Abstract: We investigated the effect of 60μg of corticosterone administered to domestic chicks either before or after hatching on the behavioural response to isolation in a novel arena and performance in a task involving the simultaneous identification of food and detection of a predator (overhead silhouette of a hawk moving overhead). Following release into a novel arena, chicks treated with corticosterone at 18 days of incubation emitted more distress vocalizations. In contrast, no difference in the number of vocalizations was found between chicks treated with corticosterone at day 1 post-hatching and controls. Behaviour in the home cages was generally similar across treatments, though chicks treated with corticosterone at 18 days of incubation slept more than control chicks. While searching for grain against a background of pebbles, chicks treated with corticosterone at embryonic day 18, but not chicks treated on day 1 post-hatching, took longer to detect the overhead image of a predator than did controls. Corticosterone treatment at both ages increased the rate of pecking at grains and pebbles. Our findings support work on other birds indicating that corticosterone treatment during incubation influences stress reactivity. The impairment in predator detection in chicks treated with corticosterone on day 18 of incubation appears to be caused by the known effects of corticosterone treatment at this age in preventing the development of lateralization of the thalamofugal visual projections. This further support the hypothesis that brain lateralization provides an advantage in performing more than one task simultaneously.

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Pre- and post-hatching effects of corticosterone treatment on behavior of the domestic chick.

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ABSTRACT

We investigated the effect of 60μg of corticosterone administered to domestic chicks either before or after hatching on the behavioral response to isolation in a novel arena and performance in a task involving the simultaneous identification of food and detection of a predator (overhead silhouette of a hawk moving overhead). Following release into a novel arena, chicks treated with corticosterone at 18 days of incubation emitted more distress vocalizations. In contrast, no difference in the number of vocalizations was found between chicks treated with corticosterone at day 1 post-hatching and controls. Behavior in the home cages was generally similar across treatments, though chicks treated with corticosterone at 18 days of incubation slept more than control chicks. While searching for grain against a background of pebbles, chicks treated with corticosterone at embryonic day 18, but not chicks treated on day 1 post-hatching, took longer to detect the overhead image of a predator than did controls. Corticosterone treatment at both ages increased the rate of pecking at grains and pebbles. Our findings support work on other birds indicating that corticosterone treatment during incubation influences stress reactivity. The impairment in predator detection in chicks treated with corticosterone on day 18 of incubation appears to be caused by the known effects of corticosterone treatment at this age in preventing the development of lateralization of the thalamofugal visual projections. This further support the hypothesis that brain lateralization provides an advantage in performing more than one task simultaneously.
INTRODUCTION

Corticosterone levels fluctuate during development for various reasons and in this respect modulation of corticosterone may play a critical role in establishing brain structures and behavioral patterns that persist into adult life (Wellberg and Seckl, 2001). For example, maternal corticosterone levels at the time of laying correlate with corticosterone in the yolk in Japanese quail, and birds from eggs with high corticosterone grow more slowly and the adult birds are more responsive to stressors than birds from low corticosterone eggs (Hayward and Wingfield, 2004), implying that the mother’s condition at the time of laying alters the phenotypic profile of her offspring. In canaries, corticosterone secretion of the hatchlings is influenced by hatching order (Schwabl, 1999), raising the possibility that the systematic variation of corticosterone deposited in the egg by the mother or secreted by the developing animal may be adaptive. Indeed, Kitaysky et al. (2003) showed that corticosterone-implanted kittiwake chicks increased food begging and intake and the authors suggested that this provided a trigger for brood reduction and therefore reduced competition for food. Kitaysky et al. (2003) also found that corticosterone-treated chicks were poorer than controls at forming a cue-food association 10 days after removal of the corticosterone implant, and they performed less well on a detour task 8 months later, showing long-term effects of having high levels of corticosterone during early life.

In the domestic chick, corticosterone levels generally increase throughout the incubation period (Kalliecharan and Hall, 1976) with the pituitary-adrenal axis becoming active about 6 days before hatching (Kalliecharan and Hall, 1974). Corticosterone administration during incubation has been shown to lead to behavioral and neurological changes. For example, injection of 60ng of corticosterone into the egg at the later stages of incubation improves recall of a passive avoidance
task (Sui et al, 1997), possibly by facilitation late-phase fucosylation of glycoproteins (Sandi and Rose, 1997). Another effect of corticosterone treatment during incubation is prevention of the development of asymmetry in the thalamofugal visual projections of the chick (Rogers and Deng, 2005).

There is considerable evidence indicating that there are left-right differences in brain functioning and responding that are wide-spread in vertebrate species (Rogers, 2002; Rogers and Andrew, 2002). It would appear that being lateralized, particularly for a response such as predator avoidance, would be disadvantageous since it reduces responding to predators on one side of the body. Hence the reasons for maintenance of at least this type of side bias is likely to be counteracted by some benefits. One possibility is that lateralization improves the ability to perform more than one task simultaneously, and indeed dark-incubated chicks (which lack asymmetry of the thalamofugal visual projections and of some types of visual behavior; Rogers and Bolden, 1991) are poorer at detecting an overhead predator while feeding than light-exposed, lateralized chicks (Rogers, 2000; Rogers et al, 2004; Dharmaretnam and Rogers, 2005). Although the above differences in response in light-exposed and dark-incubated chicks were not due to nonspecific effects caused by light-exposed chicks being more distressed by the stimulus (Rogers, 2000), it would be useful to provide an additional control for other possible effects, beyond the effects of dark incubation, on the development of visual lateralization. Injection of 60μg of corticosterone into the egg at 18 days of incubation prevents the light-stimulated development of asymmetry in the visual projections from the thalamus to the Wulst regions in the left and right hemispheres of the forebrain (Rogers and Deng, 2005), thus providing a means of removing the light-stimulated lateralization of the visual projections, which results from the embryo’s orientation within the egg so that it occludes its left eye. Therefore, in addition to
revealing the role and timing of corticosterone injection on behavioral development, comparison of the response of corticosterone treated and control chicks in a dual task was done to test the hypothesis that one advantage of brain asymmetry is that it facilitates the ability to perform more than one task at once.

Sui et al (1997) found that improvement of memory following a passive avoidance task was maximal when corticosterone was injected at days 19 and 20 of incubation, rather than just before this stage of incubation or after hatching. Clearly, there appears to be a sensitive phase of corticosterone sensitivity, at least for performance in the passive avoidance learning task. One aim of the present experiment was therefore to examine the effects on behavior of corticosterone injection before and after hatching.

In the following experiments we examined the effect of corticosterone injection on general behavior, response to isolation in a novel arena and performance in a dual task involving the simultaneous identification of food and detection of a predator (overhead image of a hawk). The dual task was based on two known lateralizations of the chick: the use of the right eye (left hemisphere) for pecking at food (Rogers, 1997) and the left eye (right hemisphere) to monitor for predators (Rogers, 2000). Corticosterone was injected on either day 18 of incubation or day 1 post-hatching to examine the possible age-related fluctuations in corticosterone sensitivity.
EXPERIMENT 1: Injection before hatching

Materials and methods

The subjects were 23 Black Australorp/White leghorn cross chickens obtained as fertile eggs from a commercial hatchery (SF Barter and Sons, Huntingwood, NSW, Australia). The eggs were initially incubated mainly in the dark but with some intermittent light, and transferred to a light incubator at 18 days of embryonic development. On day 18 of incubation, 0.5 ml of either a corticosterone (N=13) or a vehicle solution (N=10, controls) was injected into each egg using a 30G needle and delivered next to the embryo. The corticosterone solution contained corticosterone (Sigma number 05H0092 and 092k1255) dissolved in absolute ethanol at 12 mg/ml and diluted in sterile 0.9% saline to a concentration of 120μg/ml (hence each egg received 60μg of corticosterone). The vehicle solution contained 0.9% sterile saline and 1% ethanol. Within 24 hours of hatching, the chicks were housed in groups of 2 or 3 in metal cages (22 x 22 x 30 cm) with one transparent side. They were group housed at this age to facilitate learning to eat and drink. The room temperature was kept at 25±2°C with additional heating provided by 25 W bulbs placed 45 cm above each cage. Chick starter crumbs were scattered on the paper floor and water provided ad libitum. At the end of the second day after hatching (day 2) chicks were housed individually.

At 5 days of age, each chick was placed in the centre of a novel circular pen (diameter 85 cm, height 30 cm) and remained in this open-field for 5 min. The walls were made of sheet metal and the floor was covered with woodshavings. Behavior was recorded by an overhead video camera and the videos were analyzed using an observational logging program (ODlog 1.2.1,
Macropod Software, 2001). We recorded the latency to vocalize (peeps), the number of peeps, the time spent walking or running, the number of jumps, the number of pecks and the number of defaecations. Peeps are defined as short vocalizations in descending tone emitted when chicks are stressed. The open field was divided into 30 squares (17 cm) and the number of times that a chick entered a square (i.e. at least half the body over the line) was counted. Additionally the duration that chicks spent in the centre of the arena (45 cm diameter) was recorded.

At 7 days of age, behavior in the home cages was recorded by instantaneous sampling of each chick every minute for 40 minutes starting at 0900 hrs, 1200 hrs and 1500hrs. We recorded standing, sitting, sleeping (i.e. at least one eye closed), pecking (which included eating), drinking, walking, preening, jumping and “other” behavior (such as stretching, scratching and defecating).

At 8 and 9 days of age, the chicks were tested in a task requiring them to search for food grains on a background of pebbles adhered to the floor while, at the same time, monitoring overhead for the image of a predator (task described in detail by Rogers et al., 2004 and referred to as the pebble floor task). The testing apparatus consisted of a 75x 20 x 30cm apparatus, made from wood and painted grey. The ends of the apparatus were partitioned with transparent perspex sheets, preventing access to 23cm sections at each side. Mirrors angled at 45° were positioned in the end sections to allow the experimenter to view the chick (see diagram in Rogers et al., 2004). Glued to the floor of the central section (20 x 29cm) were small pebbles, approximately the size of starter crumbs. The chick could be observed through a small window in the side and at floor level to record the number of pecks at pebbles and crumbs. A flat TV screen (Panasonic TX-66PW150A with a refresher rate of 50 Hz) was placed 40cm above the floor of the apparatus, and displayed a silhouette approximating a hawk or kite (8cm in width, Fig. 1) on a white background moving from right to left at a speed of 12 cm/s. The moving image was presented every 18s. This
video presentation had been used successfully by Dharmaretnam and Rogers (2005) to reveal differences between strongly and weakly lateralized chicks.

The testing procedure involved placing the chick on the pebble floor and, when the chick had pecked 5 times, the video of the moving hawk was presented. Observations continued until the chick had pecked 60 more times, or after 20min, whichever came first. Pecks were scored as being made at either the food grains or the pebbles. On the following day (day 9), each chick was again placed in the apparatus and the first 25 pecks were recorded with the overhead image of the hawk presented after the chick had pecked 5 times.

The responses of the chick to the hawk image were recorded on video. Latency to respond to the hawk and response to each presentation of the hawk were scored on a scale of 0-5: 0=no reaction, 1= look up or tilt head, 2= peep, turn body or walk, 3= startle call, sideways step or circle, 4= freeze, head shake or jump and 5= crouch or run.

**Analysis**

Behavior in the open field was analyzed in a 2x2 (sex and treatment as the factors) ANOVA. Behavior in the home pens was analyzed using a repeated measures ANOVA with the time of day as a within subject-factor and treatment and sex as between-subject factors. Since drinking and other behavior did not meet the requirements for parametric analysis (as these activities were quite rare), a mean for each individual was taken from the three observation periods and, because these data met requirements for parametric analysis, analyzed in a 2x2 ANOVA. Jumps were infrequent and were not analyzed.

The first five pecks in the pebble floor test were recorded in the absence of the overhead image of the hawk, to ensure that chicks started to peck. One chick did not peck at all, and was
omitted from the analysis (hence N=12 for the corticosterone treatment). The number of pecks at pebbles in the first 5 pecks and latency to carry out the first 5 pecks were analyzed in an ANOVA. The number of pecks at pebbles and duration to peck twenty times were analyzed in a repeated measures ANOVA, with block of pecks (first 20, 21-40 and last 20 pecks) as a within-subject variable, and treatment and sex as the between-subject variables. The latency to respond to the hawk (i.e. the trial in which a response to the hawk was first detected) and the magnitude of this response was analyzed in Mann-Whitney tests.

Results

Open field test

Corticosterone-treated chicks emitted more peeps than control chicks (Fig 2: ANOVA, $F_{1,19}=16.9$, $P<0.001$). Additionally, males emitted more peeps than females in the open field test (Fig 2: ANOVA, $F_{1,19}=10.8$, $P=0.004$), but there was no significant interaction between treatment and sex (ANOVA, $F_{1,19}=0.9$, $P=0.33$). Despite the above differences, no significant differences in the latency to begin vocalization were found (treatment effect, ANOVA, $F_{1,19}=0.75$, $P=0.40$; sex effect, ANOVA, $F_{1,19}=2.5$, $P=0.13$; interaction, ANOVA, $F_{1,19}=0.09$, $P=0.76$).

Males tended to spend more time walking than females (103.4±17.7 s and 83.6±14.9 s respectively, ANOVA, $F_{1,19}=4.2$, $P=0.054$) and to enter more squares than females (87.6±20.4 and 72.0±22.2 squares entered respectively, ANOVA, $F_{1,19}=3.3$, $P=0.08$). Neither the treatment/sex interaction (time spent walking, ANOVA, $F_{1,19}=2.6$, $P=0.12$; squares entered, ANOVA, $F_{1,19}=2.4$, $P=0.14$) nor treatment alone (time spent walking, ANOVA, $F_{1,19}=2.3$, $P=0.14$; squares entered, ANOVA, $F_{1,19}=3.0$, $P=0.09$) were found to influence activity in the open
field test. There was no difference in the total time spent in the centre (treatment effect, ANOVA, F\(_{1,19}=0.4\), P=0.53; sex effect, ANOVA, F\(_{1,19}=0.02\), P=0.89; interaction, ANOVA, F\(_{1,19}=0.4\), 0.51).

Lastly, in the open field, there were no significant differences in the number of pecks (treatment effect, ANOVA, F\(_{1,19}=1.3\), P=0.27; sex effect, ANOVA, F\(_{1,19}=1.3\), P=0.27; interaction, ANOVA, F\(_{1,19}=1.2\), P=0.29) or in the number of defaecations (treatment effect, ANOVA, F\(_{1,19}=1.9\), P=0.18; sex effect, ANOVA, F\(_{1,19}=0.04\), P=0.85; interaction, ANOVA, F\(_{1,19}=0.04\), P=0.85).

Behavior in the home cages

Corticosterone-treated chicks slept less than control chicks (Fig 3; Treatment effect, ANOVA, F\(_{1,19}=5.1\), P=0.04; though contrary to the indications of Fig 3, the treatment/time of day interaction was not significant, ANOVA, F\(_{2,38}=1.2\), P=0.32). Treatment had no effect on standing (ANOVA, F\(_{1,19}=1.35\), P=0.26), sitting (ANOVA, F\(_{1,19}=0\), P=0.98), pecking (ANOVA, F\(_{1,19}=1.4\), P=0.25), walking (ANOVA, F\(_{1,19}=0\), P=0.98), preening (ANOVA, F\(_{1,19}=0.8\), P=0.78), drinking (ANOVA, F\(_{1,19}=1.3\), P=0.26), or any other behavior (ANOVA, F\(_{1,19}=2.0\), P=0.18), and there were no significant interactions involving treatment.

In conjunction with the increased sleeping in the afternoon (Fig 3; ANOVA, F\(_{2,38}=7.2\), P=0.002), there was also less walking (3.8±1.0\% at 0900hrs, 4.0±0.8\% at 1200hrs and 1.4±0.4\% at 1500hrs; ANOVA, F\(_{2,38}=4.1\), P=0.02) and less preening (5.7±1.0\% at 0900hrs, 9.7±1.5\% at 1200hrs and 3.8±0.9\% at 1500hrs; ANOVA, F\(_{2,38}=7.5\), P=0.002) in the afternoon. No significant interactions involving the time of day were observed. Time of day had no effect on the amount of time spent sitting (ANOVA, F\(_{2,38}=0.8\), P=0.47) or pecking (ANOVA, F\(_{2,38}=1.7\), P=0.20).
Males spent more time standing than females in the morning but less than females in the afternoon (morning and afternoon respectively; males 23.6±4.0% and 7.6±2.9%, females 14.3±1.8% and 14.6±2.4%, time/sex interaction, ANOVA, F2,38=3.4, P=0.04). Additionally, females sat more than males (8.3±1.7% and 4.1±1.4% of the time respectively; ANOVA, F1,19=8.6, P=0.009). No other significant interactions involving sex were observed. Sex had no effect on sleeping (ANOVA, F1,19=1.5, P=0.23), pecking (ANOVA, F1,19=0.05, P=0.81), walking (ANOVA, F1,19=0.02, P=0.88), preening (ANOVA, F1,19=0.08, P=0.83), drinking (ANOVA, F1,19=0.2, P=0.63) or any other behavior (ANOVA, F1,19=1.7, P=0.20).

Pebble floor and overhead image of a hawk

*Pecking behavior*

In the first five pecks on day 8 (i.e. without the overhead model predator) corticosterone-treated chicks pecked 3.2±0.3 times at pebbles and control chicks pecked 2.7±0.6 times at pebbles (ANOVA, F1,18=0.5, P=0.47; sex effect, ANOVA, F1,18=0.5, P=0.47; treatment/sex interaction, ANOVA, F1,18=0.7, P=0.42). Likewise, there was no significant effect on the time taken to perform the first five pecks (treatment effect, ANOVA, F1,18=1.1, P=0.32; sex effect, ANOVA, F1,18=0.22, P=0.65; treatment/sex interaction, ANOVA, F1,18=1.6, P=0.22).

As expected, chicks were quicker in performing each of the blocks of 20 pecks as the task progressed (ANOVA, F2,28=4.4, P=0.02; with no significant interactions) and pecked the pebbles less as the task progressed (Fig 4; ANOVA, F2,28=4.8, P=0.02; with no significant interactions). There were no significant effects of treatment or sex on the number of pecks at pebbles (Fig 4; treatment effect, ANOVA, F1,14=1.3, P=0.27; sex effect, ANOVA, F1,14=0.1, P=0.72; treatment/sex interaction, ANOVA, F1,14=0.9, P=0.36) or on the time taken to perform the pecks
As expected, on day 9 chicks pecked at the pebbles less often (ANOVA, F_{1,14}=12.9, P=0.003; with no significant interactions) and pecked 25 times in a shorter period of time (ANOVA, F_{1,14}=22.3, P<0.001) than they had done on day 8. Corticosterone-treated chicks were faster than control chicks at performing the first 25 pecks on day 9 (68.0±31.0s and 189.2±46.2s respectively, ANOVA, F_{1,14}=6.9, P=0.02), though there was no difference between the two treatments in the number of pecks at pebbles on day 9 (ANOVA, F_{1,14}=0, P=0.93). The sex of the chicks had no effect on the time taken to perform 25 pecks (ANOVA, F_{1,14}=1.7, P=0.22; treatment/sex interaction, ANOVA, F_{1,14}=0.6, P=0.44) or on the number of pecks at pebbles on day 9 (ANOVA, F_{1,14}=0.2, P=0.66; treatment/sex interaction, ANOVA, F_{1,14}=0.2, P=0.66).

**Response to the image of a hawk**

Corticosterone-treated chicks were less responsive to the overhead predator than were controls: their first response to the image of a hawk occurred in a later presentation than it did for control chicks (Fig 6; Mann-Whitney, U=40.0, N_1=10, N_2=12, P=0.04, one-tailed; there was no sex effect, Mann-Whitney, U=57.5, N_1=N_2=11, P=0.31). Once they had responded to the image of a hawk, however, their subsequent responses were the same as those of control chicks (51.0±4.6% and 49.3±4.5% of the presentations respectively, Mann-Whitney, U=59.0, N_1=10, N_2=12, P=0.95; there was no sex effect, Mann-Whitney, U=45.0, N_1=N_2=11, P=0.72). No evidence was found to indicate that corticosterone-treated chicks reacted more or less strongly than controls to the overhead predator in either the first response (mean response rank of 2.04±0.2 and 2.25±0.2 respectively, Mann-Whitney, U=49.5, N_1=10, N_2=12, P=0.49; there was
no sex effect, Mann-Whitney, U=55.0, N₁=N₂=11, P=0.72) or in the mean of all responses (mean response rank of 2.3±0.3 and 2.7±0.4 respectively, Mann-Whitney, U=55.0, N₁=10, N₂=12, P=0.72; there was no sex effect, Mann-Whitney, U=57.0, N₁=N₂=11, P=0.80).

**EXPERIMENT 2: Injection after hatching**

**Materials and methods**

The procedure was identical to that used in Experiment 1 except that the 20 subjects (again Black Australorp/White leghorn cross chickens) were obtained at one day of age. These chicks had been exposed to light during incubation at the hatchery and so were visually laterialized. On arrival, ten chicks were injected subcutaneously into the leg with 60μg of corticosterone in 0.5 ml of the sterile saline and ethanol vehicle and the remaining 10 chicks were injected with 0.5ml of sterile saline and ethanol vehicle (control). The chicks were housed and behavior recorded in an open field test, the home cages and in the dual task as in Experiment 1.

**Results**

**Open field test**

We found no evidence that corticosterone treatment on day 1 influenced the number of peeps emitted in the open field test (Fig. 2; treatment effect, ANOVA, F₁,₁₆=0.4, P=0.51; sex effect, ANOVA, F₁,₁₆=0.2, P=0.63; treatment/sex interaction, ANOVA, F₁,₁₆=0.5, P=0.47). No difference was found between corticosterone treated chicks and controls in the latency to begin
vocalization (treatment effect, ANOVA, F_{1,16}=1.3, P=0.27; sex effect, ANOVA, F_{1,16}=2.8, P=0.11; treatment/sex interaction, ANOVA, F_{1,16}=0.2, P=0.69).

Additionally, no evidence was found of a treatment or sex effect on the time spent walking (treatment effect, ANOVA, F_{1,16}=0.2, P=0.69; sex effect, ANOVA, F_{1,16}=0.5, P=0.50; treatment/sex interaction, ANOVA, F_{1,16}=0.2, P=0.67) or on the number of squares entered (treatment effect, ANOVA, F_{1,16}=0.17, P=0.68; sex effect, ANOVA, F_{1,16}=0.7, P=0.42; treatment/sex interaction, ANOVA, F_{1,16}=0.4, P=0.54). Corticosterone-treated chicks, however, spent more time in the centre of the open field than control chicks (163.9±21.3s and 80.3±8.3s respectively, ANOVA, F_{1,16}=13.6, P=0.002; sex effect, ANOVA, F_{1,16}=0.5, P=0.49; treatment/sex interaction, ANOVA, F_{1,16}=0.8, P=0.39).

Lastly, there were no significant differences between corticosterone-treated chicks and controls in the number of pecks (treatment effect, ANOVA, F_{1,16}=3.6, P=0.08; sex effect, ANOVA, F_{1,16}=2.2, P=0.16; treatment/sex interaction, ANOVA, F_{1,16}=1.5, P=0.24) or in the number of defaecations (treatment effect, ANOVA, F_{1,16}=0.1, P=0.73; sex effect, ANOVA, F_{1,16}=0.1, P=0.73; treatment/sex interaction, ANOVA, F_{1,16}=0.1, P=0.73).

**Behavior in the home cage**

Corticosterone treatment had no significant effect on the duration of standing (ANOVA, F_{1,16}=3.6, P=0.08), sitting (ANOVA, F_{1,16}=1.4, P=0.25), sleeping (ANOVA, F_{1,16}=0.3, P=0.61), walking (ANOVA, F_{1,16}=0.02, P=0.89) or preening (ANOVA, F_{1,16}=1.1, P=0.30) and it had no effect on the number of pecks (ANOVA, F_{1,16}=1.9, P=0.19). There were no significant interactions involving treatment, and drinking and “other” behavior were so rare that they were not analyzed statistically.
As in Experiment 1, chicks slept more in the afternoon than earlier in the day (Fig. 3; 26.7±4.3% at 0900hrs, 29.9±5.1% at 1200hrs and 45.6±6.1% at 1500hrs; ANOVA, F2,32=4.0, P=0.03 with no significant interactions involving the time of day). However, time of day had no significant effect on other activities (standing, ANOVA, F2,32=0.3, P=0.71; sitting, ANOVA, F2,32=3.0, P=0.06; walking, ANOVA, F2,32=0.7, P=0.50; preening, ANOVA, F2,32=0.8, P=0.45; pecking, ANOVA, F2,32=1.9, P=0.17; with no significant interactions involving the time of day).

The sex of the chicks had no significant effect on the duration of standing (ANOVA, F1,16=1.7, P=0.21), sitting (ANOVA, F1,16=0.7, P=0.42), sleeping (ANOVA, F1,16=0.3, P=0.56), walking (ANOVA, F1,16=1.9, P=0.18), preening (ANOVA, F1,16=1.2, P=0.28) or on the number of pecks (ANOVA, F1,16=0.6, P=0.47). There were no significant interactions involving sex.

**Pebble floor and overhead image of a hawk**

**Pecking behavior**

Corticosterone-treated chicks performed the first five pecks over a shorter interval than control chicks (84.7±25.4s and 190.1±36.0s respectively, ANOVA, F1,15=8.7, P=0.01; treatment/sex interaction, ANOVA, F1,15=3.9, P=0.07) and the same was true for the next 20 pecks (corticosterone 165.2±55.6s and control 496.0±105.0s, ANOVA, F1,14=11.1, P=0.005; treatment/sex interaction ANOVA, F1,14=2.0, P=0.18). However, no evidence was found that corticosterone treatment influenced the number of pecks at pebbles either in the first five pecks (ANOVA, F1,15=0.001, P=0.93; treatment/sex interaction, ANOVA, F1,15=0.4, P=0.52) or in the next 20 pecks (Fig 4; ANOVA, F1,14=0.08, P=0.78; treatment/sex interaction, ANOVA, F1,14=1.4, P=0.26). No further analysis was performed on pecking since the sample size was considerably
reduced due to five chicks (4 males and 1 female, all corticosterone-treated) not delivering more than 40 pecks.

Male chicks pecked at the pebbles more than female chicks in the first five pecks (ANOVA, $F_{1,15}=6.6$, $P=0.02$) though the sex of the chicks had no influence on the number of pecks at pebbles in the next 20 pecks (ANOVA, $F_{1,14}=0.3$, $P=0.62$). The sex of the chicks did not influence the time taken to deliver either the first five pecks (ANOVA, $F_{1,15}=0.8$, $P=0.37$) or the next 20 pecks (ANOVA, $F_{1,14}=0.3$, $P=0.62$).

As in Experiment 1, on day 9 chicks pecked at the pebbles less often (4.8±0.9 and 10.4±1.0 respectively, ANOVA, $F_{1,12}=21.2$, $P=0.001$) and delivered 25 pecks in a shorter interval of time (126.7±36.6s and 347.9±63.0s respectively, ANOVA, $F_{1,12}=10.4$, $P=0.009$) than they had done on day 8. No evidence was found to indicate that corticosterone treatment influenced the number of pecks at pebbles (ANOVA, $F_{1,12}=0.06$, $P=0.82$) or the duration of 25 pecks (ANOVA, $F_{1,12}=0.22$, $P=0.64$) on day 9. Lastly, the sex of the chicks had no effect on pecking behavior (pecks; ANOVA, $F_{1,12}=0.05$, $P=0.83$; duration; ANOVA, $F_{1,12}=1.2$, $P=0.30$) or the treatment/sex interaction on day 9 (pecks; ANOVA, $F_{1,12}=0.2$, $P=0.64$; duration; ANOVA, $F_{1,12}=1.3$, $P=0.29$).

*Response to the image of a hawk*

All except one chick responded to an image of the hawk on the first presentation (Fig 6; Mann-Whitney, $U=40.0$, $N_1=10$, $N_2=9$, $P=0.29$; sex effect, Mann-Whitney, $U=40.5$, $N_1=10$, $N_2=9$, $P=0.34$). There was also no difference between treated and control chicks in their ability to detect the image of the predator on subsequent presentations (Mann-Whitney, $U=25.0$, $N_1=10$, $N_2=9$, $P=0.10$; sex effect, Mann-Whitney, $U=38.5$, $N_1=10$, $N_2=9$, $P=0.60$). No evidence was found of a difference between treated and control chicks in the level of response to an image of a
hawk in the first presentation (Mann-Whitney, U=37.5, N₁=10, N₂=9, P=0.83; sex effect, Mann-Whitney, U=40.5, N₁=10, N₂=9, P=0.31). Females responded more strongly to the image of a hawk than males (mean scores of 2.8±0.2 and 1.8±0.2 respectively (Mann-Whitney, U=9.0, N₁=10, N₂=9, P=0.006) but there was no treatment effect on the mean response score (Mann-Whitney, U=37.5, N₁=10, N₂=9, P=0.82).

DISCUSSION

Chicks injected with corticosterone on day 18 of incubation peeped more in an open field test than control chicks, indicating that they were more distressed in this test than the controls. Interestingly, injection of corticosterone post-hatching had no effect on peeping, or any other activity in the open field test, suggesting that the embryo is more susceptible to elevation of corticosterone levels than the young chick. Somewhat similarly to our findings, Hayward and Wingfield (2004) found that Japanese quail chicks hatched from eggs with high corticosterone levels showed a stronger physiological stress response to capture and restraint than chicks from eggs with lower levels of corticosterone. However, we did not find a difference between corticosterone treated and control chicks in response to the hawk. Lay and Wilson (2002) also found that the application of 60ng of corticosterone onto the egg of domestic chicks at 16 days of incubation had no effect on vocalizations during isolation in a novel arena similar to the one we used, though the dose used in their study was considerably smaller than that used in the present study. One possibility for this lack of consistency in stress reactivity in corticosterone treated chicks may be that the stress response is context specific, i.e. it is observed in some situation but not others. In view of the known effects of maternal care on stress reactivity in the rat (Francis et
al, 1999; Francis and Meaney, 1999), and the effect of the mother hen’s stress level on the levels of corticosterone in the egg outlined in the introduction (Hayward and Wingfield, 2004), it would be useful to determine whether in birds the mother’s state of stress around the time of ovulation serves as a form of nongenomic transmission of individual differences in stress reactivity.

In Experiment 1, male chicks walked more than female chicks in the open field test. This, together with the lower amount of time that males spent sitting when they were in the home cage, suggests that males were generally more active. These results were, however, not replicated in Experiment 2. In Experiment 2, females responded more strongly than males to the overhead image of a hawk, though again this was not found in Experiment 1. Although we have shown that corticosterone affects the behavior of male and female chicks differently, the absence of similar sex differences in Experiment 1 and 2 suggest that there may be sensitive periods at which these corticosterone-induced sex-dependent alterations of behavior occur.

As has been reported previously, chicks learned to avoid pecking at pebbles and pecked at food crumbs with increasing experience of the pebble-floor test, and this learning was preserved until the following day. Although corticosterone treatment had no significant effect on the number of pecks at pebbles, chicks injected with corticosterone on day 18 of incubation pecked more rapidly than controls on day 9, and chicks injected with corticosterone at 1 day of age post-hatch pecked more rapidly than control chicks on day 8. These findings raise the possibility that corticosterone treatment increased motivation to peck, though this is not supported by the lack of a significant difference between the treatments in pecking in the home cages. Additionally, Sui et al (1997) found that the injection of 60ng of corticosterone into the egg at 19, 20 and 21 days of embryonic age, but not on the days before or after this period, had no effect on the motivation to peck at differently colored beads. Again, the precise method and timing of
corticosterone administration may be important in modulating the effects of corticosterone treatment on the rate of pecking. Alternatively, a period of food deprivation is required before the effects of corticosterone on motivation to peck are manifested.

As predicted, chicks injected with corticosterone on day 18 of incubation initially failed to respond to the image of a hawk while they were engaged in discriminating food crumbs from pebbles, whereas control chicks both pecked and detected the overhead predator. Corticosterone prevents the development of asymmetry in the thalamofugal visual projections normally caused by light experience during the pre-hatching period (see Rogers 1990 for the role of light on development of the visual projections, and Rogers and Deng, 2005), but cannot have this effect when it is administered post-hatching since the asymmetry has already developed by this age (Deng and Rogers, 2002). In this respect, the post-hatching injection of corticosterone provides a useful control for the effects of corticosterone on behavior other than that stemming from lateralization of the thalamofugal visual projections. However, comparison between Experiments 1 and 2 is limited since there were differences in the incubation procedure, such as the amount of light received by the embryo, which may have lead to behavioral differences beyond those caused by the timing of the corticosterone injection. It could also be argued that the doses of corticosterone received by the embryo and the post-hatched chick were somewhat different and that this caused the different effects. However, it is likely that the dose post-hatching was effectively higher than that received by the embryo since it was given subcutaneously compared to indirectly as in the case of the embryo. The lesser effect post-hatching therefore emphasizes the evidence for a sensitive period pre-hatching during which corticosterone modifies brain development. These findings, therefore, lend support to the hypothesis that lateralization improves the animal’s ability to perform more than one task simultaneously. The ability to
perform more than one task simultaneously may be an important advantage of being lateralized (Vallortigara and Rogers, 2005).

In conclusion, chicks injected with corticosterone on day 18 of embryonic development, but not chicks injected with corticosterone at 1 day of age, appeared to be more distressed following release into a novel arena than controls suggesting that there may be a sensitive period during which corticosterone alters stress reactivity. No difference was found between corticosterone-treated and control chicks in learning to discriminate food from non-food, or remembering this task. Corticosterone treated chicks, however, were faster at pecking than controls chicks suggesting that the former may have a higher pecking motivation than the latter. Lastly, we found that chicks injected with corticosterone at 18 days of incubation, a procedure that prevents development of lateralization of the visual pathways, were less likely than controls to detect an overhead image of a predator, supporting the hypothesis that an advantage of lateralization may be to allow the animal to perform more than one task simultaneously.

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Figure 1: Image of the overhead hawk used in the dual task.

Figure 2: The number of peeps emitted in 5 minutes in the open field test for pre- and post-hatching injected chicks. Means (±SE) are presented. The black bars are the scores for corticosterone-treated chicks and the white bars for vehicle-treated chicks. *** P<0.001.

Figure 3: The percentage of time spent sleeping in the home cages for pre- and post-hatching injected chicks. The black bars are the scores for corticosterone-treated chicks and the white bars for vehicle-treated chicks. Corticosterone-treated chicks slept less than control chicks (P<0.05), though no such effect was seen in post-hatching injected chicks. Generally, chicks slept more in the afternoon than earlier in the day (P<0.01 and P<0.05 for pre- and post-hatching injected chicks respectively).

Figure 4: The number of pecks at pebbles in the pebble-floor test for pre- and post-hatching injected chicks. The black squares are the scores for corticosterone-treated chicks and the white squares for vehicle-treated chicks. Chicks pecked at the pebbles fewer times with increasing time on the pebble floor (both P<0.05).
Figure 5: The time taken to perform 20 pecks on day 9 for pre- and post-hatching injected chicks. The black bars are the scores for corticosterone-treated chicks and the white bars for vehicle-treated chicks. * P<0.05.

Figure 6: The average number of presentations required for the chicks to notice the overhead image of the predator. The black bars are the scores for corticosterone-treated chicks and the white bars for vehicle-treated chicks. * P<0.05, one-tailed. Note that the chicks injected with corticosterone (black bars) on day 18 of incubation had higher scores, meaning that they were less likely to notice the overhead predator.