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Exposing avian embryos to light affects post-hatch anti-predator fear responses

Gregory S. Archer*, Joy A. Mench

Department of Animal Science, University of California, Davis, United States

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ABSTRACT

Environmental stimuli present during incubation can impact the behavior of birds post-hatch. To determine the effect of exposing broiler chicken embryos to light on fear-related behavior post-hatch, we conducted two experiments in which we incubated eggs under various light schedules, and then measured fear responses when the chickens (N = 720) were 3–6 wk of age. In Expt. 1, the incubation photoperiods were 0L:24D, 12L:12D, and 24L:0D, and tonic immobility (TI) and inversion (INV) tests were administered. In Expt. 2, the incubation photoperiods were 0L:24D, 1L:23D, 6L:18D, and 12L:12D; and an approach test (APPR) and an emergence (EMRG) test were administered in addition to TI and INV. In Expt. 1, both 12L:12D and 24L:0D had shorter latencies to right during TI (213.5 ± 23.7 and 231.8 ± 24.2 s, respectively) than 0L:24D (305.5 ± 26.1 s) and also wing flapped less intensely during INV (12L:12D 5.0 ± 0.1 wing flaps; 24L:0D 5.4 ± 0.2) than 0L:24D (5.7 ± 0.1). In Expt. 2, the 12L:12D birds once again had shorter latencies to right during TI (120.0 ± 16.5 s) and wing flapped less intensely during INV (4.7 ± 0.1 wing flaps) than 0L:24D (201.4 ± 24.9 and 5.5 ± 0.1, respectively). They also had shorter latencies to exit the dark box in EMRG (28.9 ± 3.3 s), and were less active (28 ± 2%), vocalized less (178.8 ± 9.3 times/3 min) and spent more time closer to the observer during APPR (63 ± 3%) than 0L:12D (42.9 ± 5.0 s, 35 ± 3%, 211.2 ± 10.4 times/3 min, 51 ± 3). The 1L:23D and 6L:18D showed some reductions in fearfulness compared to 0L:24D, but these were not consistent across tests. The 6L:18D and 12L:12D birds demonstrated lateralization in the direction to leave the box in EMRG, whereas 1L:23D and 0L:12D exited left or right at chance levels. The results of these experiments indicate that providing at 12 h of light stimulation daily during embryogenesis results in long-term reductions in fearfulness as measured by multiple tests, and that this may be related to cerebral lateralization. In conjunction with other research, these findings show that light exposure during embryogenesis has important implications for behavioral phenotypes and welfare in chickens.

1. Introduction

Avian behavioral phenotypes are influenced by both the internal and external environment during embryogenesis. Maternal deposition of androgens into the eggs can alter offspring phenotypes (*e.g.* related to boldness or alertness) such that the offspring are better suited for current environmental conditions (Groothuis et al., 2005). Avian embryos also respond to external stimuli such as olfactory, auditory and photoperiodic cues (Reed and Clark, 2011). For example, exposure to species specific call during embryonic development is important for post-hatch species recognition in some avian species (Gottlieb, 1976; Gottlieb, 1985). Olfactory stimulation can also shape later behavior. Bertin et al. (2010) observed that chickens exposed to odors during incubation preferred food bearing those odors post-hatch.

Photoperiodic cues are particularly important factors influencing post-hatch phenotypes. Embryos receive regular brief light exposures when their parent(s) leave the nest to feed (Buschmann et al., 2006; Mrosovsky and Sherry, 1980) or respond to distress calls from the embryos by rising to turn the eggs (Rogers, 1996). This light can penetrate the eggshell and reach the embryo where it is sensed either by developing retinal cells or the pineal gland (Cooper et al., 2011), resulting in melatonin synthesis. Melatonin affects embryonic growth rates as well as the development of the visual, skeletal and immune systems (see Reed and Clark, 2011).

Prenatal light exposure has also been shown to affect post-hatch behavior. Domestic fowl chicks exposed to light during incubation have a different diurnal rhythm of feeding activity than dark-incubated chicks (Archer et al., 2009), and also differ from dark-incubated chicks in terms of discrimination learning ability (Rogers, 1990), memory retention for a passive avoidance task (Sui

* Corresponding author at: Poultry Science Department, 101 Kleberg Building, MS 2472 TAMU, College Station, TX 77845, United States.
E-mail address: garcher@poultry.tamu.edu (G.S. Archer).

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and Rose, 1997), and aspects of social behavior including social exploration, social recognition, and competitive success (Riedstra and Groothuis, 2004; Rogers and Workman, 1989). Chicks exposed to light during incubation also show more avoidance of an unfamiliar imprinting object than dark-incubated chicks during the first 12 h post-hatch (Dimond, 1968).

The mechanisms underlying the behavioral effects of light stimulation during incubation in birds probably involve both melatonin effects on behavioral rhythms and the direct effect of embryonic light stimulation on brain development via lateralization of the thalamofugual and tectofugal visual pathways (Rogers, 1982). The latter results in hemispheric specialization, with the left hemisphere specialized for visual discrimination tasks, food-searching and vocal production and recognition, while the right is specialized for situations that involve a strong negative affective component, such as fear or aggression (Phillips and Youngren, 1986; Rogers, 2008). It has been hypothesized that strong lateralization is adaptive in that it facilitates dual processing of sensory information, allowing individuals to efficiently perform two tasks simultaneously. This hypothesis has been tested in domestic fowl (Dharmaretnam and Rogers, 2005) using simultaneous presentations of food acquisition tasks and predator stimuli. These studies showed that lateralized light-incubated chicks were able to more efficiently obtain food while continuing to maintain vigilance than less lateralized individuals.

Despite the presumed adaptive value of the patterns of lateralization found in light-incubated chicks, there is actually limited evidence that the behaviors observed at young ages persist throughout development. Shifts in lateralization for fear behavior in domestic chicks have been reported to occur between 5 and 15 days of age post-hatch (Andrew and Brennan, 1983), but the effects of providing light during incubation on lateralization and fear responses in older birds have not been evaluated.

Our previous studies suggest that providing light during incubation can have beneficial effects on the welfare of broiler chickens 3–6 weeks post-hatch (Archer et al., 2009; Archer and Mench, 2009), decreasing composite asymmetry, reducing the corticosterone response to crating stress, and enhancing antibody response to challenge. We conducted two experiments to evaluate whether providing light during incubation also had developmentally persistent effects on fearfulness. Since anti-predator responses have been shown to be the most reliable measures for assessing fear (Miller et al., 2005, 2006), we utilized four different fear tests involving anti-predator responses (inversion, tonic immobility, emergence, and approach). We also assessed the degree of laterality in light and dark-incubated chicks by determining the direction of movement in the emergence test.

2. Materials and methods

2.1. Animals and husbandry

We obtained fertilized chicken eggs (Cobb 500 strain) from a commercial hatchery, and randomly assigned them to be incubated under different lighting schedules. In Experiment 1 (N = 664 eggs) we used three lighting schedules: 0 h of light and 24 h of darkness (0L:24D), 24 h of light and 0 h of darkness (24L:0D), or 12 h of light and 12 h of darkness (12L:12D). In Experiment 2 (N = 1512 eggs) we used four lighting schedules: 0 h of light and 24 h of darkness (0L:24D), 1 h of light and 23 h of darkness (1L:23D), 6 h of light and 18 h of darkness (6L:18D), or 12 h of light and 12 h of darkness (12L:12D). The lighting durations in Experiment 1 were used to determine if there was an effect of light at the extremes (constant or no light) and/or the mid-point. Experiment 2 used shorter lighting durations to determine if there was a minimal amount of light needed during incubation to obtain the results observed in Experiment 1. The intensity of light at the egg level within the incubators was 550 lx as measured with a photoreceptor sensor of a light meter (LT Lutron, model LX-100™; Das Distribution Inc., East Granby, CT). In Experiment 1 lighting was controlled manually by covering or uncovering the clear incubator top with cardboard to block any light entering, while in Experiment 2 each treatment was contained within a ventilated environmentally controlled light-tight box that allowed the photoperiod to be automatically controlled by timers. Temperature and humidity inside the incubators (Hovabator; G.Q.F. Manufacturing Co., Savannah, GA) were monitored to insure that the conditions in the different incubators were similar. We candled eggs (Cool-Lite tester, GQF, Savannah, GA) once a week and removed non-viable eggs. Each experiment was conducted in three trials, with three incubators per treatment per trial.

Following completion of hatch all chicks were moved to a housing room at the Hopkins Avian Research Facility at the University of California, Davis. They were managed according to the guidelines set forth in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

In the housing room we placed chicks (N = 506 in Experiment 1, N = 1006 in Experiment 2) from each trial in one of nine pens (Experiment 1) or one of twelve pens (Experiment 2) by incubator, with treatments randomized by block (incubator) throughout the room. Each pen was 6.0 m^2 and was bedded with wood shavings (approximately 10 cm deep). Chicks were given access to feed and water *ad libitum* throughout the study. They were fed a pre-starter mash (23.21% crude protein, 3.10 kcal/g metabolizable energy) for the first 3 weeks, and then Purina Mills Flock Raiser Sunfresh Crumble™ (St. Louis, MI; 20% protein, 3.00 kcal/g metabolizable energy) for the remainder of the 6 week experiment.

The birds were raised under a 12L:12D photoperiod with a photophase light intensity of 250 lx. Light intensity was measured along a horizontal plane at 25 cm above the floor with the photoreceptor sensor of the light pointed toward the light sources. There were no dawn-dusk transitions between the light and the dark phase of the photoperiod. Light intensity during the dark phase was 0 lx (complete darkness).

2.2. Fear measurements

We used tonic immobility (TI) and inversion (INV) tests to measure anti-predator responses in both experiments. Tonic immobility testing was carried out as described by Archer and Mench (2014) on 10 randomly selected birds (5 males and 5 females; determined by secondary sex characteristics) per pen when the birds were five weeks old. In brief, we placed birds on their backs in a wooden cradle and held them there for 15 s. If the bird righted before 10 s TI was re-induced up to three times. If the bird could not be induced in three attempts it was scored as 0. We recorded latency to first head movement, latency to right, and number of induction attempts. The test was terminated in 600 s if a bird failed to right, and that bird was scored as 600.

When the birds were 42 days old we subjected the same ten birds that had been used for TI testing to an INV test, as described by Newberry and Blair (1993) and Archer and Mench (2014). We caught each bird and then inverted it by holding it by its legs with one hand until the bird ceased to wing flap, or for 30 s. We determined the duration of and number of wing flaps, the number of vocalizations, and the number of body curls from video recordings (Cannon, ZR900, Melville, NY, USA; 24 frames per second).

In Experiment 2 we administered two additional fear tests, the approach test (APPR) and the emergence test (EMRG), when the birds were three weeks of age. Ten randomly selected birds (5 males

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