The behavioral and neurological effects of hypoxia during the embryonic development of domestic chicks (*Gallus gallus)*

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Abstract:

Previous research suggests that hypoxia during critical periods of development leads to the impairment of cognitive ability and brain structure early on in the life of the domestic chick. However, it is not known what, if any, effect these hypoxic insults cause in the life of the chick if induced during non-critical periods. We tested this question in developing chicken embryos by half-wrapping eggs with a membrane impermeable to oxygen. Eggs were forced hypoxic during two periods for 24h at incubation day 9 and 48h at day 13 (n=7; the traditional critical periods) or for 24h at day 11 and 48h at day 15 (n=5; a delayed period) and compared to a control without hypoxia (n=6). The goal was to determine to what degree delaying hypoxic insult eliminates its consequences, in relation to critical period exposure and no hypoxic exposure. Chicks were assessed for spatial, working memory and cognitive function 8 days after hatching using multiple behavioral tests. We also measured differences in brain structure through volumetric analysis of the hippocampus, which plays an important role in memory function. Our results suggest that delaying hypoxic insult may have more pronounced effects on cognitive disability, but may eliminate negative effects on spatial memory and fear, although no structural differences in the brain were found.

Introduction:

The developing embryo is notably affected by a lack of oxygen, called hypoxia, especially in regards to growth of the brain. Oxygen is a necessary substrate in the processes of developing neurons and synapse formation. It has been shown that, in humans, restricted neurodevelopment in uterine can have lasting cognitive effects in children up to three years of age (Scherjon et al, 1998).

The chick embryo has been used extensively as a model for the adverse effects of oxygen deprivation on the developing brain (Camm et al, 2005; Rodricks et al, 2010). Chicks are ideal for these experiments because they develop independently of maternal effects, preventing compensation for any adverse conditions applied to the embryos. They are also precocial at birth, allowing for early behavioral assessments.

One behavioral dysfunction previously found in developmentally hypoxic chicks was deficits to memory. It is believed in both mammal and avian brains that memory formation relies heavily on proper functioning of the hippocampus (Hp). Previous work has shown that acute hypoxia for 24h at incubation days 10 and 14 lead to impairments in long-term memory. Hypoxia at day 10 also affected short-term memory in chicks (Rodricks et al, 2010). E10 and E14 have thus been identified as the critical periods in development during which hypoxia can have damaging effects on memory function.

Methods:

Hypoxia was induced by wrapping half of the egg, lengthwise, in Parafilm M. Chicks were subject to hypoxia for 24 hours from day E9-10 and 48 hours from E13-15 (called Early Hypoxia, or EH) or for 24 hours from day E11-12 and 48 hours from E15-17 (called Delayed Hypoxia, or DH).

Cognitive function - Chicks were habituated to metal washers with a plastic covering during days 5 to 8 post hatch. Using the same holeboard as described above, chicks were presented with a board full of worms. The holes were covered with the washers in such a way that the chicks could see the worms, but they had to remove the washer to reach one.

Fear - The hesitation of a chick to eat a worm from a novel object was measured in comparison to a mealworm presented independently to the worm. If the worm was not consumed after 10 minutes, the object was removed.

Spatial Memory - A board measuring 10in x 7in with 6, ½ in-holes in a 3 x2 arrangement was used to test the spatial memory of the chicks. Chicks were habituated for one hour every day and tested on day 9 after hatching. Individuals were moved into the testing tank and a board where all holes were uncovered, but only one contained a worm were placed in from of them. After the chick consumed this worm, the board was immediately removed. Another worm was placed in the same location, but all holes were covered and the board was returned to the chick.

Results – Cognitive Function

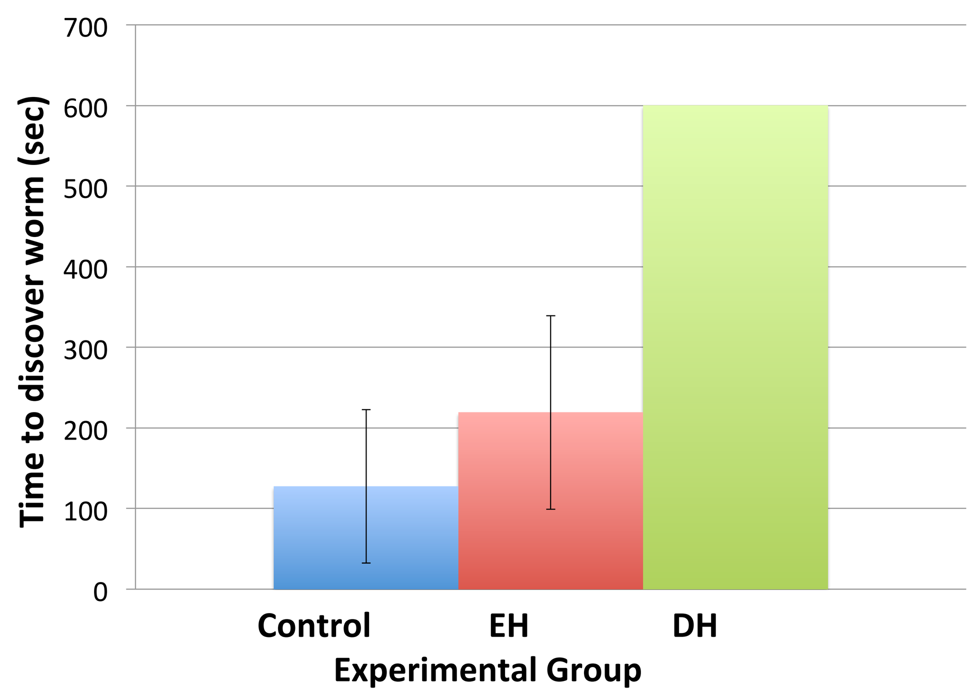

Figure 1. The time it took for each chick to uncover and eat one worm in the washer test was recorded as a test of cognitive ability. The test was ended after 10 minutes if no worm was eaten. No chicks in DH completed the task. Data presented is mean with error bars representing 95% confidence interval. Control (n=6), EH (n=6), DH (n=5). One-way ANOVA, f=6.61, df_{Group}=2, df_{error}=14, p=0.010

Results – Fear Assessment

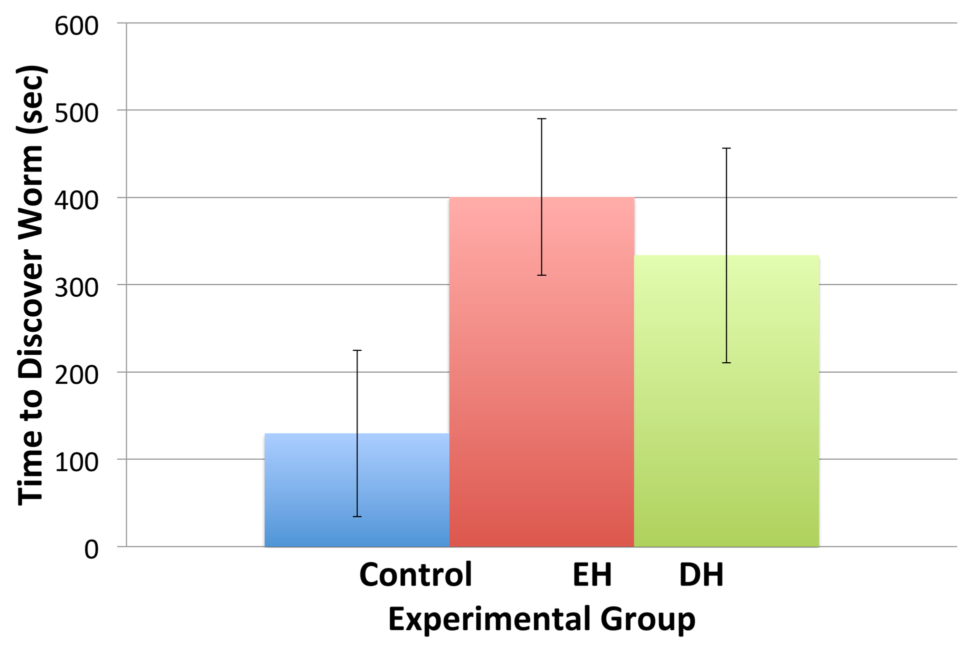

Figure 2. The ability of a chick to discover a worm in a novel object was tested as a measure of fear, assuming fear would lead to longer approach time. The test was ended after 10 minutes. Data presented is mean with error bars of 95% confidence interval, but differences are not significant. Control (n=6), EH (n=6), DH (n=5). One-way ANOVA, f=2.02, df_{Group}=2, df_{error}=14, p=0.169

Results – Spatial Memory

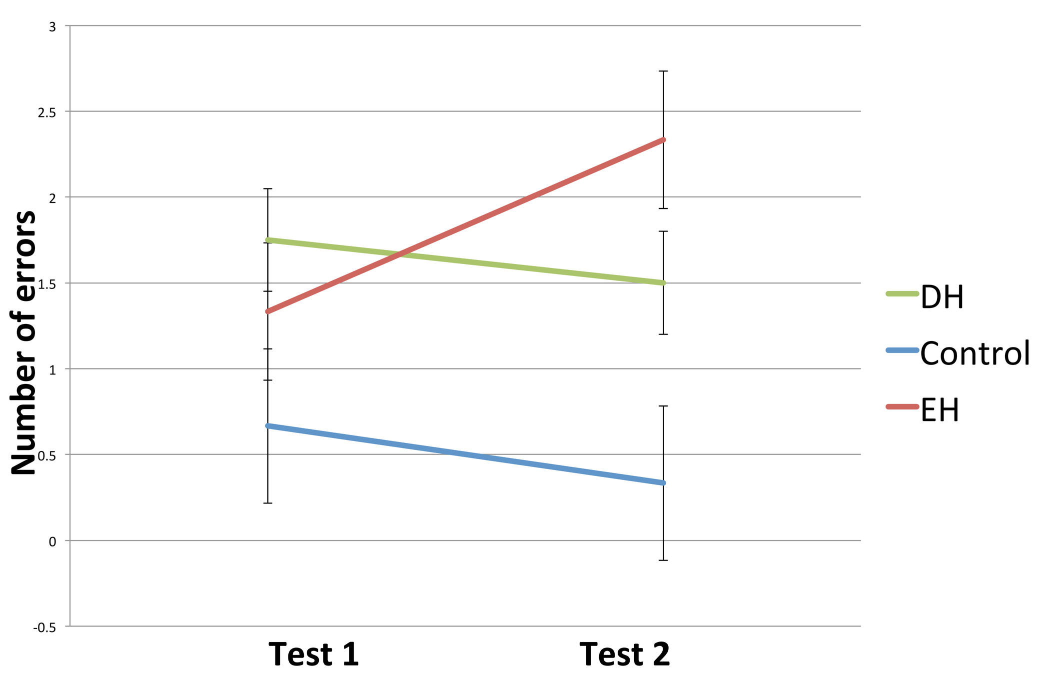

Figure 3. All groups were trained and tested in successive trials on the holeboard task for spatial memory. The number of errors per test was recorded and a decrease in errors was used as a signifier of memory retention. Chicks that did not peck during the training trials or failed to uncover a worm were excluded. Data presented are means with error bars of SEM. Control (n=4), EH (n=6), DH (n=4). GLM, $f_{test\ 1,\ test\ 2}$=1.19, 6.07, df_{Group}=2, 2, df_{error}= 11, 11, p= 0.339, 0.017.

Identifying the Hippocampus:

After extraction, brains were post-fixed in 4% paraformaldehyde then frozen at -80°C. Tissue was cut into 40 µm coronal sections, mounted on slides and stained with thionin Nissl stain. The right and left hippocampi are outlined in red.

Results – Volumetric Analysis

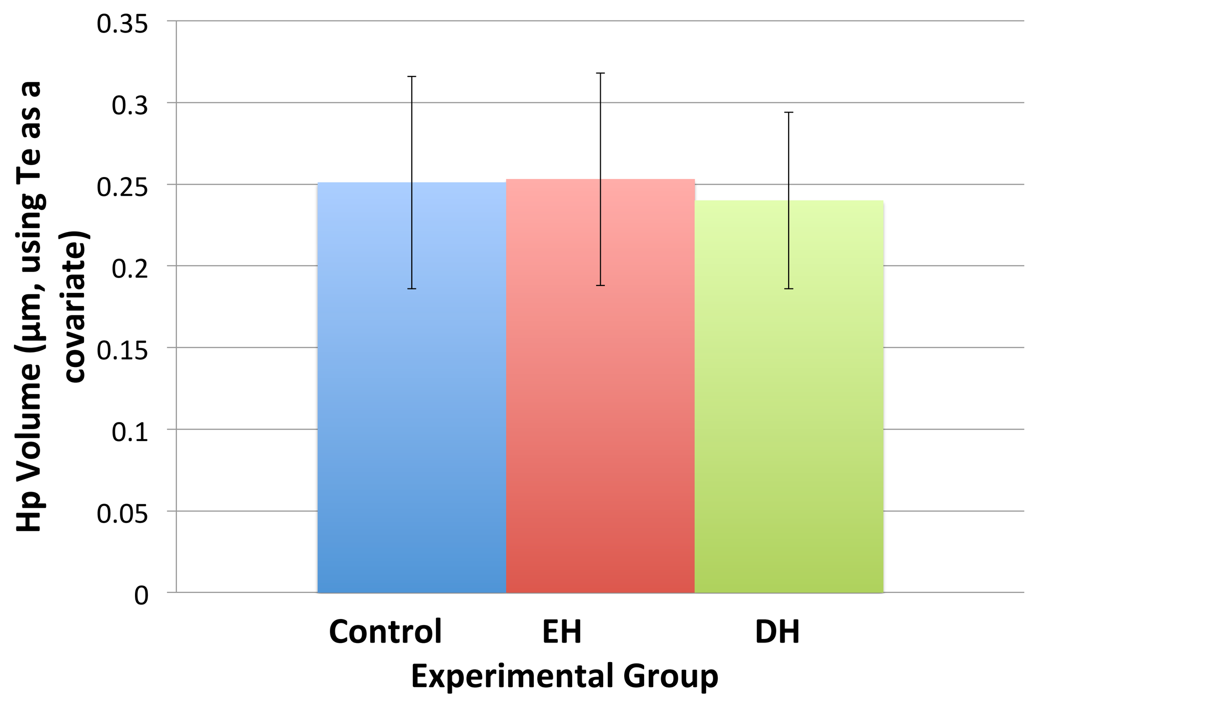

Figure 4. Hp volumes were measured to determine if any structural differences existed between the experimental groups. Data presented are means with error bars of Standard Deviation, using the telencephalon volume as a covariate. No significant differences were seen between groups. Control (n=6), EH (n=6), DH (n=5). GLM , f=0.07, df_{Group}=2, df_{error}=14, p=0.929

Discussion:

These results suggest that delaying hypoxic insult causes cognitive deficits above normal, but may eliminate some negative effects on memory and fear. There were no structural differences between the groups. It may be possible that deficits in structure and some behaviors that are caused by gestational hypoxia, as previously found at 2 days post-hatch, are ameliorated after several more days of life. Future experiments should involve further structural analyses to determine if other areas of the brain are affected, such as the medial striatum, another memory center, or the amygdala, which is associated with fear. Also, the effects of hypoxia on other parts of the chick, such as immune function, could be beneficial to learning the total effects of gestational hypoxia.

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