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Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers

Raul H. Marin[a], Paula Freytes[a], Diego Guzman[a], R. Bryan Jones[b,*]

[a]*Centro Regional de Investigaciones Científicas y Transferencia Tecnológica (CRILAR-CONICET), Calle Mendoza y Entre Ríos, Anillaco 5301, Argentina*

[b]*Welfare Biology Group, Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, Scotland, UK*

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Abstract

Genetic selection for appropriate levels of sociality (motivation to be with conspecifics) could benefit poultry welfare and performance. Runway tests that require chicks to traverse a corridor in order to reach other chicks in a goal box are commonly used to measure this behavioural trait. However, we need to determine if the chicks' responses in such tests are sensitive to certain experiential variables before we can recommend possible selection criteria for future breeding programmes. The present study focused on fear and on the identity of the stimulus birds. Broiler chicks either remained undisturbed or were exposed to an acute stressor (mechanical restraint) before their tonic immobility fear responses were measured 1 h later in Experiment 1. Exposure to the stressor significantly prolonged tonic immobility and, hence, presumably, underlying fear levels. In Experiment 2, the responses of stressed chicks and undisturbed controls were assessed when they were tested individually in a runway with a goal box containing either familiar or unfamiliar chicks of the same age. Our finding that stressed chicks emerged from the start box sooner and spent longer near the stimulus birds suggests that exposure to a frightening event increased social reinstatement motivation. Furthermore, social affiliation was more pronounced when the goal box contained familiar cagemates rather than strange chicks, regardless of prior treatment. This finding demonstrates that broiler chicks that were housed in groups of twelve can discriminate between familiar and unfamiliar conspecifics encountered in novel surroundings. Thus, sociality was positively associated with fearfulness and broilers clearly showed social discrimination in runway tests. These findings highlight the dangers of disregarding variables, such as fear and the capacity for social recognition in tests of social motivation. We strongly recommend that exposure to frightening events prior to test should be avoided and that the identity of the birds in the runway

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[*] Corresponding author. Tel.: +44-131-527-4466; fax: +44-131-440-0434.
E-mail address: bryan.jones@bbsrc.ac.uk (R. Bryan Jones).

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goal box should be standardized, i.e. either familiar or unfamiliar, and noted.

Keywords: Chicken; Fear; Sociality; Social discrimination; Stress; Tonic immobility

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1. Introduction

The chicken is a highly social animal that would normally live in small family groups or in larger mixed groups with a relatively stable social structure; indeed close attachments are often formed between brood mates or members of a group (Duncan, 1981; McBride et al., 1969). However, modern farming practice often imposes several deviations from what might be considered the natural situation; these include crowding, the alteration of group membership, loss of contact with familiar flock-mates, and exposure to strangers. Disrupting the birds' social environment or confining them in an inappropriate one can cause them intense and prolonged distress (Jones and Harvey, 1987; Mills and Faure, 1990; Jones and Mills, 1999). Not only can this contribute to the development of depression (reduced vitality or even hopelessness), social withdrawal and cognitive impairment but it may also seriously damage the birds' health and productivity (Duncan, 1981; Mills and Faure, 1990; Jones, 1996; Mendl, 1999).

The birds' underlying levels of sociality (motivation to be with conspecifics) can exert profound effects on all aspects of social interaction, including affiliation, aggression, dispersal, mating and social structure, as well as on the birds' behavioural and physiological responses to social disruption (Jones, 1984; Vallortigara, 1992; Jones, 1996; Jones and Mills, 1999). Therefore, a mis-match between a bird's underlying sociality and its social environment could elicit either a series of acute stress responses or chronic social distress (Mills et al., 1993; Jones and Hocking, 1999; Jones and Mills, 1999). For instance, low-sociality birds might be ill-suited for housing in social groups, particularly very large or very confined ones.

We already know that sociality in Japanese quail is sensitive to selective breeding and that its expression can be influenced by certain environmental manipulations, such as sudden isolation or the need to interrupt social contact to gain access to food and water (Mills and Faure, 1991; Faure and Mills, 1998; Jones and Mills, 1999). We also know that there is considerable individual variation in sociality in domestic chicks (Jones et al., 1999). If such phenotypic variation translated to genetic variability there might be considerable scope for genetic selection for levels of this behavioural trait that are considered appropriate for particular housing systems. However, we need to improve our understanding of a number of variables that might influence our assessment of sociality before we can make recommendations about test criteria, selection programmes, or optimum levels of sociality for different housing systems. In the present study, we focused on the potential effects of manipulating fear levels and the identity of the stimulus birds for the reasons described below.

Runway tests have been widely used to measure social reinstatement behaviour (and thereby underlying sociality) as well as the ability to make social discriminations in birds (Suarez and Gallup, 1983; Vallortigara et al., 1990; Mills et al., 1995; Jones et al., 1999).

For example, 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). Runway tests involve removing a bird from its home environment, placing it at one end of a corridor, and then measuring the time it takes to approach a small group of conspecifics held in a goal box at the opposite end as well as how long it spends near them. This test incorporates exposure to frightening events (capture by the experimenter and exposure to a novel environment) as well as social stress (transient separation from companions). Therefore, it follows that the birds' runway responses might be sensitive to their underlying levels of fearfulness; i.e. the propensity to be easily frightened by a wide range of potentially alarming events (Jones, 1996).

Prior exposure to a known stressor can markedly affect animals' responses in subsequent test situations by increasing fearfulness (Suarez and Gallup, 1983; Jones, 1997; Satterlee et al., 1993). More specifically, brief mechanical restraint is a powerful stressor (Satterlee and Johnson, 1988; Jones et al., 2000) and exposing Japanese quail to this procedure immediately before the induction of tonic immobility (TI) significantly increased the duration of this fear-potentiated anti-predator reaction (Satterlee et al., 1993). Therefore, in Experiment 1, we determined whether brief restraint in a crush cage would also heighten fearfulness in broiler chicks by measuring its effects on their TI responses 1 h later.

Chickens are attracted to familiar stimuli in otherwise unfamiliar surroundings (Bolhuis, 1991; Jones, 1996) and the presence of conspecifics is thought to reduce fear in such situations (Jones and Merry, 1988). It is also generally accepted that young domestic chicks, or at least those of laying strains, can differentiate between cagemates and strange chicks. 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). Fear-related responses in a novel open field were also less pronounced when chicks were tested in pairs consisting of cagemates rather than strangers (Jones, 1984). Moreover, Japanese quail from genetic lines selected for low or high sociality (Mills and Faure, 1991; Jones and Mills, 1999) readily discriminated between cagemates and strange quail in a two-choice runway test (Jones et al., 1996). In Experiment 2, previously untested chicks either remained undisturbed or were exposed to brief mechanical restraint 1 h before their sociality levels were assessed in a runway when the goal box contained either familiar or unfamiliar chicks. This enabled us to address two main questions. Firstly, are the affiliation responses of domestic broiler chicks in a runway sensitive to the identity of the stimulus birds? Secondly, are social reinstatement responses exaggerated after exposure to a frightening event?

2. General methods

2.1. Animals and husbandry

We used mixed-sex Cobb broiler chicks throughout the present study. Two separate batches of 48 and 120 mixed-sex Cobb broiler chicks were obtained from a commercial supplier (INDACOR, Argentina) at 1 day of age for Experiments 1 and 2, respectively.

Upon receipt, they were randomly allocated to groups of 12 and housed in one room in wooden brooders painted white and measuring 85 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 with a photoperiod of 07.00–20.00 h. Food (Cargill, broiler BB, 20% min crude protein, 12.34 MJ of metabolisable energy/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.

3. Experiment 1

3.1. Methods

At 10 days of age, the TI reactions of 40 chicks were recorded after their exposure to one of two treatments. Twenty unstressed chicks (controls, CON) remained undisturbed in the brooder until they were individually captured and gently carried approximately 8 m by hand to an experimental room for TI testing (see below). The remaining 20 chicks comprised the stressed (STR) treatment group; these were also individually captured and then restrained for 5 min in a wooden crush cage (20 cm × 20 cm × 20 cm) painted white. As soon as the bird was placed in the cage a moveable interior wall was fixed in place so that it prevented all but slight movements of the chick's head and legs and those associated with respiration. This procedure is known to elicit fear and to elevate circulating levels of corticosterone (Satterlee and Johnson, 1988; Jones et al., 2000). Each of the STR chicks was then marked on the head with black ink to facilitate later identification and returned to its home cage before its TI responses were measured 1 h later. Equal numbers of CON and STR chicks were taken from each home cage and two chicks in each group of 12 remained untested.

Each bird was tested individually and once only. TI was induced by restraining the chick on a table top for 15 s; it was held on its left side facing away from the experimenter with one hand on its sternum and one lightly cupping its head. We measured the number of inductions (15 s periods of restraint) necessary to attain TI lasting at least 20 s and the duration of TI, i.e. the latency until the bird righted itself. If the bird failed to meet the 20 s immobility criterion the induction procedure was repeated immediately. If TI could not be induced by the fifth attempt, the chick was deemed to be non susceptible and a TI duration of 0 s was given. A test ceiling of 10 min was set and any chick that failed to right itself before this time elapsed was given a maximum score of 600 s. All testing was carried out between 9.00 and 15.00 h. Twenty chicks were tested in each treatment group and test order was random. A one-way ANOVA was used to analyse the untransformed TI scores which all met the assumptions of the test.

3.2. Results and discussion

A numerical tendency for stressed chicks to require fewer inductions before adopting TI than controls failed to reach significance. However, those chicks that had been exposed

Table 1
Tonic immobility responses of 10-day-old broiler chicks that had either remained undisturbed (CON) or that had been exposed to a mechanical restraint stressor 1 h earlier (STR); means ± standard errors ($N = 20$)[a]

Measures	CON	STR	$F_{1,38}$	P value
Inductions (no)	2.60 ± 0.38	1.85 ± 0.26	2.63	0.113
TI duration (s)	181.0 ± 42.7	327.9 ± 52.1	4.73	0.036

[a] No: number; F values were derived from a one-way analysis of variance.

to the restraint stressor showed significantly longer durations of tonic immobility than did the undisturbed controls (Table 1). This finding clearly indicates that fear levels were heightened in broiler chicks that had been exposed to an acute restraint stressor 1 h earlier.

4. Experiment 2

4.1. Methods

The social reinstatement responses of eighty previously unused chicks were tested in a runway when they were 9 or 10 days old. Eight of the 12 chicks in each of the 10 home cages were tested in the runway and the remaining four birds in each cage were used as test stimuli in the goal box (see below). Half of the tested chicks were exposed to the crush cage stressor (stressed, STR) described above for 5 min. They were then marked and returned to their home cages before their runway responses were recorded 1 h later. The remaining 40 chicks (controls, CON) remained undisturbed in their home cages until runway testing began. Four CON and four STR chicks were taken from each cage. Each of these groups was then further subdivided at test. Twenty of the 40 CON chicks were exposed to cagemates in the runway goal box whereas birds from different cages (strangers) were used as stimuli for the other 20 chicks. Birds housed in different home boxes were visually isolated from each other during rearing but we cannot state equivocally that some degree of familiarity between them might not have been imparted through ‘‘social transmission’’ of auditory or of airborne olfactory cues. There were four test groups of 20 birds each (control + cagemates, stressed + cagemates, control + strangers and stressed + strangers).

We used two runways so that two chicks could be tested simultaneously by two experimenters. Each test apparatus consisted of an unpainted wooden corridor measuring 200 cm × 40 cm × 40 cm (length × width × height). This was divided into three compartments using removable wire-mesh partitions. The compartments situated at opposite ends of the runway were each 20 cm long and they comprised the start box and the goal box, respectively. Thus, the actual runway was 160 cm long. Each runway was situated in a separate experimental room and the temperature and illumination were maintained at similar levels to those in the room in which the chicks were reared with the addition of a 60 W light bulb suspended above the goal box. Two age-matched chicks were used as stimulus birds in the goal boxes. These were taken either from the same home cage as the test chick (cagemates) or from another cage (strangers) and they were allowed 2 min to acclimatise before testing began. Because it was considered likely that the stimulus birds would be more attractive to the test chick if they were active or standing than if they were immobile or asleep they were changed after each block of four tests. Each chick was tested individually and once only. It was placed in the start box of one of the runways and allowed 2 min to acclimatise; it could see the stimulus birds at this time. The wire mesh door was then raised and we measured the latencies for the chick to leave the start box and to enter the 20 cm 'close' zone nearest the goal box as well as the accumulated time spent in the close zone over the 10 min test period. This particular size of the close zone was chosen because it was considered sufficiently small to allow a rigorous test of social proximity. The chick was scored as being in the zone if it contained the major part of its body. The chicks could not see the experimenters who sat behind hides and observed the chicks through small holes. Testing began at 08.00 h and was completed by 16.00 h on both of the test days. Each chick was marked with ink after test to facilitate identification of untested chicks and then returned to its home cage. Each consecutive block of four tests comprised representatives from each of the four treatment groups and test order was random within blocks.

The data were subjected to analyses of variance (ANOVA) with a 2×2 factorial arrangement. The factorial was made on the effects of stress treatment (control versus stressed) and type of stimulus birds (cagemates versus strangers). The latencies for the chick to leave the start box and to enter the 20 cm 'close' zone nearest the goal box were transformed to logarithms in order to fit the assumptions of the ANOVA. A P value of <0.05 was considered to represent significant differences.

5. Results and discussion

Although there were no detectable effects of the identity of the stimulus birds (cagemates, strangers) on the latency scores ($F_{1,76} = 0.07$, $P = 0.786$ and $F_{1,76} = 0.48$, $P = 0.490$ for the latencies to leave the start box and to reach the close zone, respectively), broiler chicks spent significantly longer ($F_{1,76} = 4.59$, $P < 0.036$) in the close zone when the goal box contained cagemates than when it contained strangers (Table 2). Prior

Table 2
Means and standard errors of the runway responses of undisturbed (CON) and stressed (STR) broiler chicks tested with either cagemates or strange chicks in the goal box[a]

Measures (s)	Cagemates		Strangers	
	CON	STR	CON	STR
Latency to leave	21.7 ± 12.2	4.5 ± 1.3	10.5 ± 3.0	13.2 ± 6.0
	(0.79 ± 0.12)	(0.46 ± 0.09)	(0.76 ± 0.10)	(0.54 ± 0.13)
Latency to enter CZ	33.7 ± 17.2	8.3 ± 1.5	27.2 ± 9.8	27.8 ± 14.6
	(1.12 ± 0.11)	(0.84 ± 0.06)	(1.11 ± 0.10)	(0.99 ± 0.13)
Time in CZ	494.8 ± 24.1	568.0 ± 14.9	446.2 ± 14.8	531.8 ± 23.2

[a] CZ: close zone (within 20 cm of stimulus birds). Logarithmic values are shown in parentheses where transformation of data was required.

exposure to the crush cage stressor decreased the latency to leave the start box ($F_{1,76} = 5.69$, $P < 0.020$) and significantly increased ($F_{1,76} = 15.86$, $P < 0.001$) the time spent near the stimulus birds. A tendency for stressed chicks to enter the close zone sooner than the controls narrowly failed to reach significance ($F_{1,76} = 3.38$, $P < 0.070$). The effects of prior stress treatment appeared to be more pronounced in the presence of cagemates rather than strangers. However, there were no significant interactions between the effects of treatment and the type of stimulus birds ($F_{1,76} = 0.21$, $P = 0.649$; $F_{1,76} = 0.60$, $P = 0.442$; $F_{1,76} = 0.078$, $P = 0.781$ for the latencies to leave the start box and to reach the close zone and the time spent there, respectively).

The present findings suggest firstly that broiler chicks are capable of discriminating between familiar cagemates and strange chicks and secondly that the expression of social reinstatement responses in a runway test is positively associated with fearfulness.

6. General discussion

The duration of the tonic immobility response to manual restraint is widely considered to be positively related to the antecedent fear state and to thereby represent a useful behavioural index of fear (Gallup, 1979; Jones, 1996; Faure and Mills, 1998). In Experiment 1, broiler chicks that had been restrained for 5 min in a crush cage showed significantly longer TI responses when tested one hour later than did their undisturbed controls. This finding is consistent with a previous report that exposure to a similar immobilisation stressor prolonged the tonic immobility reactions of Japanese quail that were tested immediately after the stressful event (Satterlee et al., 1993). The present finding also indicates that the heightened fear levels induced by mechanical restraint persisted for at least one hour after the event in broiler chicks.

The results of Experiment 2 showed that exposure to this immobilisation stressor also affected the social reinstatement responses of previously untested broiler chicks when they were observed in a runway test one hour later. Stressed broilers emerged from the start box significantly sooner and then spent longer near the stimulus birds in the goal box than did the undisturbed control birds. Given the results of Experiment 1, these findings strongly suggest that social reinstatement motivation becomes more pronounced in broiler chicks after their exposure to a frightening event. This may reflect the fact that chicks are strongly attracted towards any familiar stimulus in otherwise unfamiliar and frightening surroundings (Bolhuis, 1991; Jones, 1996). Interestingly, genetic selection programmes using Japanese quail suggest that the behavioural traits of fearfulness and sociality can be manipulated independently of each other; thus, quail selected for short or long TI fear reactions showed similar social reinstatement responses in a treadmill test and vice versa (Faure and Mills, 1998).

Social affiliation and proximity were also significantly greater here when the goal box contained cagemates rather than strange conspecifics, regardless of prior treatment. Interestingly, despite the absence of significant treatment × identity interactions, social reinstatement responses after exposure to restraint stress were more pronounced when the goal box contained cagemates rather than strangers. These results demonstrate that the ability to discriminate between familiar and unfamiliar conspecifics encountered in a novel environment generalises from Japanese quail (Jones et al., 1996) and domestic chicks of layer strains (Jones, 1984; Cailotto et al., 1989) to include those of a broiler (meat-type) strain. The fear-reducing effects of the presence of a cagemate rather than a stranger in an open field was lost when chicks of a layer strain were housed in groups of ten rather than in pairs (Jones, 1984); this suggests that there may be a critical number between 2 and 10 above which those chicks could not learn and remember the individual characteristics of their cagemates. However, the present findings indicate that the ceiling could be raised because social discrimination was apparent in broiler chicks housed in groups of 12.

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, recent findings concerning the behavioural and physiological correlates of contrasting T-maze responses in broiler chicks sound a proactive and positive note. Thus, chicks that traversed a T-maze and thereby reinstated social contact quickly subsequently grew faster in laboratory (Marin et al., 1997) and commercial (Marin et al., 1999) conditions, showed lower adrenocortical responses to an acute stressor (Marin and Jones, 1999), and exhibited markedly greater sociality in home cage as well as runway tests (Jones et al., 1999) 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 for responding to social and/or other stressors. Interestingly, the introduction of a novel balloon into the home cage elicited a lower plasma corticosterone response in Japanese quail genetically selected for high (HSR) rather than low (LSR) social reinstatement behaviour (Faure and Mills, 1998). Regardless of the precise mechanisms, these findings suggest that a selection programme based on a sociality criterion, like runway or T-maze behaviour, could have a number of beneficial effects. Indeed, it has already been suggested that poultry with high levels of sociality would probably be better able to adapt to high stocking densities (Faure and Mills, 1998), though Magnolon (1994) had sounded a cautionary note by suggesting that high sociality quail were more aggressive than low sociality ones. However, it was thought that this observation may have simply reflected an increased opportunity for fighting afforded by greater social proximity in HSR than LSR quail rather than line differences in aggressiveness (Jones and Hocking, 1999). Furthermore, it was recently shown that higher levels of pecking at intruder birds by HSR than LSR quail actually represented non-aggressive exploration (Francois et al., 2000).

In conclusion, our findings confirm that brief mechanical restraint can elevate underlying fear levels and that this effects persists for at least one hour after the event. Our findings also show that exposure to this frightening event subsequently increased chicks' affiliation responses in a runway and that social reinstatement was sensitive to the familiarity or otherwise of the stimulus birds. In other words, the expression of sociality is positively related to fearfulness and broilers can distinguish between familiar and unfamiliar chicks. Collectively, these findings illustrate the potential dangers of disregarding certain variables, such as fear and social discrimination, in studies of social motivation. Therefore, we make two strong recommendations for tests of sociality requiring transient isolation and voluntary locomotion along a novel, predetermined path. Firstly, effort should be made to prevent the birds from being frightened prior to test. Secondly, the identity of the birds in the runway goal box, (i.e. familiar or unfamiliar), should be standardized and noted.

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