

# Are parental care induced gene expression patterns affect more by internal cues or external stimuli?

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## Abstract

Are parental care induced gene expression patterns affect more by internal cues or external stimuli? We test two alternative hypothesis: internal clock cues versus external offspring stimuli by manipulating the timing of normal transitions during parental care. When we removed offpsring, we find the most pronounces chagnes in gene expression later during incubation or right after hatch, compared to early removal of eggs. Given the similarity in gene expression the days immediately preceeding and immedately following hatch, we do not have the power to say weether gene expression patters are more like hatch or incuabtion when dummmmy egg are used to extend incubation. This matters because...

## Introduction

## Materials and Methods and Results

Using egg and chick removal and replacement experiments we will determine if neural transcription and translation during parental care transitions are based on external sensory information (the presence/absence of eggs or chicks) or on an internal clock-timing mechanism (natural biological rhythms unaffected by changes in external sensory information). As rock doves are known to cross-foster eggs and chicks, our working hypothesis is that the transition to parental care behaviors is based on external sensory input, and specific predictions are explained in conjunction with each manipulation below.

We will place eggs in nests of birds lacking them and sample brains the following day. Prediction: birds will transition into incubation behavior, and neural transcription and translation will mirror that of birds sampled on the day their first egg was laid. This would support our working hypothesis that sensory perception of the environment drives these changes. If we do not observe changes in behavior, transcription, and translation, or they only occur in males, one of many interpretations is that males respond to environmental cues while females are cued by an internal clock-timing mechanism regulating transitions into parental care.

We will prolong incubation by removing eggs from actively incubated nests on the day before hatching (Day 17) and immediately replace them with infertile, ‘dummy’ eggs. We will then sample brains 3 days after normal hatch time (Day 21). Collaborator Silver and colleagues have shown sex differences in the endocrine profiles of male and

female doves in response to prolonged incubation, in that prolactin levels are maintained in incubating females but not males (Ramsey et al. 1985). Because of this, we predict that if changes in behavior, neural transcription, and translation occur in females because of sensory information related to chicks hatching (supporting our working hypothesis), then females that experience this prolonged incubation will mirror the changes of incubating birds sampled on Day 17 of incubation. However, if changes in behavior, transcription and translation are brought on by internal clock mechanisms rather than external sensory information, as predicted at this stage in males, bird behavior and biology will resemble that of birds collected on Day 3 post-hatching.

We will prolong incubation by removing eggs from nests undergoing active incubation on Day 17 (the end of incubation) and immediately replacing eggs with ‘dummy’ eggs. On Day 21 (3 days post-natural hatch time) we will offer hatchlings. Doves are known to care for nestlings placed in their nest that are not their own (Klinghammer and Hess 1964, Hasen 1971). Brains will be sampled the following day. Prediction: Behavior, transcription and translation will be similar to birds naturally caring for chick(s) one day post-hatch of the first chick, supporting our working hypothesis. Alternatively, if behavior, transcription and translation are similar to birds caring for hatchlings on Day 3 post-hatching, which would have been the normal time course for parental care if chicks had hatched naturally, this would support regulation by an internal clock mechanism.

We will remove eggs during incubation on Day 1 (beginning), Day 9 (middle), or Day 17 (end) and sample brains on the following day. Note: This is not a repeated measures sampling, and three groups of male-female pairs will be independently sampled at each time point. Prediction: Birds will revert to a behavioral, transcriptional and translational pattern characteristic of the “Nesting, prior to lay” sampling point, lending support to our working hypothesis. If birds maintain a profile characteristic of birds collected at similar time points whose eggs have not been removed, this lends support to an internal clock mechanism.

We will remove newly, naturally hatched chick(s) on Day 2: second chick hatches and sample brains from parents the following day (Day 2 post (second) egg hatching). Prediction: Behavior, transcription and translation are sensory driven and will revert to a pattern characteristic of the ‘Nesting, prior to lay’ point, supporting our working hypothesis. Alternatively, if an internal clock mechanism is at play, we would expect to see a pattern similar to birds caring for chicks on Day 2 post (second) egg hatching.

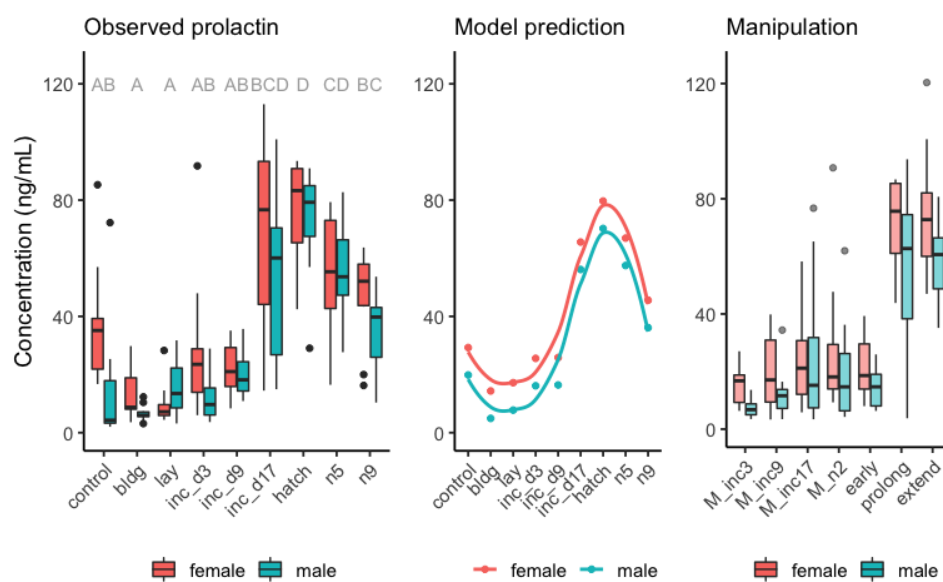
We will remove eggs on Day 8 (middle) of incubation and immediately replace them with newly hatched chicks. We will sample brains on the following day (Day 9). Prediction: Changes in behavior, transcription and translation are externally sensory driven, and these will be similar to birds sampled on ‘Day 1: 1st egg hatches, supporting our working hypothesis. Alternatively, if profiles remain similar to birds sampled on Day 9 that are actively incubating eggs, this would support the presence of an internal clock mechanism.

## Results

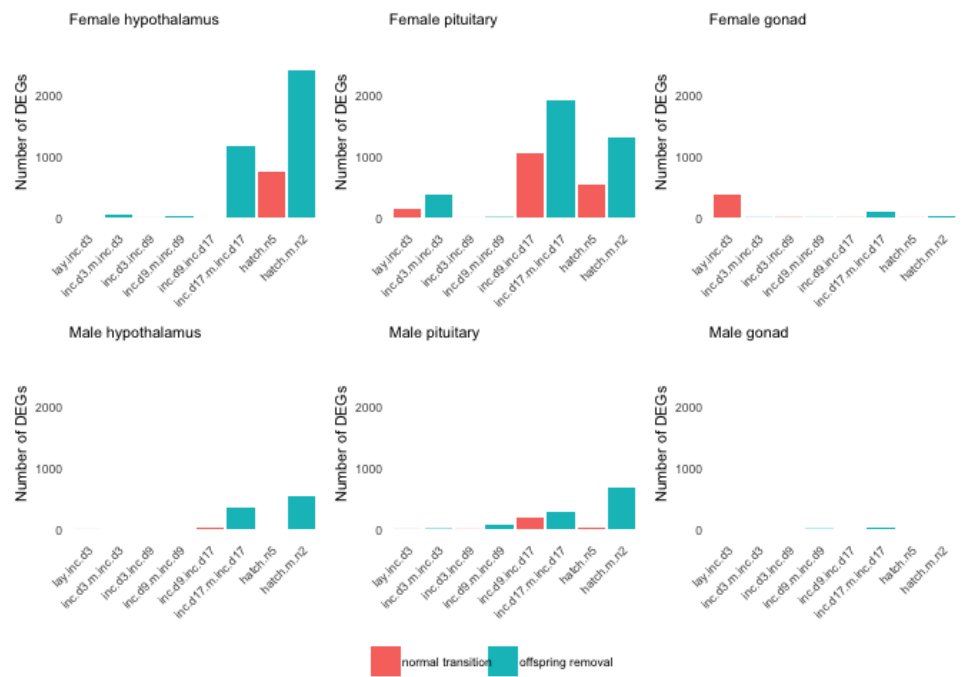
## Supplementary Figures

## Acknowledgments

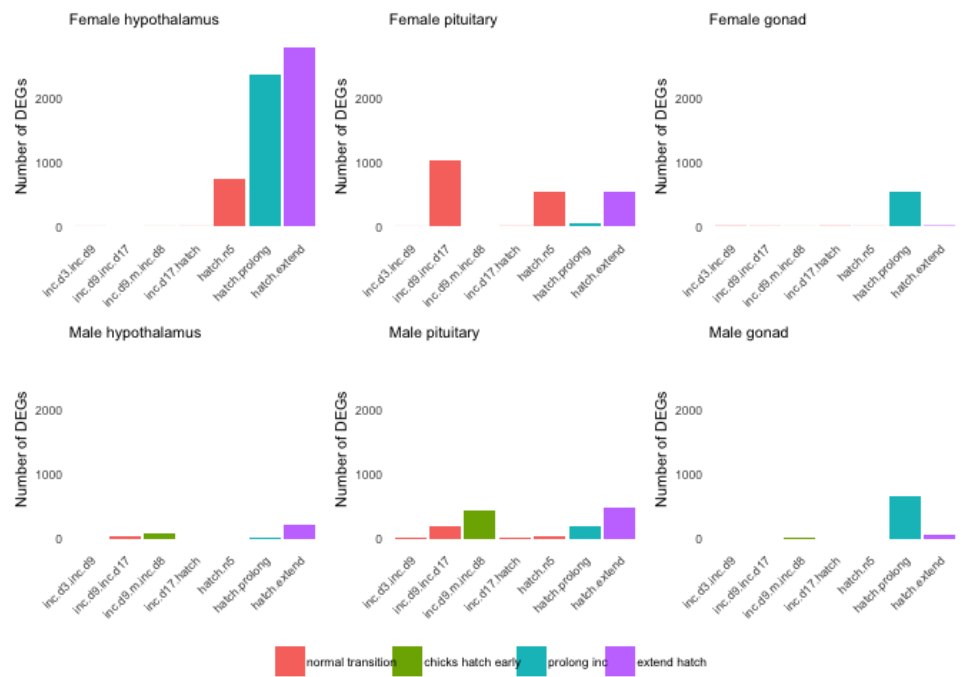
This project is a synergistic collaboration between the PI, Rebecca Calisi-Rodríguez (expertise in avian behavior, parental care and neurobiology), co-PI, Matthew MacManes (expertise in next-generation sequencing, transcriptome assembly, and gene



**Fig 1.** Circulating prolactin levels with an without manipulation.



**Fig 2.** Offspring removal.



**Fig 3.** Manipulation of timing of hatch with dummy eggs and foster chicks.

expression analyses), and Collaborator, Rae Silver (expertise in neurobiology, dove behavior, and decades of successful breeding and maintenance of dove colonies at Barnard College).

**References**