

The manuscript titled "Disturbance history alters the development of the HPA axis in altricial nestling birds" explores the effects of chronic stress on nestling birds, specifically focusing on the hypothalamic-pituitary-adrenal (HPA) axis development in the Noisy Miner, an altricial bird species. The study aims to understand how daily handling, as a form of stress, influences corticosterone levels and pre-fledging body mass in comparison to control groups that were not subjected to daily handling.

The manuscript is well-written, and I am happy to have had the chance to review it. It provides a comprehensive introduction and background that successfully situates the study within the broader context of ecological and physiological research on birds. This will be an important contribution to understanding the physiological impacts of chronic stress on bird development.

Introduction:

The introduction is concise and clear and well organized. It provides excellent background information about glucocorticoid function and HPA axis development in nestling birds.

Line 62-63: You may want to consider adding the follow reference for the influence of GCs on memory. To the best of my knowledge, it is the only study that links HPA axis function and long-last memory in a bird species. To fully disclose, this is my own work.

Jones, Blake Carlton, Sara E. Bebus, Stephen M. Ferguson, Philip W. Bateman, and Stephan J. Schoech. "The glucocorticoid response in a free-living bird predicts whether long-lasting memories fade or strengthen with time." *Animal behaviour* 122 (2016): 157-168.

Methods:

For treatment broods please provide how long the average nest visit was for daily visits, from first disturbance to leaving the area. There is a potential confound between chronic activation of the HPA axis, and less feeding by parents due to nest disturbance. Estimates of provisioning rates between control and treatment nests before during and after nest visits could elucidate this possibility. In its absence, I think you should recognize this possibility, with perhaps any explanation for why you may think the possible confound is unlikely to explain the results.

Please provide the range of % recovery for the cort assay.

Line 247: I'm wondering why brood size, hatch order, and sex are included as random effects rather than fixed effects. Random effects are typically used when the levels of a variable are seen as a sample of a larger population of possible levels, like nest or brood id. Further, the partial pooling (using a shrinkage estimator) that is employed by a mixed model for random effects makes sense particularly for samples with poorly represented levels with a relatively large overall sample size, such as brood id, but not sex or hatch order (Gelman & Hill 2006). How many levels are there for brood order and brood size? Can you elaborate on your decision for include these variable as random effects rather than fixed ones?

Gelman, A. and Hill, J., 2006. *Data analysis using regression and multilevel/hierarchical models*. Cambridge university press.

Results:

Thank you for including individual data points on the boxplots.

I think it would be valuable to see the data visualized for comparing body mass between controls and treatment nests.

With the morphometric data you collected, can you see if structural size or feather development differs between control and treatment nestlings?

Are you able to test differences in fledgling timing between controls and treatments?

Conclusions:

I thought the conclusions were well discussed. I wonder if we know much about the possibility of how chronic handling stress during nestling stage might influence adult phenotype. Perhaps from any studies employing a different type of stressor during the nestling phase and tracking individuals longitudinally.

Can you provide any discussion on potential mitigation strategies for reducing handling stress in ecological studies?

I found some lines that need spaces.

52 — space need between “axis” and “(Romero...”

103 — space needed between “...2016)” and “and even...”

259 — space need between “11” and “and 14...”