	192
Significance[5]	*p*<0.001	*p*<0.05	*p*<0.05	ns	*p*<0.05	*p*<0.01	ns	ns	ns	*p*<0.05	*p*<0.05	ns	p=0.07

[1]Tint: time (minutes) post introduction of the chick in the shuttle
[2]S9p: 5sec after the stress
[3]Stot: cumulated 360 observations
[4]COM: MHUSA and placebo combined
[5]Significance: comparison between the number of animals in N compared to COM

in zones A and B, but experimenters were blinded with respect to which block contained the active principle.

Procedure

Experiments were carried out on two batches of 15 chicks. The experiment was performed on one chick at a time, and each chick was tested once. For the first batch of 15 chicks, a MHUSA block was placed at end A of the shuttle box and a placebo block at end B. For the second batch of chicks exactly the same design was used but the location of the blocks was reversed (MHUSA block at end B and control block at end A). Chicks were not able to make physical contact with the blocks, as these were placed behind a grille at the extreme end of the shuttle box. Birds were tested consecutively, with the same blocks remaining in position throughout the batch of experiments. Video recording (CAMSET 13, Velleman©) commenced immediately after a chick was introduced in the shuttle box. Scan sampling was recorded using a video cassette recorder (DAEWOO© SV-813S) to record the spatial location of the chick every 60 seconds. The isolation paradigm followed that which was described by Feltenstein *et al.* (2003), and involved isolation of the group-raised chick from its social companions. After 9min 30sec, chicks were then exposed to a stressful event using a sudden loud noise. This was achieved by noisily opening and closing the door of the testing room in a manner that was standardized for all the tests. The position of the chick within the shuttle box was recorded immediately after this event. Each test lasted 11 minutes from the introduction of the chick in the shuttle box, so that the position of each chick was recorded 12 times (each 60sec and after event). This gave a total of 360 location measurements for the whole test group of 30 chicks.

Statistical analysis

Statistical analysis was performed using SYSTAT 10 software. For each sampled time point the position of the chick under test was recorded as A, B or N, according to the section of the shuttle box it was in at that time. Thus there were 30 observations for each of the 12 time points. A Chi-squared test was then performed for each time point and for the entire data set (n=360). The position of chicks was noted in a yes/no design for each time point and for each chick. Three sets of results were therefore obtained: MHUSA vs other (placebo and Neutral cumulated), placebo vs other (MHUSA and Neutral cumulated), and Neutral vs other (MHUSA and placebo cumulated). We then compared computed observations to theoretical ones. We tested for equality between computed and theoretical values (5% risk, df=1), focusing on our main parameter: scan of the total observations after the two batches. Results were considered significant if $p<0.05$.

RESULTS

As shown in Table 1, chicks were significantly more frequently observed in the MHUSA zone compared to placebo for the cumulated 360 scan samples (main parameter) (S_{tot}, $p<0.05$). This was also the case at 8 minutes post introduction of the chick (S_8, $p<0.05$). There was a trend for chicks to be observed more often in the MHUSA zone, compared to placebo, immediately after the stressful event (S_{9p}, $p=0.1$) and one minute after it (S_{10}, p=0.1). For all other observations, there was no significant difference. During the time immediately after introduction to the shuttle box (0–3 minutes after introduction) birds were recorded significantly more often in zone N than in both the MHUSA and placebo zones combined (denoted as COM). While we observed that for time points S_1 (p<0.001), S_2 (p<0.05) and S_3 (p<0.05), more chicks were recorded in the neutral zone compared to COM (Table 2), the reverse was true at S_5 (p<0.05) and S_6 (p<0.01) , with more chicks being in COM. For time points S_7, S_8, S_9 and S_{11}, we found no statistical difference between the number of chicks in N compared to COM. Immediately after the stressful event, as well as 60 seconds after it, chicks were significantly more commonly observed in COM compared to N (S_{9p}, p<0.05 and S_{10}, p<0.05). For the cumulative data for all 360 time points chicks were more frequently recorded in COM than in N (S_{tot}, p=0.07).

DISCUSSION

Since Collias (2000) has demonstrated that chicks are able to recognize their siblings at 3 days of age, we would expect that after four days in the barn with the same companions, chicks from our trial were used to their surroundings and social contact with each other. After the age of four days, age and sex may influence the chick's response to a component similar or equivalent to a semiochemical (Fluck *et al*., 1996). Thus, the decision was made to use MHUSA on naïve chicks at four days of age in order that the results would not to be influenced by these biological factors. In the barn, chicks were used to their environment, and still naive to any of the experimental stressors. They were also old enough to imprint on other chicks, as demonstrated by Collias (2000). Since the whole trial was performed in a single day there cannot have been any time related effects.

Apart from being imprinted upon them, chicks may also have become the subject of similar bonds of attachment from their siblings. Thus, as shown by Fisher-Mamblona (2000), the chicks may have been both imprinted and imprinted upon. Knowing this, we may hypothesize that when chicks were separated from their group, they experienced panic not only because of novelty, but also because of the sudden loss of attachment. Our experimental design is close to the one by Warnick *et al*. (2006), which proposed that a separation stress paradigm better modelled panic disorder than it models generalized anxiety disorder. The experience of being handled, even for a few seconds, is also able to create stress (Grandin, 1997). The chicks used in this study experienced a number of stressful events, including handling, a move from their original crate and then their introduction to a new environment (the shuttle box) representing a period of isolation.

The main studied parameter (S_{tot}) shows that MHUSA plays an attractant effect on naive chicks when exposed to isolation stress. We can then split the response of the chicks into three phases, with regard to the events that happen to them and how they responded. During the first phase, which lasted from the moment of introduction into the shuttle box until the fourth minute, individuals were adapting to the new environment. This would explain the tendency for chicks to remain in the neutral zone of the shuttle box, where they were initially introduced. This is in accordance with results by Feltenstein *et al*. (2002), which showed that birds exhibited more distress vocalization than change of location in a social separation test. In the present study vocalisation was not analysed and it would have been interesting to look for correlations between vocalisation and location in the shuttle box. Our results are also in accordance with those of Feltenstein *et al*. (2003), which involved isolation periods, followed by observation of stress parameters. The second phase could be characterized as the "choice period", lasting from S_4 (included) until S_9 (inclusive). During those six minutes, the chick tended to choose to be in the MHUSA zone, compared to placebo, which may indicate an attempt to reach its group or something associated with attachment. The end of this second period is reached when the stressful event arises (S_{9p}). From that moment, it seemed that the isolated chick lost its reference points or its confidence in the environment. These results are in accordance with those of Henderson *et al*. (2001) who attested of the importance of noise in danger perception in birds. In our experiment, the event appears to be stressful enough to produce significant results.

After S_{10} (inclusive), it seems that the chicks return to the same state as at time point S_1, with individuals returning to the neutral area as if compelled to reassess the environment. It would have been of interest to observe the reaction of the chicks from S_{11} onward, in order to compare behaviour with that during time points S_1 to S_3, but this data was not collected in this experiment. Nevertheless, the effect of the stress event was of interest since it has been reported that stress response reaches its maximum before 10 min since corticosterone level does not increase further after this interval (Hazard *et al.,* 2008). We hypothesize that because MHUSA plays a role in the chick's response to stress and danger, it will have attractant properties, and that this attractiveness may be either learned (odour dependant) or innate (semiochemical) (Brennan and Zufall, 2006).

In this experiment the chick's response to MHUSA appears to be innate and specific for two main reasons. First, the chicks had been exposed neither to the presence of a hen nor to MHUSA. Second, if learning were significant, we would expect a faster reaction after the appearance of the stressor. According to Brennan & Zufall (2006), these findings support the description of MHUSA as a semiochemical. In contradiction to this, chicks spent three days with the same conspecifics and MHUSA may in some way remind them of their siblings. Indeed, Bonadonna *et al*. (2004) showed that chicks are able to find their own nipple using odours. Nevertheless, these observations may also result from imprinting due to mutual imprinting in the resting barn during the period before introduction into the tunnel (Fisher-Mamblona, 2000). Thus, it is more likely that MHUSA is innate since the ability to imprint decreases within one day after hatching (Collias, 2000). The main conclusions from this test are that MHUSA plays a role in the chick's reaction to a stressful event, that it has an attractant-like effect on naïve chicks, and that this effect may be innate and not learned.

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