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Abstract

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Author summary

One of the central questions of animal behavior is how biological mechanisms mediate and respond to behavior. Successful rearing of offspring involves a shift from aggressive and sexual behaviors to more caring and nurturing ones, but what neural substrates mediate this transition? How flexible are these changes in response to unpredictable environmental perturbations, and how is behavior altered because of them? In a rapidly changing world, understanding how the environment affects the brain and how, in turn, the brain affects the behavioral transition into parental care will shed light on how changes in environment can ultimately affect fitness. Previous discoveries have implicated particular hormones that play a role in the maintenance of parental care behavior in vertebrates, such as: oxytocin and vasopressin (avian homologs: mesotocin and vasotocin), vasoactive intestinal peptide, and prolactin. However, we know very little about when and how the brain transitions into parental care behaviors in any vertebrate, and this knowledge is fundamentally important to our understanding of the mechanisms mediating parental care. Here, we propose to expand our knowledge by using classic offspring replacement and removal manipulations to uncover the role of GnIH during the transition to parental care behaviors. In addition to this targeted approach, we propose a powerful untargeted approach to explore what other changes in the brain may be occurring during this transition. We will use high-throughput sequencing technology and immunohistochemistry to uncover all genetic changes and specific proteomic changes in the brain, along with their relationship with GnIH, which occur during the transition to parental care. Our long-term goal is to understand the interplay of genes, proteins, and parental care. Specifically, we aim to understand the genetic and physiological mechanisms driving parental care behaviors in all vertebrates, and to document this knowledge in a rich, open database. This knowledge would enable manipulation of specific parental care behaviors and their activation using emerging powerful genetic technologies.

Introduction

Understanding the mechanisms underlying parental care are critical to circumventing issues with parent-newborn bonding as well, where ultimate explanations are obvious, but specific mechanisms remain elusive.
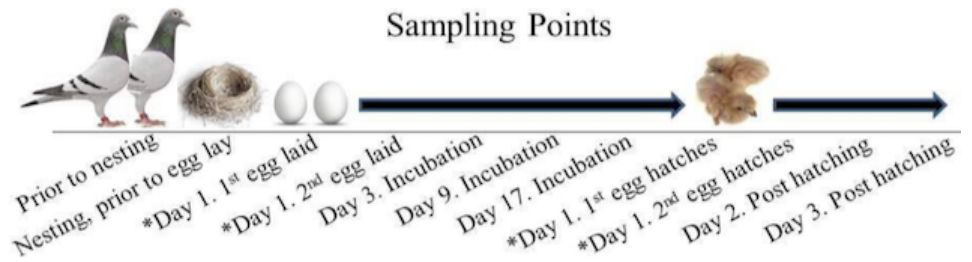
The rock dove (*Columba livia*) is an ideal system to characterize changes in genetic expression during parental care transitions because: 1) ample genomic resources are available, including a complete annotated genome assembly (Gillespie et al. 2013; Shapiro et al. 2013) and methodology concerning reproductive physiology and behavior (Dong et al. 2012); and 2) rock doves are prolific, year-round breeders that thrive in captivity, making observation, manipulation, and sampling highly feasible year-round.
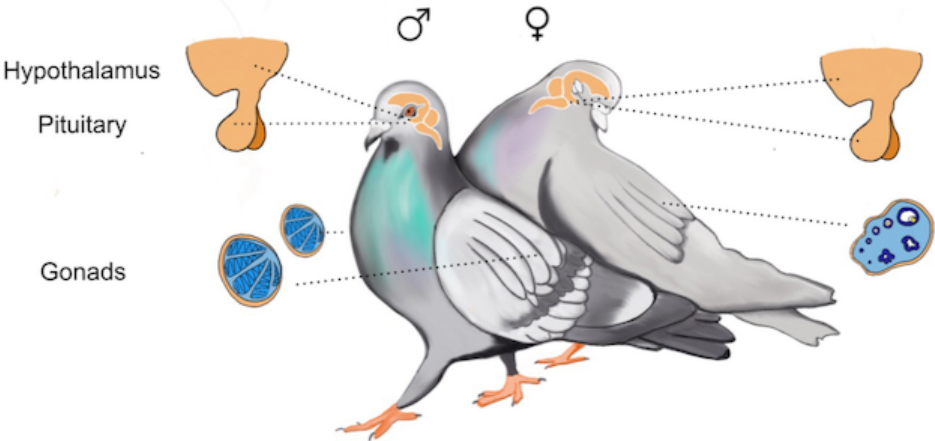
Rock doves are socially monogamous and offer bi-parental care, making inter- and intra-sexual comparisons possible. Birds offer two important behavioral transition points into parental care: the incubation of eggs and the caring for chicks. This produces two unique opportunities to study how the brain transitions into two different suites of parental care behaviors. Additionally, rock doves exhibit a parental care strategy analogous to mammals in that they, too, 'lactate' to feed their young (Gillespie et al. 2011, 2012). This lactation, unlike simple regurgitation of food, consists of the production and sloughing off of skin cells inside the crop sac of females and males, creating a protein-rich milk-like substance on which they rear their chicks. Many functional similarities between rock dove and mammalian lactation exist concerning the mediation of this event by the hormone prolactin (Dumont 1965). Additionally, like mammalian milk, rock dove milk delivers essential immunoglobulins and nutritional benefits to young, aiding in their immune function and development of microbiota (Gillespie et al. 2012). Thus, because rock doves incubate eggs and exhibit mammalian-like mediation and function of lactation for young, they have the potential to serve as a powerful theoretical bridge to understand the neurobiology of both avian and mammalian transitions into parental care.

Materials and Methods

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Sampling Points

Prior to nesting · Nesting, prior to egg lay · *Day 1. 1st egg laid · *Day 1. 2nd egg laid · Day 3. Incubation · Day 9. Incubation · Day 17. Incubation · *Day 1. 1st egg hatches · *Day 1. 2nd egg hatches · Day 2. Post hatching · Day 3. Post hatching

Elucidate cause of transcription & protein changes during parental care transitions

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.

Give birds eggs

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.

Prolong incubation

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.

Extend time of 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.

Remove eggs

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.

Reduce time to 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.

Remove chicks

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.
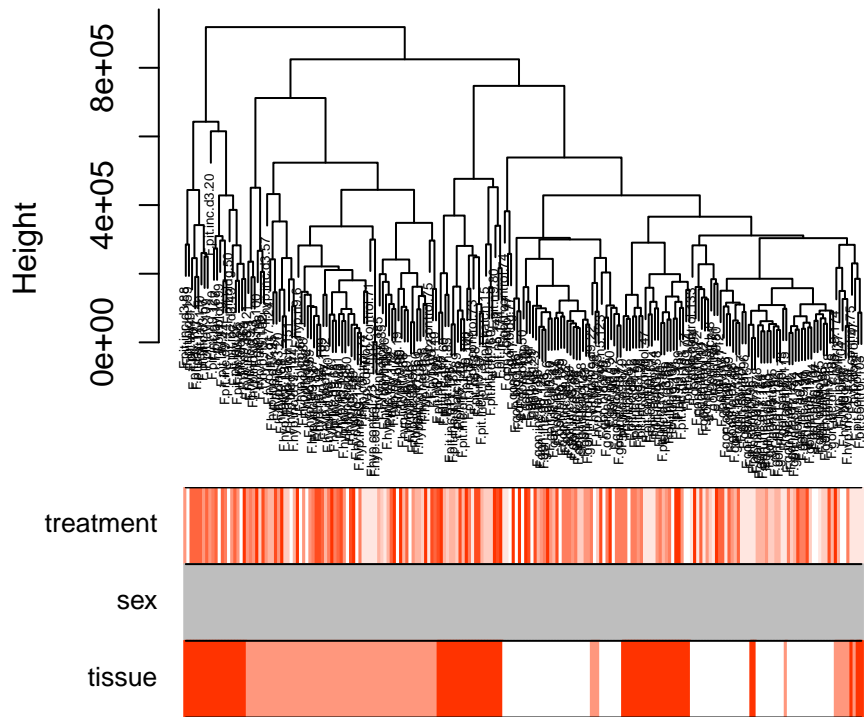
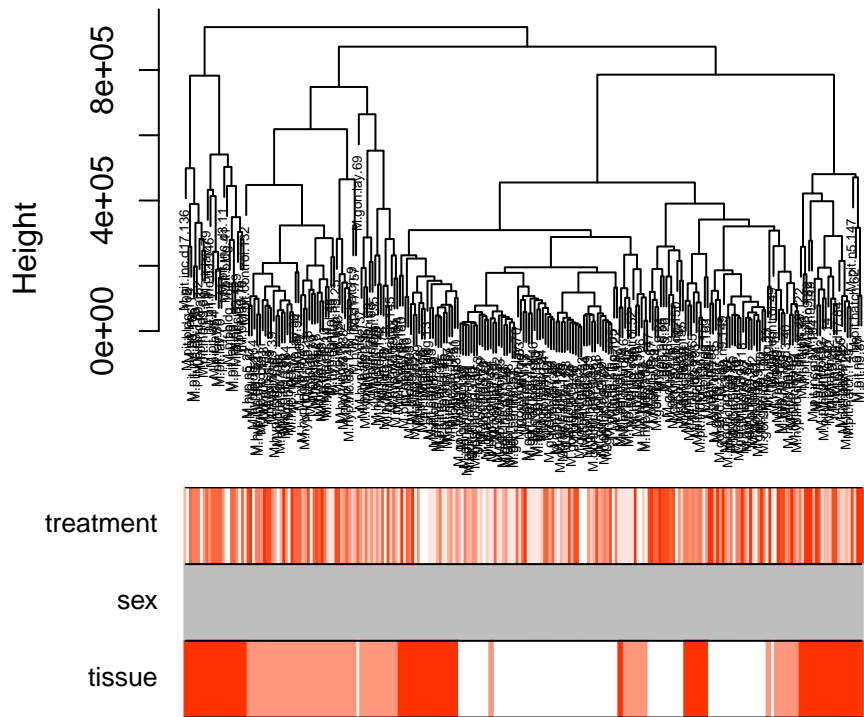## Results

Here are two sample references: [1,2].

Characterization dendrogram with trait heatma
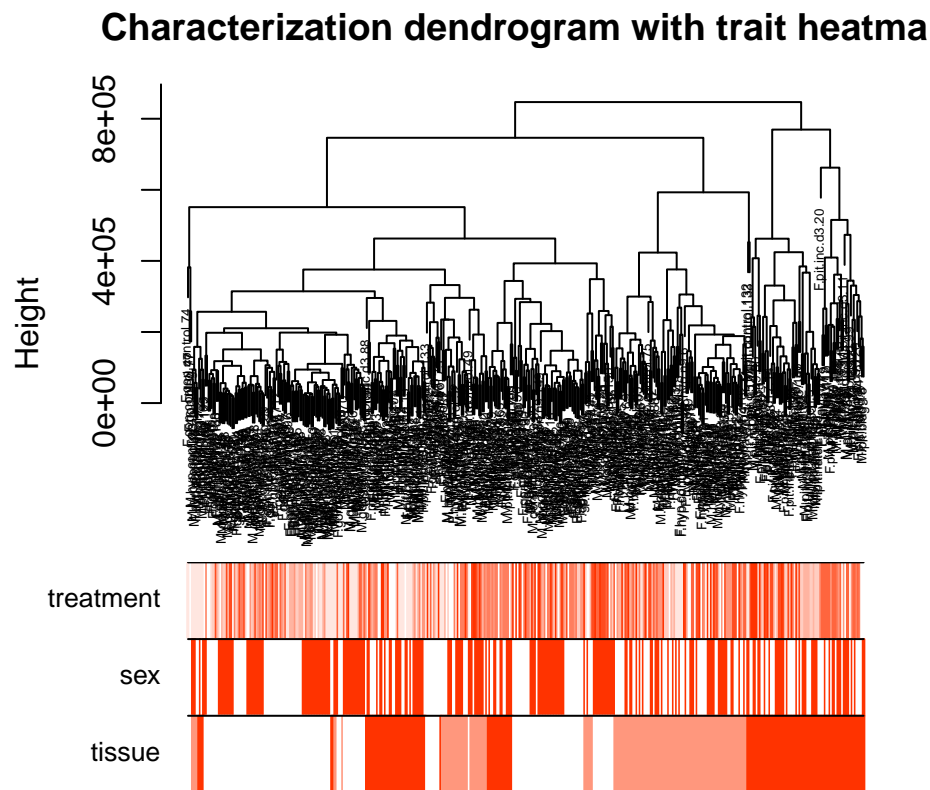
Fig 1. Characterization dendrogram with trait heatmap.. Most
hypothalamus and pituitary samples separte form the gonad samples, but some are
clusters contain samples from all tissues.The top panel is a dendrogram showing
Euclidean distance of sample. Females (white), males (red), gonad (white),
hypothalamus (pink), pituitary (red).

Female sample dendrogram with trait heatmap

Fig 2. Female characterization dendrogram with trait heatmap.. Most
pituitary samples form a single cluster. More hypothalamus samples cluster with gonads
than with pituitary. The top panel is a dendrogram showing Euclidean distance of
sample. Females (white), males (red), gonad (white), hypothalamus (pink), pituitary
(red).

Male sample dendrogram with trait heatmap

Fig 3. Male characterization dendrogram with trait heatmap.. As with females, most pituitary samples form a single cluster, while more hypothalamus samples cluster with gonads than with pituitary.

Acknowledgments

Metadata and extras

Funding Statment

References

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doi:10.1016/0003-4916(63)90068-X

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