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VOLUME 110 ISSUES 3-4 APRIL 2008 ISSN 0168-1591

APPLIED ANIMAL BEHAVIOUR SCIENCE



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Social reinstatement responses of meat-type chickens to familiar and unfamiliar conspecifics after exposure to an acute stressor

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Accepted 27 April 2007

Available online 5 June 2007

Abstract

Runway tests are considered indicative of underlying sociality in birds and their ability to make social discriminations. We evaluated whether experience of a prior stressor alters the subsequent affiliation responses of 9 or 10-day-old chicks simultaneously exposed to familiar (cagemates) and unfamiliar conspecifics placed in goal boxes at opposite ends of a runway. Birds were housed in groups of eight in home cages. Half of the birds in each home cage were used as either familiar or unfamiliar social stimuli in the goal boxes. The other half of the birds were randomly assigned either to a control (CON; $n = 51$) group that remained undisturbed until testing or to a stress-treatment (STR; $n = 52$) group that was exposed to a 5-min restraint stressor, returned to its home cage and then tested 1 h later. Birds were individually tested in the runway for 5 min and the behaviours video-recorded. During revision of tapes, the projected floor image of the runway was divided into squares and zones. The stressor decreased ($P < 0.01$) the time spent in close proximity (close zone; CZ) to conspecifics regardless of the familiarity of the stimulus birds. Regardless of treatment, test chicks showed shorter latencies to enter ($P < 0.05$) and spent longer time ($P < 0.02$) in the familiar than in the unfamiliar CZ suggesting that young chicks can discriminate between familiar and unfamiliar conspecifics encountered in novel surroundings. While in close proximity to familiar conspecifics, STR birds showed a reduced ($P < 0.05$) number of squares entered compared to CONs. This reduced locomotor activity was not accompanied by an increased activity in other zones of the runway. At the end of the trial, both CON and STR birds showed a reduced ($P < 0.05$) locomotor activity in the unfamiliar CZ and an increased ($P < 0.05$) activity in the central zone of the runway. Interestingly, no differences were detected between CON and STR birds in the total number of squares entered during the

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trial. These results suggest that prior stressor exposure did not affect the overall amount of locomotion but altered the spatial distribution of it. Collectively, our findings suggest that exposure to an acute stressor event subsequently affects chicks' affiliation responses in runway tests. The way a bird will react depends on the identity (familiar or unfamiliar) of the conspecifics in its close environment.

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Keywords: Meat-type chickens; Stress; Social discrimination; Runway

1. Introduction

Many of our intensively farmed poultry are social species that, in nature, would normally live in groups with a relatively stable social structure (Duncan, 1981). Indeed close attachments are often formed between brood mates or members of a group (McBride et al., 1969). Social bonding among flock members and establishment of a rank order are based on recognition and discrimination (Doyen, 1987; Zayan, 1987; Jones et al., 1996). However, modern farming practices can impose several deviations from what might be considered the natural situation. Crowding, alteration of group membership and large groups exceeding the social recognition capacity often disturb or completely prevent natural social relationships between the birds and cause potentially harmful effects (Jones et al., 1996; Hughes et al., 1997). A mis-match between chickens' underlying levels of sociality and their social environment could elicit either a series of acute or chronic stress responses with associated negative effects on performance (Mills et al., 1993; Jones and Hocking, 1999; Jones and Mills, 1999). Not only could this contribute to the development of depression and social withdrawal but it could also seriously damage the birds' health and productivity (Duncan, 1981; Mills and Faure, 1990; Jones, 1996). For instance, low-sociality birds might be ill-suited for housing in social groups, particularly very large or very confined ones. Therefore, a continued study on sociality of domestic fowl may have important fundamental and practical relevance.

Runway tests have been widely used to study social reinstatement responses; these are considered to be indicative of underlying sociality in birds as well as of their ability to make social discriminations (Vallortigara et al., 1990; Jones et al., 1999; Väisänen and Jensen, 2004). Both domestic chicks and Japanese quail approached conspecifics more readily than an empty goal box or one containing members of different avian or mammalian species (Suarez and Gallup, 1983; Mills et al., 1995; Jones and Mills, 1999). Moreover, it is generally accepted that young domestic chicks can recognise a cagemate from a stranger even after very brief social experience. For instance, the presence of strange chicks elicited heightened rates of inter-subject pecking, disrupted feeding, and increased fear reactions (Rajecki et al., 1976; Zajonc et al., 1988; Vallortigara, 1992). Ten-day-old chicks showed a more pronounced social affiliation (indicated by an increased time spent near conspecifics) when the goal box contained familiar cagemates rather than unfamiliar chicks (Marin et al., 2001). Japanese quails genetically selected for low or high social reinstatement motivation were also able to discriminate between familiar cagemates and unfamiliar birds in a runway with two goal boxes (Jones et al., 1996).

Different stressful stimulations (e.g., electric shock, loud noise, restraint or social separation) may affect behaviour when animals are subsequently exposed to a threatening or stressful situation (Gallup and Suarez, 1980; Satterlee et al., 1993; Jones, 1997). Social reinstatement evaluation in runways involves removing a bird from its home environment, placing it in a novel one, and then measuring its responses to a small group of conspecifics held in a goal box (e.g., the

time it takes to approach them, how long it spends near them). Therefore, these tests clearly incorporate stress-inducing elements (capture by the experimenter, exposure to a novel environment and transient separation from companions). The behavioural changes that a prior stress may induce will depend on the characteristics and conditions of the testing. For example, in an open field or novel environment, increased silence, inactivity and/or decreased exploration would be expected (Jones, 1987). In a tonic immobility (TI) fear reaction test (Gallup, 1979; Jones, 1986), the consequences of a prior stressor has been shown to be an increased TI duration and a reduction in the number of periods of restraint required to obtain a TI response (Satterlee et al., 1993). It has been suggested that the presence of conspecifics in an otherwise unfamiliar situation serves to reduce fear (Jones, 1987; Jones and Merry, 1988). In a runway test with only one goal box (holding either familiar or unfamiliar conspecifics), the application of an acute stressor prior to testing reduced the time the test birds took to approach their conspecifics and increased the time spent near them. The latter response was also more pronounced when the goal box contained familiar rather than unfamiliar conspecifics (Marin et al., 2001). The present study evaluated whether experience of a prior stressor alters the subsequent affiliation responses of chicks simultaneously exposed to familiar (cagemates) and unfamiliar conspecifics placed in goal boxes at opposite ends of a runway.

2. Materials and methods

2.1. *Animals and husbandry*

Two-hundred-and-eight mixed-sex meat-type (Cobb-500) chicks were obtained from a commercial supplier (INDACOR, Córdoba, Argentina) at one day of age and were randomly allocated to groups of eight and housed in 26 wooden home cages painted white and measuring 42 cm × 45 cm × 50 cm (length × width × height). The wire-mesh floor (1 cm grid) was raised 2 cm to allow the passage of excreta. Ambient temperature was maintained between 28 and 32 °C and lighting was provided by fluorescent lamps from 06:00 to 20:00 h. Food (Cargill, Córdoba, Argentina, broiler BB, 20% minimum crude protein, 2950 kcal/kg) and water were supplied ad libitum.

The experimenters always took care to move slowly and steadily when carrying out maintenance chores or when removing and replacing chicks during testing.

Although gender differences in sociality have been reported in domestic chicks (Vallortigara, 1992) we were unable to control for this variable because of difficulties to accurately sex the birds at the early ages used here and unavailability of the facilities to maintain them till adulthood.

2.2. *Runway apparatus*

The apparatus (Fig. 1) consisted of an unpainted wooden runway measuring 200 cm × 40 cm × 40 cm (length × width × height), that was divided into three compartments by wire-mesh partitions. The middle compartment (measuring 160 cm long) was the actual runway. The other two compartments (each 20 cm long), situated at opposite ends of the runway, were termed familiar and unfamiliar goal boxes. The familiar goal box housed two birds (familiar social stimuli) taken from the same home cage as the test bird, while the unfamiliar goal box housed two conspecifics (unfamiliar social stimuli) from a different home cage than the test bird. To allow the passage of excreta during testing, the runway apparatus was fitted with a wire-mesh floor (1 cm grid) that was raised 2 cm above the surface upon which the apparatus was placed. Temperature

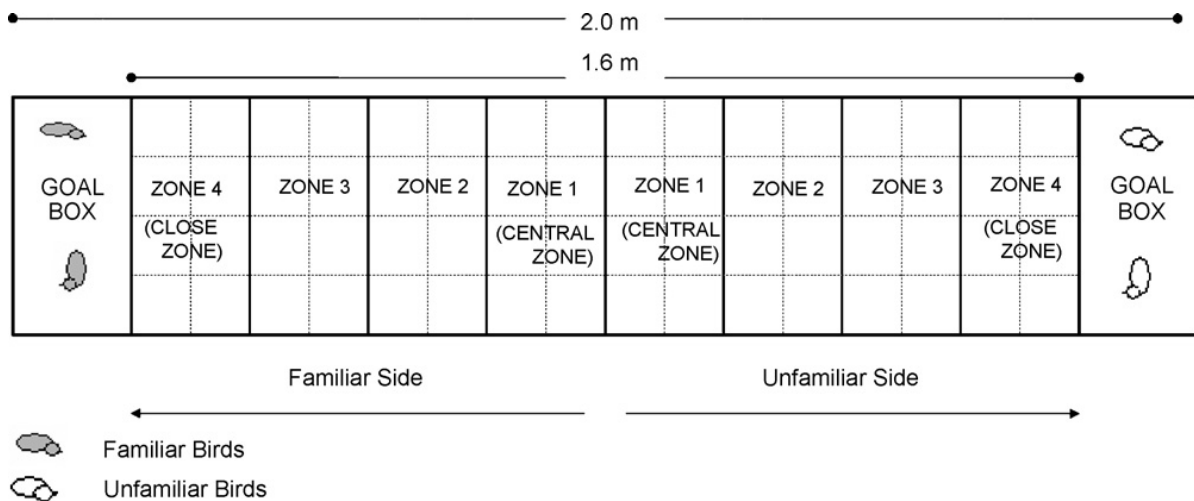


Fig. 1. Diagram of the runway apparatus. Lines dividing squares and zones were not marked on the floor of the runway, but only on the television screen during video-tape analysis. Placement of the familiar and unfamiliar conspecifics respectively on the left and right side of the runway is presented as an example. Close zone (within 20 cm of conspecifics).

and illumination were maintained at similar levels to those in the rearing room with the addition of a 60 W light bulb suspended above each of the goal boxes.

2.3. Treatments and procedure

The social reinstatement responses of 9 or 10-day-old chicks were individually tested during 5 min in the runway apparatus. Four of the eight chicks in each of the 26 home cages were randomly selected and tested in the runway, while the remaining four birds in each cage were used as test stimuli in the goal boxes. During the trials, one chick escaped from the home cage, thus in all 103 chicks were tested. Birds were assigned to either a control (CON) group or a stress-treatment (STR) group. Control chicks ($n = 51$) remained undisturbed in their home cages until they were individually captured and hand-carried approximately 4 m to a separate room for testing. Birds from the STR group ($n = 52$) were similarly captured and immediately exposed to a 5-min restraint stressor. The stressor consisted of individually placing the bird in a wooden crush cage (20 cm × 20 cm × 20 cm) painted white, with a moveable interior wall fixed in place so that it prevented all but slight movements of the chick's head and legs and those associated with respiration. Following STR treatment, each bird was faintly marked on top of the head with a fast drying inert dye to facilitate later individual identification. The bird was immediately returned to its home cage before its runway responses were recorded 1 h later. Two CON and two STR chicks were taken from each home cage.

Runway testing was conducted between 08:00 h and 16:00 h over two consecutive days (approximately half of the birds being tested each day). Each bird was tested only once. At the end of the 5-min test period the bird was faintly re-marked to facilitate identification of untested individuals, and returned to the home cage. Testing order of individuals was randomized over the two treatment groups and two test days.

Since feeding chicks showed a right-foot preference for scratching at the ground (Rogers, 1995) and hens chose the left arm of a Y-maze faster than the right (Petherick et al., 1993), it is conceivable that hemispheric specialization and/or pre-existing right-left preferences might decelerate running or affect the first direction of travel in the runway. Therefore, in order to

control for any possible positioning effect, test chicks were gently placed near the midpoint of the runway floor facing towards a sidewall (same sidewall for all test birds), and the assignation of the familiar and unfamiliar conspecifics to the goal boxes at either end of the runway (right or left side according to a lateral view) was maintained during two consecutive tests and then swapped.

The behaviour of each chick was recorded using a closed circuit television system with a video camera suspended approximately 2.5 m directly above the runway apparatus. This arrangement made certain that the experimenter was completely hidden from the chick's view during testing. During subsequent re-play of the video recordings on a television screen, the projected floor image of the runway was divided on the screen into 64 imaginary squares of 10 cm × 10 cm each (Fig. 1). The floor image was also divided in two halves related to either the familiar or unfamiliar conspecifics side of the runway. Each half was further subdivided in four zones of 20 cm each, numbered consecutively 1–4 from the midpoint to each goal box. Zone 1 was termed central zone and zone 4 was termed “close” zone (CZ; i.e., the 20 cm zone nearest to either familiar or unfamiliar conspecifics) (Fig. 1). The following measures were taken: (1) latency to ambulate (s): time elapsed from beginning of the test till the chick takes at least two consecutive steps in any direction; (2) latencies to start approaching conspecifics (s): latencies to first entry to either familiar or unfamiliar zone 2; (3) latencies to enter CZ (s); (4) initial direction of travel: whether the bird first entered the familiar or the unfamiliar zone 2; (5) first CZ entered; (6) time in CZ (s): accumulated time spent in either familiar or unfamiliar CZ during the 5-min test period; (7) percentage of time in CZ (PCZ): the amount of time spent in a CZ was also expressed as a percentage of the time available after first entry using the following formula: $PCZ = (\text{Time spent in CZ} / (\text{Total time of the trial, 300 s} - \text{Latency to enter CZ})) \times 100$. PCZ is regarded as one of the most sensitive measures of affiliation in runway tests because it minimizes the potentially confounding effects of individual differences in locomotor ability and in fear-induced immobility in the start box (Jones et al., 2002). (8) Squares entered: the number of squares entered per min in each zone of the runway. The total number of squares entered during the 5-min test period was also registered.

2.4. Statistical analysis

Latencies to start approaching conspecifics, and to enter CZ, time in CZ and PCZ were analyzed by a split-plot ANOVA with treatment (CON versus STR) as the between-subject variable and identity of the social stimuli (familiar versus unfamiliar) as a within-subject variable. Assumptions of the ANOVA were verified. The number of birds that initiated their travel towards either familiar or unfamiliar conspecifics and that entered first a CZ (familiar or unfamiliar) within each experimental group (CON and STR) were analyzed using the Proportion Test (Analytical Software, 2000). Squares entered were analyzed by a split-plot ANOVA with treatment (CON versus STR) as the between-subject variable and side of the runway (familiar and unfamiliar), proximity to either familiar or unfamiliar conspecifics (central zone, zones 2, zone 3, and CZ within each side of the runway), and testing time (1st, 2nd, 3rd, 4th and 5th min) as the within-subject variables. Number of squares entered were transformed to ranks (Shirley, 1987) in order to fit better the ANOVA assumptions. Measures of the latency to ambulate and the total number of squares entered were subjected to a one-way ANOVA that evaluated the effect of treatment (CON versus STR). Post-hoc treatment group comparisons were conducted using the Fisher LSD test. A *P* value of <0.05 was considered to represent significant differences.

3. Results

Prior exposure to the restraint stressor did not significantly affect the latencies to start approaching conspecifics and to enter CZ ($F_{1,101} = 2.96$, $P < 0.09$ and $F_{1,101} = 3.41$, $P < 0.07$, respectively). However, restraint stress significantly decreased ($F_{1,101} = 6.65$, $P < 0.01$) the time in CZ regardless of the identity of the stimulus birds (Table 1). No significant differences ($F_{1,101} = 1.63$, $P < 0.21$) between CON and STR birds were found in the PCZ. When the identity of the social stimuli was evaluated, no significant differences were found in the latency to start approaching familiar or unfamiliar conspecifics ($F_{1,101} = 1.79$, $P < 0.18$). However, test chicks showed significantly shorter latencies ($F_{1,101} = 3.82$, $P < 0.05$) to enter the familiar CZ than to enter the unfamiliar CZ and spent significantly longer times ($F_{1,101} = 6.45$, $P < 0.013$) and showed higher PCZs ($F_{1,101} = 4.63$, $P < 0.03$) in the familiar CZ than in the unfamiliar CZ (Table 1). There were no significant interactions between the effects of treatment and identity of the social stimuli for the variables measured herein.

Neither CON nor STR groups showed significant differences in the number of individuals that initiated their travel towards familiar or unfamiliar conspecifics (Table 2). However, within the CON group, a higher number ($P < 0.04$) of individuals entered first the familiar CZ than the unfamiliar one. No differences were found in the number of STR birds that entered first either the familiar or the unfamiliar CZ (Table 2).

No significant differences were detected between CON and STR birds in the latency to ambulate (29.38 ± 4.57 s and 41.50 ± 6.92 s, respectively) or in the total number of squares entered (40.86 ± 4.24 s and 37.71 ± 4.99 s, respectively). However, when the locomotor activity

Table 1

Means and standard errors of the runway responses of 9 or 10-day-old control (CON) and stressed (STR) meat-type chickens tested with familiar and unfamiliar conspecifics placed in goal boxes at opposite ends of a runway

Measures	CON		STR		Probability	
	Familiar	Unfamiliar	Familiar	Unfamiliar	T	I
Lat. start approaching						
Conspecifics (s)	131.04 ± 17.94	181.54 ± 17.78	164.19 ± 17.44	188.36 ± 15.70	0.09	0.18
Lat. enter CZ (s)	159.44 ± 16.56	209.04 ± 15.21	190.28 ± 15.07	215.74 ± 14.52	0.07	0.05
Time in CZ (s)	120.90 ± 16.21	71.48 ± 13.84	96.58 ± 14.97	53.49 ± 11.08	0.01	0.02
PCZ	0.50 ± 0.07	0.36 ± 0.07	0.52 ± 0.07	0.29 ± 0.06	0.21	0.03

Test for the effects of treatment (T) and identity (I) of conspecifics. Lat.: latency to; (s): seconds; CZ: “close” zone (within 20 cm of conspecifics); PCZ: (Time in CZ)/(Total duration of the trial, 300 s – Lat. enter CZ) × 100.

Table 2

Number of 9 or 10-day-old control (CON) and stressed (STR) meat-type chickens that initiated their travel towards either familiar or unfamiliar conspecifics and entered either “close” zone first (within 20 cm of conspecifics; CZ)

Treatment	Initial direction of travel towards		
	Familiar	Unfamiliar	Probability
CON	28	23	0.16
STR	28	24	0.28
First CZ entered			
CON	30	21	0.04
STR	27	25	0.42

in the runway was temporally and spatially evaluated, that is, taking into account the number of squares entered per min in each zone of the runway, a significant interaction ($F_{12,1224} = 3.65$, $P < 0.001$) among treatment, familiar or unfamiliar side of the runway, proximity to conspecifics and testing time was found. Results are summarized in Fig. 2. Fisher LSD post-hoc test showed a time dependent decrease in the number of squares entered in the central zone of the runway during the first 4 min of the test while the opposite was evidenced in the CZs. In the familiar side of the runway, during the 5th min of the test, CON birds maintained the locomotor activity in the CZ shown previously (2nd, 3rd, and 4th min), while the STR group showed a significant reduction in the number of squares entered in the CZ without a concomitant increase in the number of squares entered in the other zones (central zone, zone 2 or 3) of the apparatus. On the other hand, in the unfamiliar side of the runway at the end of the trial (5th min), both CON and

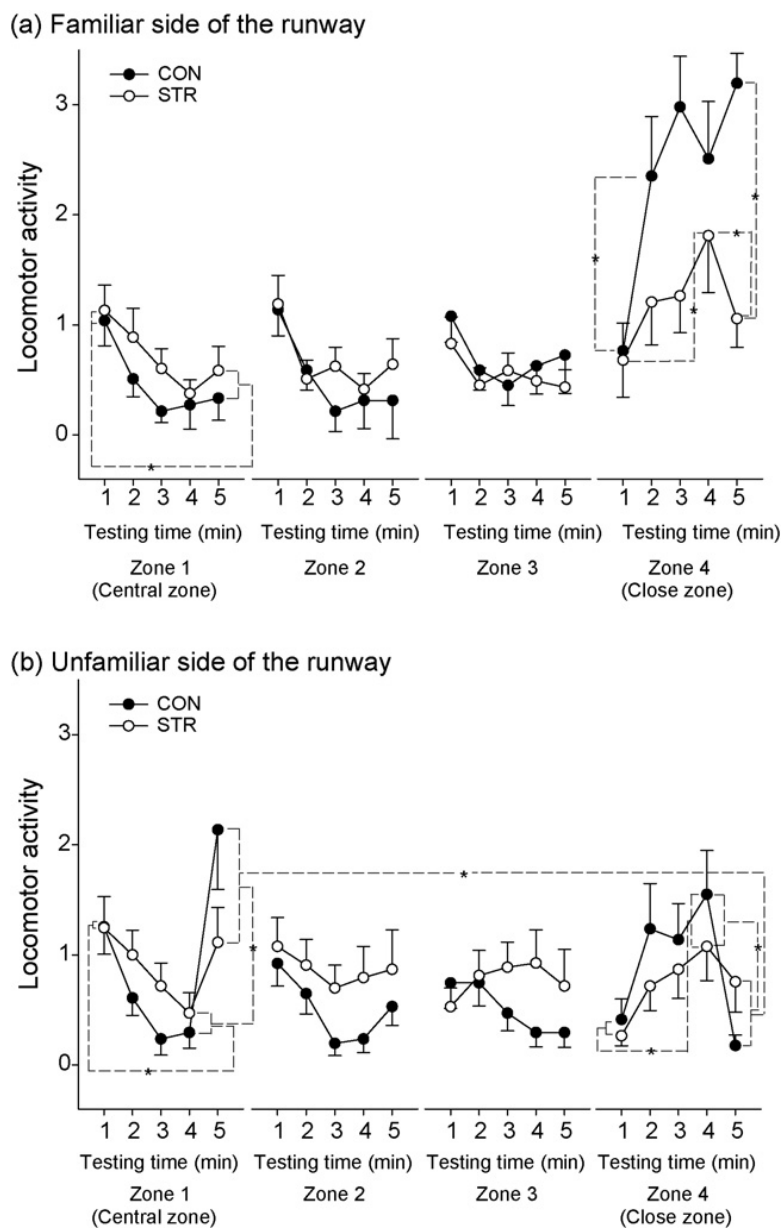


Fig. 2. Means and standard errors of runway locomotor activity (number of squares entered) of 9 or 10-day-old control (CON) and stressed (STR) meat-type chickens. * $P < 0.05$. Central zone (within 20 cm of the midpoint of the runway); close zone (within 20 cm of either familiar or unfamiliar conspecifics); zones 2 and 3 (intermediate zones).

STR birds reduced their locomotor activity (number of squares entered) in the CZ and increased their locomotor activity in the central zone of the runway.

4. Discussion

The results in this study show that exposure to a restraint stressor affects the subsequent affiliation responses of young meat-type chickens simultaneously exposed to familiar (cagemates) and unfamiliar conspecifics placed in goal boxes at opposite ends of the runway. STR chicks showed a significant reduction in the time in CZ regardless of the identity of the stimulus birds. At first glance, this result suggests that stressor exposure reduces the need to be in close social contact with conspecifics. However, the lack of differences between STR and CON birds found in the PCZs (percentage of the time spent in CZ once test bird arrived at that area) may indicate that the reduced time in close proximity to conspecifics was mainly due to a delayed arrival to that area and not to a reduced sociality. Additional support for the latter explanation would be gained if we consider that the stressor exposure narrowly failed ($P < 0.07$) to significantly increase the latency to enter CZ. A study that evaluates the effect of prior stress exposure on the affiliation responses during longer test periods would be worthy to evaluate this hypothesis.

Exposure to a restraint stressor has been also shown to affect the social reinstatement responses of young chickens when evaluated in a runway with only one goal box (Marin et al., 2001). However, in that study STR chicks spent significantly longer times in CZ than their CON counterparts. Some considerations may provide an explanation for the differences observed in the two studies. Firstly, even though both runways represent a novel and therefore frightening situation, the position of the experimental bird at the beginning of the test with regard to the stimulus birds is different. In the single runway, the test bird has just one option to find social protection in close proximity to conspecifics. In the two goal box runway, the test chick may be considered to be “surrounded” by birds and the consequences of ambulation towards one end of the runway drives the bird further away from the birds at the opposite end. Secondly, the bird in the single box runway was exposed to one stimulus group at a time (either familiar or unfamiliar), while in our study the bird was presented with two different options simultaneously (both familiar and unfamiliar). Regardless of the ability to discriminate conspecifics at a distance (see other considerations below), in the two goal box runway, a further conflicting situation may arise when the bird decides towards which group of stimulus birds it is going to ambulate. In support of that, test birds arrived at the close zone area about five times sooner in the runway with only one goal box (Marin et al., 2001) compared to the runway with two goal boxes (Table 1), although the distance to travel from the start area was twice as long in the former study. Taken together, the results of both studies suggest that the way a bird will react in a runway depends on the characteristics of the apparatus (i.e., the presence of one or two goal boxes).

The CON group entered a higher number of squares in the familiar CZ than in the unfamiliar CZ. Although open to debate, that behaviour may reflect an increased exploratory activity in order to enter the goal box and make a closer contact with their familiar conspecifics in an otherwise unfamiliar surrounding (Bolhuis, 1991; Jones, 1996; Jones et al., 1996). While in close proximity to familiar conspecifics, STR birds showed a reduced locomotor activity at the end of the trial (5th min) compared to CONs and compared to its own activity shown in the previous min of the trial (4th min). The reduced locomotor activity in CZ was not accompanied by an increased locomotor activity in other zones of the runway. This response is consistent with the inhibitory effect that increased fear usually induces on other behaviours including locomotion (Hogan, 1965; Jones,

1987, 1996). On the other hand, when the locomotor activity was evaluated on the unfamiliar side of the runway at the end of the trial, both CON and STR birds reduced the number of squares entered in close proximity to the unfamiliar conspecifics (unfamiliar CZ) and increased the squares entered in the central zone of the runway. The results obtained within the STR group suggest that the manifestation of the fear-induced inhibition on locomotion depends on the characteristics of social stimuli (either familiar or unfamiliar) found in its close environment. Interestingly though, no significant differences were detected between CON and STR birds in the total number of squares entered during the trial. These locomotor activity results suggest that prior stressor exposure did not affect the overall amount of locomotion but altered the spatial distribution of the locomotor behaviour depending on the identity or social condition (familiar or unfamiliar) of the conspecifics in its close environment. The suggestion is currently been further tested in a runway with two goal boxes study on the affiliation responses of two lines of Japanese quail genetically selected for either a reduced (low stress) or an exaggerated (high stress) plasma corticosterone response to a restraint stressor (Satterlee and Johnson, 1988). These lines have been proposed as useful animal models for studying the relationships between behaviour, stress, welfare and production performance (Marin et al., 2002; Jones et al., 2004) because they show important differences in fearfulness, non-specific reduction in stress responsiveness, sociality and productivity traits (Jones and Satterlee, 1996; Jones et al., 1992, 2002; Marin et al., 2002).

Regardless of treatment, test chicks showed shorter latencies to enter and spent longer time in the familiar than in the unfamiliar CZ suggesting that young chicks can discriminate between familiar and unfamiliar conspecifics encountered in novel surroundings. These results are consistent with previous observations in a runway with only one goal box, where the time spent in close proximity to conspecifics was longer when the goal box contained familiar compared to unfamiliar birds (Marin et al., 2001). Previous findings using other testing procedures also support the contention that poultry chicks are able to discriminate between partner and stranger birds at a very low age (Jones, 1984; Cailotto et al., 1989; Jones et al., 1996).

In another two goal box runway study with red jungle fowl and White Leghorns, Väisänen and Jensen (2004) reported that the avoidance versus affiliation reaction measured as a tendency to stay in each close zone (which is based on social discrimination) is clear only when the chick had once approached the social stimuli. This finding is in agreement with our results showing a lack of significant differences in the latencies to start approaching familiar or unfamiliar conspecifics and the absence of differences between the number of birds that initiated their travel (first 20 cm ambulated) towards familiar or unfamiliar conspecifics. It has been reported that visual recognition in hens is possible only at a very close distance of 8–30 cm (Dawkins, 1995) therefore distinguishing the social identity (familiar or unfamiliar) of the conspecifics in the goal boxes might have been difficult for the test chicks from the midpoint of the runway (where they were placed at the beginning of the test). On the other hand, test chicks showed significantly shorter latencies to enter the familiar CZ than to enter the unfamiliar CZ regardless of treatment. Moreover, a higher number of CON birds entered first the familiar CZ than the unfamiliar one, suggesting that social discrimination might occur at a distance between 20 and 60 cm. Interestingly that discriminating effect was not evident within the STR group. This suggests that stressor exposure either altered the ability of the birds to discriminate at a distance or made both groups of stimulus birds (familiar or unfamiliar) equally attractive (or rewarding), at least when the first close approach to companions is being made. Another possibility is that STR chicks set off towards the first group seen, which could be chance, depending on which direction they were facing when starting ambulation, without discriminating. At the end of the trial, once in close proximity to conspecifics, birds reduced their locomotor activity in the unfamiliar CZ and

increased it in the central zones of the runway. This indicates that birds, instead of remaining with unfamiliar conspecifics, continued exploring the apparatus. Taken together, the results suggest that both CON and STR birds were able to clearly discriminate stimulus birds at a very short distance and that their affiliation responses were influenced by the identity (familiar or unfamiliar) of the stimulus birds.

We have already described how a mis-match between underlying sociality and the birds' social environment could damage welfare and productivity (see Section 1). In this respect, the findings concerning the behavioural and physiological correlates of contrasting T-maze responses in meat-type chickens sound a proactive and positive note. Chicks that traversed a T-maze and thereby reinstated social contact quickly subsequently grew faster (Marin et al., 1997, 1999, 2003), showed lower adrenocortical responses to an acute stressor (Marin and Jones, 1999), and exhibited markedly greater sociality in the T-maze, as well as in other behavioural tests of sociality (runway and home cage) (Jones et al., 1999; Marin et al., 2003) than their slower counterparts. The improved growth in the rapid T-maze runners may have reflected their ability to devote more of their bodily resources to growth rather than to responding to social and/or other stressors. It was proposed that recording chicks' social responses in a simpler apparatus, such as a runway leading to a goal box containing visible chicks, might be as effective, if not more so, than the T-maze apparatus (Marin et al., 2003). However, the results of the present runway study (with two goal boxes) and our previous study with only one goal box suggest that affiliation responses do not totally generalize from one study to another and are highly dependent on the apparatus design and conditions of testing used in each particular case. Thus, a clear picture of the most appropriate apparatus design and the affiliation measures taken to be used as predictors of better productivity and/or welfare is yet to come.

5. Conclusion

Our findings confirm that exposure to an acute stressor or frightening event subsequently affects chicks' affiliation responses in runway tests. The way a bird will react depends on the identity or social condition (familiar or unfamiliar) of the conspecifics in its close environment. Therefore, to avoid inaccurate conclusions in studies of social motivation, efforts should be made to prevent the birds from being stressed prior to testing and the identity of the birds used as social stimuli (i.e. familiar or unfamiliar), should be carefully considered.

Acknowledgements

This research was supported by grants from CONICET, FONCYT and SECyT UNC, Argentina. DAG holds a research fellowship from the later institution. RHM is a career member of CONICET, Argentina.

References

- Analytical Software, 2000. Statistix7: A User's manual. Version 7, Analytical Software, Tallahassee, FL, pp. 154.
- Bolhuis, J.J., 1991. Mechanisms of avian imprinting. *Rev. Biol. Rev.* 66, 303–345.
- Cailotto, M., Vallortigara, G., Zanforlin, M., 1989. Sex differences in the response to social stimuli in young chicks. *Ethol. Ecol. Evol.* 1, 323–327.
- Dawkins, M.S., 1995. How do hens view other hens? The use of lateral and binocular visual fields in social recognition. *Behaviour* 132, 591–606.

- Doyen, J., 1987. Individual preference related to social rank in domestic fowl. In: Zayan, R., Duncan, I.J.H. (Eds.), *Cognitive aspects of the social behaviour in the Domestic fowl*. Elsevier Science, Amsterdam, pp. 151–172.
- Duncan, I.J.H., 1981. Animal behaviour and welfare. In: Clark, J.A. (Ed.), *Environmental Aspects of Housing for Animal Production*. Butterworths, London, pp. 455–470.
- Gallup Jr., G.G., 1979. Tonic immobility as a measure of fear in domestic fowl. *Anim. Behav.* 20, 166–169.
- Gallup Jr., G.G., Suarez, S.D., 1980. An ethological analysis of open-field behavior in chickens. *Anim. Behav.* 26, 368–378.
- Hogan, J.A., 1965. An experimental study of conflict and fear; an analysis of the behavior of young chicks towards a mealworm. Part 1; The behavior of chicks which do not eat the mealworm. *Behaviour* 26, 45–95.
- Hughes, B.O., Carmichael, N.L., Walker, A.W., Grigor, P.N., 1997. Low incidence of aggression in large flocks of laying hens. *Appl. Anim. Behav. Sci.* 54, 215–234.
- Jones, R.B., 1984. Open-field responses of domestic chicks in the presence of a cagemate or a strange chick. *IRCS Med. Sci.* 12, 482–483.
- Jones, R.B., 1986. The tonic immobility reaction of the domestic fowl: a review. *World Poult. Sci. J.* 42, 82–96.
- Jones, R.B., 1987. The assessment of fear in the domestic fowl. In: Zayan, R., Duncan, I.J.H. (Eds.), *Cognitive Aspects of Social Behaviour in the Domestic Fowl*. Elsevier, Amsterdam, pp. 40–81.
- Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications and imperatives. *World's Poultry Sci. J.* 52, 131–174.
- Jones, R.B., 1997. Fear and distress. In: Appleby, M.C., Hughes, B.O. (Eds.), *Animal Welfare*. CAB International, Wallingford, UK, pp. 75–87.
- Jones, R.B., Hocking, P.M., 1999. Genetic selection for poultry behaviour: big bad wolf or friend in need? *Anim. Welfare* 8, 343–359.
- Jones, R.B., Merry, B.J., 1988. Individual or paired exposure of domestic chicks to an open field: some behavioural and adrenocortical consequences. *Behav. Process.* 16, 75–86.
- Jones, R.B., Mills, A.D., 1999. Divergent selection for social reinstatement behaviour in Japanese quail: effects on sociality and social discrimination. *Poult. Avian. Biol. Rev.* 10, 213–223.
- Jones, R.B., Satterlee, D.G., 1996. Threat-induced behavioural inhibition in Japanese quail genetically selected for contrasting adrenocortical response to mechanical restraint. *Br. Poult. Sci.* 37, 465–470.
- Jones, R.B., Satterlee, D.G., Ryder, F.H., 1992. Open-field behavior of Japanese quail chicks genetically selected for low or high plasma corticosterone response to immobilization stress. *Poult. Sci.* 71, 1403–1407.
- Jones, R.B., Mills, A.D., Faure, J.M., 1996. Social discrimination in Japanese quail *Coturnix japonica* chicks genetically selected for low or high social reinstatement motivation. *Behav. Proc.* 36, 117–124.
- Jones, R.B., Marín, R.H., García, D.A., Arce, A., 1999. T-maze behaviour in domestic chicks: a search for underlying variables. *Anim. Behav.* 58, 211–217.
- Jones, R.B., Marin, R.H., Satterlee, D.G., Cadd, G.G., 2002. Sociality in Japanese quail (*Coturnix japonica*) genetically selected for contrasting adrenocortical responsiveness. *Appl. Anim. Behav. Sci.* 75, 337–346.
- Jones, R.B., Marin, R.H., Satterlee, D.G., 2004. A 'pebble test of anxiety' did not differentiate between Japanese quail chicks from genetic lines differing in fearfulness and stress responsiveness. *Appl. Anim. Behav. Sci.* 87, 287–291.
- Marin, R.H., Jones, R.B., 1999. Latency to traverse a T-maze at 2 days of age and later adrenocortical responses to an acute stressor in domestic chicks. *Physiol. Behav.* 66, 809–813.
- Marin, R.H., Arce, A., Martijena, I.D., 1997. T-maze performance and body weight relationship in broiler chicks. *Appl. Anim. Behav. Sci.* 54, 197–205.
- Marin, R.H., Jones, R.B., García, D.A., Arce, A., 1999. Early T-maze behaviour and subsequent growth in commercial broiler flocks. *Brit. Poult. Sci.* 40, 434–438.
- Marin, R.H., Freytes, P., Guzman, D., Jones, R.B., 2001. Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. *Appl. Anim. Behav. Sci.* 71, 57–66.
- Marin, R.H., Satterlee, D.G., Cadd, G.G., Jones, R.B., 2002. T-maze behavior and early egg production in Japanese quail selected for contrasting adrenocortical responsiveness. *Poult. Sci.* 81, 981–986.
- Marin, R.H., Satterlee, D.G., Castille, S.A., Jones, R.B., 2003. Early T-maze behavior and broiler growth. *Poult. Sci.* 82, 742–748.
- McBride, G., Parer, I.P., Foenander, F., 1969. The social organization and behaviour of the feral domestic fowl. *Anim. Behav. Monogr.* 2, 125–181.
- Mills, A.D., Faure, J.M., 1990. Panic and hysteria in domestic fowl: a review. In: Zayan, R., Dantzer, R. (Eds.), *Social Stress in Domestic Animals*. Kluwer Academic Publishers, Dordrecht, pp. 248–272.
- Mills, A.D., Jones, R.B., Faure, J.M., Williams, J.B., 1993. Responses to isolation in Japanese quail genetically selected for low or high sociality. *Physiol. Behav.* 53, 183–189.

- Mills, A.D., Jones, R.B., Faure, J.M., 1995. Species specificity of social reinstatement in Japanese quail *Coturnix japonica* genetically selected for high or low levels of social reinstatement behaviour. *Behav. Proc.* 34, 13–22.
- Petherick, J.C., Seawright, E., Waddington, D., 1993. Influence of motivational state on choice of food or a dustbathing/foraging substrate by domestic hens. *Behav. Processes* 28, 209–220.
- Rajecki, D.W., Ivins, B., Rein, B., 1976. Social discrimination and aggressive pecking in domestic chicks. *J. Comp. Psychol.* 90, 442–452.
- Rogers, L.J., 1995. *The Development of Brain and Behaviour in the Chicken*. CAB International, Wallingford, UK, pp. 104–108.
- Satterlee, D.G., Johnson, W.A., 1988. Selection of Japanese quail for contrasting blood corticosterone response to immobilization. *Poult. Sci.* 67, 25–32.
- Satterlee, D.G., Jones, R.B., Ryder, F.H., 1993. Short-latency stressor effects on tonic immobility fear reactions of Japanese quail divergently selected for adrenocortical responsiveness to immobilization. *Poult. Sci.* 72, 1132–1136.
- Shirley, E.A., 1987. Application of ranking methods to multiple comparison procedures and factorial experiments. *Appl. Statistics* 36, 205–213.
- Suarez, S.D., Gallup Jr., G.G., 1983. Social reinstatement and open-field testing in chickens. *Anim. Learn. Behav.* 11, 119–126.
- Vallortigara, G., 1992. Affiliation and aggression as related to gender in domestic chicks (*Gallus gallus*). *J. Comp. Psychol.* 106, 53–57.
- Vallortigara, G., Cailotto, M., Zanforlin, M., 1990. Sex differences in social reinstatement motivation of the domestic chick (*Gallus gallus*) revealed by runway tests with social and nonsocial reinforcement. *J. Comp. Psych.* 104, 361–367.
- Väisänen, J., Jensen, P., 2004. Responses of young red jungle fowl (*Gallus gallus*) and White Leghorn layers to familiar and unfamiliar social stimuli. *Poult. Sci.* 83, 335–343.
- Zajonc, R.B., Wilson, W.R., Rajecki, D.W., 1988. Affiliation and social discrimination produced by brief exposure in day-old domestic chicks. *Anim. Behav.* 23, 131–138.
- Zayan, R., 1987. Recognition between individuals indicated by aggression and dominance in pairs of domestic fowl. In: Zayan, R., Duncan, I.J.H. (Eds.), *Cognitive Aspects of the Social Behaviour in the Domestic Fowl*. Elsevier Science, Amsterdam, pp. 321–438.