Published in final edited form as:

Dev Psychobiol. 2015 May ; 57(4): 459–469. doi:10.1002/dev.21306.

Drift during overground locomotion in newly hatched chicks varies with light exposure during embryogenesis

Jay H. Porterfield¹, Anil Sindhurakar², James M. Finley³, and Nina S. Bradley³

¹Department of Biomedical Engineering, Viterbi School of Engineering of University of Southern California, Los Angeles, CA

²Motor Recovery Laboratory, Burke-Cornell Medical Research Institute, White Plains, NY

³Division of Biokinesiology and Physical Therapy, Herman Ostrow School of Dentistry of University of Southern California, Los Angeles, CA

Abstract

In an earlier study of newly hatched chicks we reported that continuous bright light exposure throughout incubation accelerated locomotor development and continuous dark exposure delayed it, compared to less intense, intermittent light exposure. Commonly studied gait parameters indicated locomotor skill was similar across groups. However, dark incubated chicks walked with a greater step width, raising the possibility of differences in dynamic balance and control of forward progression. In this study, we established methods to retrospectively examine the previously published locomotor data for differences in lateral drift. We hypothesized that chicks incubated in darkness would exhibit more drift than chicks incubated in light. Analyses identified differences in forward progression between chicks incubated in the 2 extreme light conditions, supporting the study's hypothesis. We discuss the significance of our findings and potential design considerations for future studies of light-accelerated motor development in precocial and non-precocial animals.

Introduction

It is well established in birds that light exposure during embryogenesis can accelerate morphogenesis (Coleman and McDaniel, 1976; Ghatpande et al., 1995), promote greater motor activity (Wu et al., 2001) and the onset of hatching (Bohren and Siegel, 1975; Fairchild and Christensen, 2000). However, investigation into the impact of extended prenatal exposure on postnatal motor development has been limited (Sindhurakar & Bradley, 2010). Studies have examined the effects of light exposure in the final days before hatching, providing evidence that light is a salient stimulus capable of influencing vision-mediated behavior (Casey & Lickliter, 1998; Rogers, 1982; Rogers & Bolden, 1991), hemispheric specialization (Casey & Martino, 2000; Casey, 2005; Rogers, 1982), and social behavior (Rogers, 1982). A few studies in domestic chicks have also shown that extended

Correspondence concerning this article should be addressed to Nina S. Bradley, Division of Biokinesiology and Physical Therapy, Herman Ostrow School of Dentistry of University of Southern California, 1540 E. Alcazar St., CHP 155, Los Angeles, CA 90033 - 9006. nbradley@usc.edu.

Anil Sindhurakar, Department of Systems Biology and Disease, Keck School of Medicine of University of Southern California

light exposure during embryogenesis can accelerate development of respiratory control (Bradley and Jahng, 2003), interlimb stepping prior to hatching (Ryu & Bradley, 2009; Sindhurakar & Bradley, 2012), and overground locomotion at hatching (Sindhurakar & Bradley, 2010). However, it has yet to be determined if this acceleration in motor development is associated with any immediate or longer term negative behavioral or other biological consequences.

In a study examining the impact of light on development of overground locomotion, chicks incubated in continuous bright light hatched and began walking 1-2 days earlier than chicks incubated in less or no light (Sindhurakar & Bradley, 2010). Despite early hatching, these chicks appeared to walk with an equivalent level of skill compared to those incubated in 12L and 24D conditions. Skill was assessed using measures derived from consecutive foot placements. Curiously, chicks incubated in continuous darkness walked with a greater step width than chicks incubated in continuous bright light. The greater step width raised the possibility that dark-incubated hatchlings had less mature dynamic balance control (Sindhurakar & Bradley, 2010). During walking, dynamic balance control is required for normal forward progression. If vision, vestibular, or somatosensory inputs for balance control are manipulated, human subjects show deficits in navigation ability and begin to drift, e.g., lateral veering from a predicted vector of forward progression (Bestaven, Guillaud, & Cazalets, 2012; Boyadjian, Marin, & Danion, 1999; Earhart et al., 2001; Fitzpatrick et al., 1999). In our first study we did not examine path trajectory. Thus it remained to be determined if navigation differed between hatchlings incubated in less or more light and if drift might be greater in chicks incubated in continuous darkness.

In this study, we examined navigation performance as an indicator of dynamic balance and specifically sought to determine if measures of drift during forward progression varied with light exposure during incubation. We hypothesized that if chicks incubated in continuous darkness had less mature dynamic balance, they would exhibit more drift compared to chicks incubated in light 12 or 24 hr daily. To this end, methods were established to quantify forward progression, e.g., path length, and the step-by-step lateral deviations in forward progression, e.g., foot placement angles. Measures of path length were examined to determine the overall efficiency of forward progression and foot placement angles to determine the magnitude, variability and potential bias of lateral drift during forward progression. We applied these methods of analysis to previously published locomotor data (Sindhurakar & Bradley, 2010) and report the findings for a novel retrospective analysis. Evidence is provided indicating that drift during overground locomotion varied with light exposure during embryogenesis and that chicks incubated in darkness navigated with the most drift, consistent with our hypothesis. The significance of our key findings and considerations for future studies are discussed.

Methods

Subjects and Incubation Conditions

Fertile Leghorn chicken (*Gallus gallus*) eggs from a local hatchery were incubated in standard poultry incubators modified to house fluorescent lighting. Incubation conditions for this study were previously described (Sindhurakar & Bradley, 2010, 2012). In brief,

incubators were configured for 1 of 3 light exposures that varied light intensity and exposure duration: 650-3000 lux, 12 hr daily (12L); 4000-7000 lux, 24 hr daily (24L); or 1 lux, 24 hr daily (24D). A total of 30 hatchlings were incubated in 1 of the 3 light exposure conditions, 10 hatchlings per condition. Hatchlings were maintained in a brooder before training and between data collection sessions. Hatchlings were euthanized by lethal injection at the end of data collection. All procedures were approved by the University Institutional Animal Care and Use Committee.

Testing Procedures

Testing procedures were previously reported (Sindhurakar & Bradley, 2010) and only key methods for analyses are reported here. Within 2-3 hr after hatching, chicks were first trained to attend to and walk toward finger taps accompanied by the tester's vocal encouragement. They were then trained to walk from end to end along a tunnel (90 cm × 9 cm × 12 cm) fabricated from black poster board and having a Plexiglas floor. The chicks were placed in a darkened chamber at the starting end of the walkway and then cued by tapping and voice to walk to the other (lightened) end when a trap door was lifted. Chicks were included in the study if they walked the full length of the walkway within 3 practice trials. The metatarsal pads of both feet were marked and foot placements were video recorded from beneath the tunnel. A total of 4 walk trials from end to end were recorded during each of 2 sessions spaced 4 hours apart. The first session was conducted immediately after training.

Kinematic Analyses

Foot placements within the central 40 cm of the tunnel were previously digitized (60 samples/s) to obtain 2-dimensional (2D) coordinates (x, y) for calculating basic spatial (e.g., stride length) and corresponding temporal parameters of gait (Sindhurakar & Bradley, 2010). In this study, the 2D coordinates were used to estimate the projected location of the center of mass (COM) when both feet were in contact with the floor and foot placement angle for each step (Fig. 1). The COM positions and foot placement angles were in turn used to quantify lateral deviation in forward progression based on 4 measures: path length, drift magnitude, drift variability and drift bias. Each measure is defined and its calculation described within the following sections.

Stride Count

A stride is typically defined as 2 consecutive placements of the ipsilateral foot and stride count is the sum of all strides taken. For example, in Figure 1A, points 1 and 2 (closed squares), identify 2 left foot placements, and together they define 1 stride for the left foot (Perry, 1992). In this study, **stride count** was operationally defined as the number of consecutive ipsilateral strides taken to complete a walk trial, e.g. 9 strides of the left foot are shown in Figure 1A. Stride count was calculated for the left and right foot separately for the first 5 experiments analyzed in each of the 3 incubation conditions, as the number of strides might differ between feet depending on the foot associated with the initial and terminal foot placements within the portion of the path video recorded. Also, a chick might briefly pause along the walkway, then resume by taking a second step with the same foot. However, 2-

way analyses of variance indicated there was no difference in total number of strides for the left and right foot in either session I ($p>0.3$) or session II ($p>0.6$), so strides of the left foot were selected for stride-related analyses. We chose the left foot because it was also selected as the reference foot for spatial parameters analyzed in the earlier study, after determining there were no significant left-right differences.

Path length

The COM location was estimated for each step to calculate 3 measures of path length: 1) straight path length, the shortest distance between the first and final step, 2) total path length, the total distance traveled between the first and final step, and 3) normalized path length, the ratio of the total path length to the straight path length. The distance midway between consecutive left-to-right and right-to-left foot placements was calculated to estimate the COM location for each step. For example, in Figure 1B, an 'X' identifies the estimated COM for 4 left-to-right foot placements. COM locations were then used to calculate the **straight path length**, defined as the longitudinal distance (x axis of walkway) between the estimated COM for the first and last steps, i.e., the horizontal distance between the 1st and 4th 'X' in Figure 1B. However, forward progression included lateral deviations that increased the total distance COM traveled. To capture all distance traveled, we used COM location estimates to calculate the **total path length**, defined as the sum of the resultant vectors formed by the forward and lateral deviations for consecutive steps. The straight path length was less than or equal to 40 cm, the length of the digitized area, and varied from trial to trial because a portion of the first and/or last step was outside the video region. The variations in first and last step also contributed to total path length variability, thus we also calculated a **normalized path length**, defined as the total path length relative to the straight path length for each trial. A normalized total path length of 1.0 indicated no lateral drift in forward progression and values >1.0 indicated drift to one or both sides during forward progression.

A Matlab® (Mathworks, Natick, MA) function was developed to automate the calculation of total path length, straight path length and normalized path length for each walk trial. Left and right foot placements were first identified by successive stride number. For example, the first left foot placement in a given walk trial was (L_{x1} , L_{y1}), as illustrated in Figure 1C. The function used the 2D coordinates for foot placements to calculate the estimated location of the center of mass (eCOM) between successive placements of the left (L_{x1} , L_{y1}) and right foot (R_{x1} , R_{y1}) (Equations 1 and 2). The function then calculated the distance between successive eCOM coordinates and summed the distances (Equation 3) to generate the total path length (PL).

$$eCOM_{xi} = \left(\frac{L_{xi} + R_{xi}}{2} \right) \quad (\text{Equation 1})$$

$$eCOM_{yi} = \left(\frac{L_{yi} + R_{yi}}{2} \right) \quad (\text{Equation 2})$$

$$PL = \sum_{i=1}^{n-1} \sqrt{(eCOM_{xi+1} - eCOM_{xi})^2 + (eCOM_{yi+1} - eCOM_{yi})^2} \quad (\text{Equation 3})$$

The two final iterations of the function computed the straight path length (SPL) between the initial and final eCOM (Equation 4), and the normalized total path length (PL_{norm}, Equation 5).

$$SPL = \sqrt{(eCOM_{xf} - eCOM_{xi})^2 + (eCOM_{yf} - eCOM_{yi})^2} \quad (\text{Equation 4})$$

$$PL_{norm} = 100 * \left(\frac{PL - SPL}{SPL} \right) \quad (\text{Equation 5})$$

Foot placement angle

Foot placement angle is a 2 dimensional (x, y) measure that can be used to quantify the step by step lateral deviation during forward progression. If each foot placement is directly in front of the previous placement (e.g., x axis of walkway), the foot placement angle is 90° relative to the y axis. In this study, **foot placement angle** was defined as the exterior angle for consecutive strides of the left foot, as represented by α in Figure 1C. The α angle is often used as an indicator of foot orientation relative to the direction of travel (Kernozek & Richard, 1990). We used foot placement angle to calculate 3 measures indicative of lateral deviation in forward progression: drift magnitude, drift variability and drift bias. The angle was calculated by first creating 3 vectors: V_X , V_S , and V_B (Figure 1C).

A forward progression unit vector (V_X), defining forward advancement in a straight path at each step, extended forward from the foot placement and was parallel with the axis (x) of the tunnel. The base vector (V_B) was a unit vector that extended through the foot placement coordinates and ran orthogonal to V_X . The stride vector (V_S) extended from one left foot placement to the next left foot placement and was the resultant of the concurrent stride length and lateral deviation in successive placements of the left foot. The foot placement angle (α) was defined as the angle between V_S and V_B at each foot placement (Zverev, 2006).

A Matlab® function was developed to automate the calculation of foot placement angles. The 2D coordinates for all left or right strides per walk trial were matrix-formatted as an ASCII file for input to the function. The function subtracted the difference in coordinates for successive foot placements (Equation 6) to generate V_S (Figure 1C). V_B was also generated for each foot placement. The function used a 1×1 column vector (i.e. [0 1]) to generate V_B at each foot placement coordinate.

$$V_S = L_{xi+1,yi+1} - L_{xi,yi} \quad (\text{Equation 6})$$

The second iteration calculated the dot product of a unit vector in the direction of V_S and V_B , and took the arccosine of the product. The third iteration subtracted the arccosine of the

product from 180° to generate the exterior angle between the base vector and stride vector (Equation 7).

$$\alpha = \cos^{-1}(\mathbf{V}_S \cdot \mathbf{V}_B) \quad (\text{Equation 7})$$

Performance of the Matlab® function was verified by comparing foot placement angles generated by the function with angles measured by protractor. All foot placement angles for 9 experiments (72 walk trials), from the first 3 experiments analyzed per condition, were manually measured. Matlab-generated calculations were then compared to the measured angles to confirm the accuracy of the algorithms. Linear regression analyses for each of the 9 experiments indicated that there was uniformly strong agreement between the measurement methods. Correlation coefficients ranged from 0.95 to 0.99 and slopes from 1.02 to 1.11 for sample sizes of 45-83 foot placement angles per experiment. Analyses for 120 walk trials (15 hatchlings, 5 per condition) also indicated that placement angles did not differ between left and right feet. Thus, left foot placement angles were used to test for between group differences in 3 measures of lateral drift with respect to the progression vector, because the left foot was used as the reference for all gait parameters in the earlier study.

Any stride creating an angle greater or less than 90° between $\mathbf{V}_S$ and $\mathbf{V}_B$ was considered a deviation from a straight path, e.g., drift. Straight ahead was defined as movement parallel to $\mathbf{V}_X$ and orthogonal to $\mathbf{V}_B$ (Figure 1C). **Drift magnitude** (DM) quantified how much foot placement deviated laterally during a single stride (Equation 8), and **drift variability** was equal to the standard deviation of drift magnitude. **Drift bias** (DB) determined the direction of lateral deviation for each stride (Equation 9). DB was calculated and averaged over all steps per session (4 walk trials) to determine if there was a drift bias within session.

$$DM = |90^\circ - \alpha| \quad (\text{Equation 8})$$

$$DB = 90^\circ - \alpha \quad (\text{Equation 9})$$

Statistical Analyses

We employed a within-subject and between group design. For each subject, trial data were combined and averages were calculated for session 1 (trials 1-4) and session 2 (trials 5-8) to perform statistical comparisons. Subject means were compared using the two-way ANOVA analysis for repeated measures. Averaged standard deviations were similarly calculated and tested as an indicator of differences in mean variability across sessions and between groups. Pearson linear regression analyses were used to determine the fit (R^2) and slope for manually measured and automated calculation of foot placement angle to confirm the performance of our Matlab function. Significance was set at $p < .05$. The Student's t-test was used for post hoc comparisons and the significance level was adjusted for multiple comparisons using a Bonferroni correction.

Results

Our kinematic findings for stride-to-stride drift summarize analyses for 240 walk trials equally representing 30 chicks, 10 chicks per incubation condition, 8 trials per chick. All chicks progressed toward the open end of the walkway, walking the full length each trial. Although chicks occasionally paused in route, no chick backtracked toward the darkened chamber. Analyses were derived from 1626 strides, 36 to 83 strides per chick. We first describe the general forward progression behavior of hatchlings based on path analyses, then the characteristics of lateral drift based on stride-by-stride analyses of foot placement angles.

Stride Count and Path Length

Chicks typically took 6 to 10 strides to complete a walk trial. On average, chicks incubated in 24L conditions took the fewest strides per trial; chicks incubated in 24D took the most (Figure 2A). The two-way ANOVA indicated that the difference across incubation conditions was significant, $F(2,54) = 8.6, p < .0006$. In addition, the main effect for session indicated that chicks took significantly fewer strides during session II than session I, $F(1,54) = 11.8, p < .001$. The ANOVA interaction was not significant, $F(2,54) = 2.7, p < .08$. Post hoc analyses for group differences and a Bonferroni correction for 3 comparisons ($p < .017$) indicated that chicks incubated in 24L took significantly fewer strides to complete a walk trial than both chicks incubated in 12L ($p < .01$) and 24D ($p < .001$).

The straight path length and total path length varied across all trials, potentially masking real differences in behavior (see Methods), whereas normalized path length identified several significant findings. Normalized path length indicated, as summarized in Figure 2B, that chicks incubated in 24L tended to walk the straightest path for any given distance and chicks incubated in 24D tended overall to walk with greater lateral deviation. The two-way ANOVA for normalized path distance revealed a significant main effect of incubation conditions $F(2,54) = 5.7, p < .002$. Also, normalized path decreased from session I to session II, $F(1,54) = 8.1, p < .002$. The interaction was not significant. Post hoc analyses for group differences (Bonferroni correction $p < .017$) indicated that normalized path length for chicks incubated in 24L was significantly less than for chicks incubated in 24D ($p < .0005$). Chicks walked a total path length of 28-42 cm and a straight path length of 28-40 cm. There was no difference in total path distance across incubation conditions, $F(2,54) = 1.8, p < .18$, or between sessions, $F(1,54) = 3.0, p < .09$, and the interaction was not significant, $F(2,54) = .68, p < .51$. Further, there was no difference in straight path distance across conditions, $F(2,54) = 2.3, p < .11$, or session, $F(1,54) = 2.0, p < .17$, and the interaction was not significant, $F(2,54) = .45, p < .64$.

Drift Magnitude and Variability

Individual foot placement angles varied from 0° to 84° and trial averages ranged from 2° to 22°. Two exemplary walk trials from a 12L experiment are shown in Figure 3. The magnitude of foot placements angles is summarized by group and session in Figure 4A. Chicks incubated in 24L conditions exhibited the smallest angles and chicks in 24D exhibited overall the greatest angles. The two-way ANOVA indicated that foot placement angles differed across conditions, $F(2,54) = 8.6, p < .0006$, and decreased from session I to

session II (Figure 4A), $F(1,54) = 11.9, p < .001$. The interaction was not significant, $F(2,54) = 2.8, p < .07$. Post hoc comparisons for group differences (Bonferroni correction $p < .017$) indicated that foot placement angles were smaller for chicks incubated in 24L conditions compared to chicks incubated in 12L ($p < .008$) and 24D ($p < .002$).

Foot placement angle variability for each walk trial ranged from 1° to 31° (Figure 4B). The two-way ANOVA indicated there was a significant difference in drift magnitude variability across conditions, $F(2,54) = 3.8, p < .03$, and that variability decreased from session I to II, $F(1,54) = 9.8, p < .003$. The interaction was also significant $F(2,54) = 5.7, p < .006$. Post hoc comparisons (Bonferroni correction $p < .0083$) indicated that chicks incubated in 24L exhibited less variability than chicks incubated in 12L during session I ($p < .006$), and chicks incubated in 12L achieved a significant reduction in drift variability from session I to session II ($p < .0004$).

Drift Bias

With few exceptions, average foot placement angle for all hatchlings fell within $\pm 3^\circ$ of the progression vector, and group averages for foot placement angle were near 0° for both sessions I and II (Figure 5A). The two-way ANOVA indicated that there were no differences in drift bias across incubation conditions, $F(2,54) = 2.5, p < .09$, or between sessions, $F(1,54) = .04, p < .84$, and the interaction was not significant, $F(2,54) = 1.8, p < .18$.

Drift bias variability, like drift magnitude and variability, generally appeared to be least for chicks incubated in 24L and greatest for chicks incubated in 24D (Figure 5B). Two-way ANOVA analyses indicated that drift bias variability differed across conditions, $F(2,54) = 6.7, p < .003$, and decreased from session I to session II, $F(1,54) = 12.2, p < .001$. The interaction was also significant, $F(2,54) = 4.4, p < .02$. The post hoc comparisons between conditions (Bonferroni correction $p < .017$) indicated that hatchlings incubated in 24L conditions progressed forward with less drift bias variability than hatchlings incubated in 12L ($p < .009$) and 24D ($p < .004$). Post hoc comparisons for the interaction (Bonferroni correction $p < .01$) indicated that hatchlings incubated in 24L conditions exhibited less drift bias variability than hatchlings incubated in 12L during session I ($p < .003$) and that hatchlings incubated in 12L conditions exhibited less variability than in 24D conditions during session II ($p < .009$). Further, hatchlings incubated in 12L significantly reduced drift bias variability from session I to session II ($p < .001$).

Discussion

Incubation in continuous bright light (24L conditions) throughout embryogenesis promotes accelerated development of interlimb stepping (Sindhurakar & Bradley, 2012) and walking with the completion of early hatching (Sindhurakar & Bradley, 2010). Conversely, incubation in the absence of light (24D conditions) throughout embryogenesis delays development of these motor milestones. Although we found no clear negative impact of dark exposure on gait at hatching in our earlier study, we observed that chicks incubated in 24D condition walked with a greater step width than chicks incubated in 24L, and proposed that the wider-based gait might indicate less optimal balance control (Sindhurakar & Bradley, 2010). In this retrospective study, we further examined their locomotor performance to

determine if there was additional evidence of subtle differences in motor skill not detected by our original analyses. We hypothesized that the dark incubated chicks would exhibit more drift, e.g., ataxia, during forward locomotion than chicks incubated in 12L or 24L conditions. We predicted there would be a relationship between step width and forward navigation because increasing step width, and thereby enlarging the base of support, has been shown to be an effective strategy for increasing stability in the medio-lateral direction (McAndrew Young & Dingwell, 2012). This strategy is commonly observed when postural stability is threatened, or when the sensorimotor processes responsible for balance control are compromised. In humans, when balance is threatened by the possibility of a trip, step width is increased in anticipation of a potentially destabilizing perturbation (Pijnappels et al., 2001). Additionally, if the sensory signals used for balance control, such as vision or proprioception, are compromised, individuals will also increase step width to expand their base of support (Bauby & Kuo, 2000; Richardson et al., 2004). Increases in step width are also observed in pathological conditions affecting the cortical or cerebellar circuits involved in balance control (Stolze et al., 2002; Chen et al., 2005).

Given the presumably slower rate of morphologic maturation (Sindhurakar & Bradley 2012) and progression towards hatching (Sindhurakar & Bradley 2010), we considered that chicks incubated in 24D conditions might take wider steps because the neural structures responsible for dynamic balance control also might be less mature. This may include, for example, an inability to use available visual cues for balance control due to delayed visual pathway development in the absence of light exposure during embryogenesis (Rogers & Bolden, 1991). In other words, light during incubation may be a necessary stimulus to enable chicks to optimize the use of visual information post-hatching, particularly during functional activities such as walking. Alternatively, the integration of balance-related sensory information may have been compromised because the vestibular system was less mature than in hatchlings incubated in light. This is consistent with observations from a recent study demonstrating that chicks incubated in 24D had greater sway amplitude and sway velocity than chicks incubated in 12L or 24L (Racz et al., 2011). Another potential source of differences in balance control could be biomechanical. Though there were no differences in egg weight, body weight or toe length, tibia length varied positively with light exposure and was significantly shorter for chicks incubated in 24D compared to 24L. Further, differences in muscle activity and practice of locomotor-related leg movements in ovo (Sindhurakar & Bradley, 2012), might have contributed to differences in balance control at hatching. Both muscle fiber development and muscle activity are enhanced by light exposure and also contribute to bone maturation (Liu, Wang, & Chen, 2010; Hall & Herring, 1990). If the observed differences in dynamic postural stability stemmed from a 1-2 day delay in maturation, one might expect that 24-48 hours after hatching, chicks incubated in 24D would perform comparably to chicks incubated in 24L upon hatching. However, this remains to be explored.

In this retrospective analysis of forward progression we identified 4 key findings relative to our hypothesis. One, chicks incubated in 24L conditions exhibited the least drift during forward navigation, consistently outperforming chicks incubated in 24D. Two, there was less apparent difference in navigation parameters between chicks incubated in 24D and 12L conditions, though small consistent trends were noted suggesting potential benefits of less

intense light relative to dark exposure. Three, all chicks acquired greater navigation skill over a 4 hr period on the day of hatching. Four, under the constraints of our experimental paradigm, chicks did not exhibit a drift bias, e.g., evidence of laterality during forward navigation. We consider these points in the sections that follow.

Chicks incubated in 24L conditions exhibited the most efficient forward navigation

Chicks exposed to continuous bright light throughout embryogenesis navigated with the greatest locomotor skill, significantly outperforming chicks incubated in 24D, thus supporting the study's hypothesis that chicks incubated in 24D would exhibit more drift. Chicks incubated in 24L conditions took fewer strides per trial and walked a shorter normalized path to cover the same total and straight line distance. The fewer strides and shorter normalized path could be partly attributable to slightly longer (approximately 1 mm on average) tibia length compared to chicks incubated in 24D. However, as previously reported, stride length did not differ between 24L and 24D, suggesting the fewer strides and shorter path were due at least in part to lateral deviations in stride. The shorter normalized path and smaller foot placement angles also indicated that chicks incubated in 24L walked with less side to side drift during forward progression than hatchlings incubated in 24D conditions. Excepting normalized path length, chicks incubated in 24L also outperformed chicks incubated in 12L. Chicks incubated in 24L hatched 1 day sooner than chicks incubated in 12L and 2 days sooner than chicks incubated in 24D, and did not appear to differ morphologically (Sindhurakar & Bradley, 2010). Thus, our new results also strengthen the earlier conclusion that 24L conditions accelerated locomotor development without any apparent cost to motor skill.

Chicks incubated in 24D and 12L conditions navigated with seemingly similar efficiency

Potential differences between 12L incubated and 24D incubated chicks were less clear. Stride count, path length parameters and foot placement angles did not differ significantly between the 2 groups, and during session I, all progression parameters appeared to be similar for the 2 groups. However, chicks incubated in 12L exhibited notable improvements between sessions on all parameters, and in 1 measure, drift bias variability, the improvement achieved significance. In contrast, 24D incubated chicks consistently exhibited the least improvement between sessions (Figures 2, 4, 5). Collectively, these trends suggest that modest light exposure may have offered some small advantage over that of dark incubation that would be more apparent in a larger and more comprehensive study of potential dosage effects. Several studies suggest that further investigation is warranted. For example, during normal chick embryogenesis, the right eye at least intermittently experiences greater light exposure than the left eye and the exposure contributes to selective visual pathway development (Rogers, 1982; Rogers & Bolden, 1991). Differential exposure of the 2 eyes also strengthens hemispheric specialization for a variety of postnatal behaviors, such as attack, copulation, foot preference, and turning bias (Casey & Lickliter, 1998; Rogers, 1982). Conversely, the absence of light exposure, as during 24D conditions, may compromise neural circuit development, hemispheric specialization and lateralized control of environmentally and socially cued behaviors (Casey & Lickliter, 1998), which could contribute to locomotor behavior.

Chicks acquired greater navigational skill over 4 hrs of walk experience

Collectively, chicks achieved significant improvements in forward progression from session I to session II, as noted by decreases in stride count, normalized path length, foot placement angle and variability and drift bias variability. The improvements in locomotor performance are consistent with our previous study of global gait parameters and suggest the day of hatching may be a particularly sensitive and therefore useful window during development for further study of motor skill acquisition more generally. We found that the improvements in forward navigation between sessions were most apparent for chicks incubated in 12L. They significantly reduced their variability in performance from session I to II. Further, by session II, hatchlings incubated in 12L exhibited navigation performance within the range of hatchlings incubated in 24L, again raising the possibility that some light exposure during embryogenesis may impart a motor learning advantage over that of dark incubation. Chicks incubated in 24L did not demonstrate substantial improvements between sessions, but this may be indicative of a ceiling effect in performance skill. Specifically, their strong performance in session I relative to the other groups may indicate they already realized the maximum benefits of light exposure on locomotor control by the time the first test trials began, attenuating the benefits of a 4 hr practice interval. Collectively these trends suggest there is some benefit of light exposure impacting locomotor development worthy of further study in a larger population sample.

Chicks did not exhibit a drift bias to either side during forward progression

Average drift bias was less than 1° to the left or right of the forward progression vector for all groups during both session I and II, and less than 4° individually, indicating that veering during any stride was adequately compensated during subsequent strides in a walk trial, as also observed in Figures 1A and 3A. We anticipated that the significant delay in onset of hatching under 24D conditions would impose a longer period of asymmetric posture in ovo during prehatching and hatching that might enhance any lateralized control of posture and stepping and produce a drift bias. During the final 3 to 4 days, the chick is deeply folded on itself with the upper spine and head rotated rightward relative to the lower segments, even as it rotates and extends the neck to press the egg tooth against the shell. Conversely, disruptions of the asymmetric hatching posture have been shown to reduce normal trends in lateralized behavior after hatching, such as turning bias and footedness during locomotion in a T-maze (Casey & Martino, 2000). Nonetheless, under our test conditions, chicks did not drift selectively in either direction during walk trials.

The absence of lateral deviation in path and foot placement angle in our study should not be interpreted as indicating an absence of lateralized behavior or brain function. Several features of our task may account for the lack of a lateral bias during walk trials. Evidence indicates lateral bias in forward navigation is best observed when subjects are blindfolded (Bestaven, Guillaud, & Cazalets, 2012; Boyadjian, Marin, & Danion, 1999). In our task, chicks were not deprived of vision and they could see low level room light at the end of the darkened tunnel. Lateral bias in forward progression is more reliably observed with or without vision if distances are sufficiently long (Boyadjian, Marin, & Danion, 1999). Our walk trail analysis was limited to a region of 40 cm in the center of a tunnel 9 cm wide. Thus, our results suggest that future studies of lateral bias during locomotion employ a

longer, wider apparatus, and include more restrictive visual conditions. Finally, our task did not require a choice in direction (turning left or right), as employed by others (Casey & Lickliter, 1998; Casey & Martino, 2000; Casey & Sleight, 2001; Casey, 2005), leading us to speculate that bias may not be as readily observed for shorter walk trials if choice is not a requirement.

In this study we sought to determine if the absence of light exposure during embryogenesis had a negative impact on locomotor navigation. We asked if the greater step width observed during walk trials in an earlier study was indicative of reduced dynamic balance skill by examining forward progression in the same group of animals. All other global temporal and spatial parameters examined in our earlier study of overground locomotion suggested locomotor skill did not vary with light exposure during embryogenesis. In contrast, findings for this retrospective analysis of forward progression revealed consistent differences between chicks incubated in 24L versus 12L or 24D conditions. Further, the significant differences between 24L and 24D incubated chicks were consistent with differences in step width between these groups. Thus we conclude that in contrast to the effects of bright light exposure during incubation, dark exposure can negatively impact locomotor navigation at hatching. To what extent this reduced competency is due to slower maturation of dynamic balance remains to be more fully examined. Further, the potential costs and benefits of light exposure on motor development in precocial, and possibly non-precocial animals, are important questions yet to be fully understood.

Acknowledgments

The kinematic findings for trial data employed in this study are an extension of a previously published study on locomotor performance (Sindhurakar and Bradley, 2010). This study was supported by NIH National Institute of Child Health and Human Development grant ROI HD – 053367 (to NSB). Research reported in this publication was also supported by the Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number K12 HD – 073945.

References

- Bauby CE, Kuo AD. Active control of lateral balance in human walking. *Journal of Biomechanics*. 2000; 33:1433–1440. [PubMed: 10940402]
- Bestaven E, Guillaud E, Cazalets J-R. Is “Circling” Behavior in Humans Related to Postural Asymmetry? *PLoS ONE*. 2012; 7:e43861. [PubMed: 22957035]
- Bohren BB, Spiegel PB. Light effects during incubation on lines of White Leghorns selected for fast and slow hatching. *Poultry Science*. 1975; 54:1372–1374.
- Boyadjian A, Marin L, Danion F. Veering in human locomotion: the role of the effectors. *Neuroscience Letters*. 1999; 265:21–24. [PubMed: 10327196]
- Bradley NS, Jahng DY. Selective effects of light exposure on distribution of motility in the chick embryo at E18. *Journal of Neurophysiology*. 2003; 90:1408–1417. [PubMed: 12761280]
- Casey MB. Asymmetrical hatching behaviors: the development of postnatal motor laterality in three precocial bird species. *Developmental Psychobiology*. 2005; 47:123–135. [PubMed: 16136548]
- Casey MB, Lickliter R. Prenatal visual experience influences the development of turning bias in bobwhite quail chicks (*Colinus virginianus*). *Developmental Psychobiology*. 1998; 32:327–338. [PubMed: 9589221]
- Casey MB, Martino CM. Asymmetrical hatching behaviors influence the development of postnatal laterality in domestic chicks (*Gallus gallus*). *Developmental Psychobiology*. 2000; 37:13–24. [PubMed: 10937657]

- Casey MB, Sleigh MJ. Cross-species investigations of prenatal experience, hatching behavior, and postnatal behavioral laterality. *Developmental Psychobiology*. 2001; 39:84–91. [PubMed: 11568878]
- Chen G, Patten C, Kothari DH, Zajac FE. Gait differences between individuals with post-stroke hemiparesis and non-disabled controls at matched speeds. *Gait & Posture*. 2005; 22:51–56. [PubMed: 15996592]
- Coleman MA, McDaniel GR. Light altered changes in the embryonic age versus incubation age of White Leghorn embryos. *Poultry Science*. 1976; 55:2483–2485.
- Earhart GM, Jones GM, Horak FB, Block EW, Weber KD, Fletcher WA. Forward versus backward walking: transfer of podokinetic adaption. *Journal of Neurophysiology*. 2001; 86:1666–1670. [PubMed: 11600630]
- Fairchild BD, Christensen VL. Photostimulation of turkey eggs accelerates hatching times without affecting hatchability, liver or heart growth or glycogen content. *Poultry Science*. 2000; 79:1627–1631.
- Fitzpatrick RC, Wardman DL, Taylor JL. Effects of galvanic vestibular stimulation during human walking. *Journal of Physiology*. 1999; 517(3):931–939. [PubMed: 10358131]
- Ghatpande A, Ghatpande S, Khan MZ. Effect of different intensities of fluorescent light on the early development of chick embryos in ovo. *Cellular & Molecular Biology Research*. 1995; 41:613–621. [PubMed: 8777441]
- Hall BK, Herring SW. Paralysis and growth of the musculoskeletal system in the embryonic chick. *Journal of Morphology*. 1990; 206:45–56. [PubMed: 2246789]
- Kernozek TW, Richard MD. Foot placement angle and arch type: effect on rearfoot motion. *Archives of Physical Medicine and Rehabilitation*. 1990; 71:988–991. [PubMed: 2241547]
- Liu WJ, Wang ZX, Chen YX. Effects of monochromatic light on developmental changes in satellite cell population of pectoral muscle in broilers during early posthatch period. *Anatomical Record*. 2010; 293:1315–1324.
- McAndrew Young PM, Dingwell JB. Voluntarily changing step length or step width affects dynamic stability of human walking. *Gait & Posture*. 2012; 35:472–477. [PubMed: 22172233]
- Perry, J. *Gait Analysis: Normal and Pathological Function*. Slack Incorporated; New Jersey: 1992.
- Pijnappels M, Bobbert MF, Van Dieen JH. Changes in walking pattern caused by the possibility of a tripping reaction. *Gait & Posture*. 2001; 14:11–18. [PubMed: 11378420]
- Rácz, K.; Sindhurakar, A.; Bradley, NS.; Valero-Cuevas, FJ. Prenatal motor development affects observed motor behavior for different incubation periods in domestic chick; Proceedings of the 35th Annual Meeting of the American Society of Biomechanics; Long Beach, CA: American Society of Biomechanics. 2011; <http://www.asbweb.org/conferences/2011/pdf/327.pdf>
- Richardson JK, Thies SB, DeMott TK, Ashton-Miller JA. A comparison of gait characteristics between older women with and without peripheral neuropathy in standard and challenging environments. *Journal of American Geriatrics Society*. 2004; 52:1532–1537.
- Rogers LJ. Light experience and asymmetry of brain function in chickens. *Nature*. 1982; 297:223–225. [PubMed: 7200573]
- Rogers LJ, Bolden SW. Light-dependent development and asymmetry of visual projections. *Neuroscience Letters*. 1991; 121:63–67. [PubMed: 1708487]
- Ryu YU, Bradley NS. Precocious locomotor behavior begins in the egg: development of the leg muscle patterns for sleeping in the chick. *PLoS ONE*. 2009; 4:e6111. [PubMed: 19578536]
- Sindhurakar A, Bradley NS. Kinematic analysis of overground locomotion in chicks incubated under different light conditions. *Developmental Psychobiology*. 2010; 52:802–812. [PubMed: 20589718]
- Sindhurakar A, Bradley NS. Light accelerates morphogenesis and acquisition of interlimb stepping in chick embryos. *PLoS ONE*. 2012; 12:e51348. [PubMed: 23236480]
- Stolze H, Klebe S, Petersen G, Raethjen J, Wenzelburger R, Witt K, Deuschl G. Typical features of cerebellar ataxic gait. *Journal of Neurology, Neurosurgery, & Psychiatry*. 2002; 73:310–312.
- Wu KC, Streicher J, Lee ML, Hall BK, Muller GB. Role of motility in embryonic development I. Embryo movements and amnion contractions in the chick and the influence of illumination. *Journal of Experimental Zoology*. 2001; 291:186–194. [PubMed: 11479917]

Zverev YP. Spatial parameters of walking gait and footedness. *Annals of Humans Biology*. 2006; 33:161–176.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Kinematic analysis of navigation. A. Digitized locations of foot placements are shown for consecutive steps progressing from right to left during 1 walk trial. Foot placements were video recorded from beneath the walkway, reversing left-right orientation for the left foot (LF, closed squares) and right foot (RF, open squares). This trial was selected from a hatchling incubated in 12L conditions. B. The estimated center of mass location, noted by an 'X or •', is shown for consecutive steps of the left foot to right (X) and right foot to left (•) during 1 walk trial for a chick incubated in 12L conditions. Center of mass locations were used to complete path length analyses (see Methods for details). C. Methods for measurement of left foot placement angle (α_1) are shown for the first of 2 foot placements, identified by the step vector (V_{S_1}). Terms (Lx_i , Ly_i) defined the 2D coordinates of each left placement. The progression vector (V_{X_1}) defined forward progression relative to the foot placement coordinates and the basis vector (V_{B_1}) extended orthogonal to V_{X_1} (see text for

details). An $\alpha < 90^\circ$ indicated the stride deviated to the left of V_{X1} during forward progression.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

Stride count and normalized path distance varied significantly across groups and between sessions. A. The group means and SD for number of strides per walk trial are plotted by session (I, II) for chicks incubated in 1 of the 3 light conditions (24L, 12L, 24D), $N = 10$ hatchlings per condition. Stride count varied significantly across incubation conditions (a, $p < .0006$) and between sessions (b, $p < .001$). Post hoc comparisons for incubation condition (a₁) indicated that hatchlings incubated in 24L took fewer steps than hatchlings incubated in 12L ($p < .01$) and 24D ($p < .001$). B. The average normalized path length is plotted by session for each incubation group. Normalized path length varied significantly across incubation conditions (c, $p < .002$) and between sessions (d, $p < .002$). Post hoc comparisons for the main group effect (c₁) indicated that the normalized path length was less for hatchlings incubated in 24L compared to 24D ($p < .0005$).

Figure 3.

Exemplary plots of 2 walk trials from a hatchling incubated in the 12L condition. A. The 11 digitized foot placements (closed squares) identify 10 (numbered) strides of the left foot for 1 trial during session I. The extent of lateral deviation (drift) during forward progression was most apparent for trials in session I, e.g., note the larger lateral deviations for foot placements for strides 6-10. B. The 8 digitized foot placements identify 7 strides for a trial in session II. Forward progression is relatively straight-ahead due to minimal drift.

Figure 4.

Comparisons for foot placement angle magnitude and variability across incubation conditions and between sessions. A. Group means and SD are plotted for absolute foot placement angles as a measure of drift magnitude in session I and II. Placement angle magnitude varied significantly across incubation conditions (a, $p < .0006$) and between sessions (b, $p < .001$). Post hoc comparisons for incubation condition (a₁) indicated placement angle magnitudes were smaller for chicks incubated in 24L compared to 12L ($p < .008$) and 24D ($p < .002$). B. Average variability of absolute foot placement angles are plotted as a measure of drift magnitude variability. Variability differed significantly across incubation conditions (c, $p < .03$), between sessions (d, $p < .003$), and the interaction (i) was significant $F(2,54) = 5.7$, $p < .006$. Post hoc comparisons indicated that during session I (i₁) chicks incubated in 24L exhibited less drift variability than chicks incubated in 12L ($p < .006$) and that chicks incubated in 12L reduced drift variability (i₂) from session I to II ($p < .0004$).

Figure 5.

Comparisons for mean drift bias direction and drift bias variability across incubation conditions and between sessions. A. Group means and SD are plotted for foot placement angle as an indication of direction in drift bias for session I and II, positive means indicate that average bias was leftward directed. No significant differences were found for drift bias across conditions or between sessions. B. Average drift bias variability is plotted. Variability differed significantly across incubation conditions (a, $p < .003$), between sessions (b, $p < .001$) and the interaction (i) was significant ($p < .02$). Post hoc results for incubation condition indicated hatchlings incubated in 24L (a_1) exhibited less drift bias variability than in 12L ($p < .009$) or 24D ($p < .004$). Post hoc results for the interaction indicated that hatchlings incubated in 24L were less variable than chicks incubated in 12L during session I (i_1 , $p < .003$), and chicks incubated in 12L were less variable than those incubated in 24D (i_2 , $p < .009$). Chicks incubated in 12L significantly reduced drift bias variability from session I to II (i_3 , $p < .001$).