**Supplementary Material**

**McNew et al. 2023**

Manipulation of a social signal affects DNA methylation of a stress-related gene in a free-living bird

**Methods:**

*Plumage manipulation*

The coloration of female breast plumage was quantified following methods described in [1,2]. We collected feathers from the center of the breast at each capture to quantify reflectance. Plumage coloration was measured with an Ocean Optics FLAMES-UV-VIS spectrophotometer with PX-2 pulsed Xenon light source and WS-1 white standard in OceanView v.1.5.2 (Ocean Optics, Dunedin, FL, U.S.A.). For each measurement, we stacked and taped four feathers on an index card and then smoothed the barbs to create a patch large enough for measurement. We used a fibre-optic UV/VIS probe in a holster that blocked external light and maintained a distance of 5 mm between the feathers and the probe. Spectra were collected with a 10 scan average, 20 nm boxcar width and 60 ms integration time. Four separate spectra were taken for each feather stack with the probe removed between measurements. For each female, we measured four sets of feathers (two from first capture and one each from second and third capture). Reflectance spectra generated by OceanView were processed in R v.3.3.3 (R Core Team, 2016) using the package ‘pavo’ (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013). We calculated mean breast brightness as the average reflectance from 300 to 700 nm (‘B2’ in the ‘pavo’ package). The four repeated measurements from each feather sample were averaged to arrive at a final brightness measure.

*Corticosterone quantification*

We measured baseline corticosterone concentration in blood plasma samples using commercially available enzyme immunoassay (EIA) kits (DetectX Corticosterone, K014eH5, Arbor Assays, Ann Arbor, MI, U.S.A.). We previously validated these kits in tree swallows, and extensive validation and protocol details are available in Taff, Zimmer et al. (2019). Briefly, we used 5 ml of plasma in a triple ethyl acetate extraction and then ran the resulting samples in duplicate following the manufacturer's protocol. Extraction efficiency was determined using samples spiked with a known amount of corticosterone; average extraction efficiency with this method was 89.7%. When starting with 5 ml of plasma, the lower detection limit was 0.8 ng/ml. Interplate variation was assessed using a plasma pool run across plates and was 5.7%. Intraplate variation was assessed using duplicate wells and averaged 10.6%.

*Pyrosequencing***:**

We extracted whole genomic DNA from frozen erythrocytes using Qiagen DNEasy Blood and Tissue Kits (Valencia, CA) following manufacturer’s protocols. We assayed DNA quantification on a Qubit 2 Fluorometer using a broad range detection standard.

**Supplemental Tables**

Table S1: Summary data for each of the CpGs characterized in this study including minimum, median, mean, and maximum methylation, and the number of samples sequenced at that site (Excel file).

Table S2: LMM model output predicting methylation of sites in the CRH gene

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **CRH** | | |
| *Predictors* | | *Estimates* | *CI* | *p* |
| (Intercept) | | -1.59 | -1.98 – -1.21 | **<0.001** |
| Treatment [Dulled] | | -0.05 | -0.17 – 0.06 | 0.353 |
| Capture [3] | | -0.02 | -0.04 – 0.00 | 0.096 |
| BibB1 | | 0.00 | -0.00 – 0.01 | 0.365 |
| **Random Effects** | | | | |
| σ2 | | 0.04 | | |
| τ00 Band | | 0.05 | | |
| τ00 cpg | | 0.26 | | |
| ICC | | 0.88 | | |
| N Band | | 68 | | |
| N cpg | | 12 | | |
| Observations | | 1401 | | |
| Marginal R2 / Conditional R2 | | 0.004 / 0.883 | | |
| Variance partitioning (repeatability): | |  | | |
|  | Band [CI] | 0.153 [0.081, 0.308] | | |
|  | CpG [CI] | 0.729 [0.491, 0.847] | | |
|  | Fixed effects [CI] | 0.004 [0.001, 0.036] | | |

Table S3: LMM model output predicting methylation of sites in the FKBP5 gene

|  |  |  |  |
| --- | --- | --- | --- |
|  | **FKBP5** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | 0.58 | -0.03 – 1.20 | 0.063 |
| Treatment [Dulled] | -0.11 | -0.36 – 0.14 | 0.382 |
| Capture [3] | -0.10 | -0.14 – -0.06 | **<0.001** |
| BibB1 | -0.00 | -0.01 – 0.01 | 0.943 |
| **Random Effects** | | | |
| σ2 | 0.13 | | |
| τ00 Band | 0.27 | | |
| τ00 cpg | 0.17 | | |
| ICC | 0.78 | | |
| N Band | 69 | | |
| N cpg | 11 | | |
| Observations | 1272 | | |
| Marginal R2 / Conditional R2 | 0.010 / 0.780 | | |
| Variance partitioning (repeatability): | |  | |
|  | Band [CI] | 0.48 [0.345, 0.624] | |
|  | CpG [CI] | 0.298 [0.121, 0.47] | |
|  | Fixed effects [CI] | 0.01 [0.004, 0.075] | |

Table S4: LMM model output predicting methylation of sites in the GR gene

|  |  |  |  |
| --- | --- | --- | --- |
|  | **GR** | | |
| *Predictors* | *Estimates* | *CI* | *p* | |
| (Intercept) | -0.94 | -1.59 – -0.28 | **0.005** | |
| Treatment [Dulled] | 0.10 | -0.01 – 0.21 | 0.075 | |
| Capture [3] | 0.11 | 0.06 – 0.15 | **<0.001** | |
| BibB1 | -0.00 | -0.01 – 0.00 | 0.698 | |
| **Random Effects** | | | | |
| σ2 | 0.19 | | | |
| τ00 Band | 0.05 | | | |
| τ00 cpg | 1.32 | | | |
| ICC | 0.88 | | | |
| N Band | 68 | | | |
| N cpg | 14 | | | |
| Observations | 1537 | | | |
| Marginal R2 / Conditional R2 | 0.003 / 0.878 | | | |
| Variance partitioning (repeatability): | |  | | |
|  | Band [CI] | 0.029 [0.015, 0.064] | | |
|  | CpG [CI] | 0.848 [0.698, 0.914] | | |
|  | Fixed effects [CI] | 0.003 [0.001, 0.013] | | |

Table S5: LMM model output predicting methylation of sites in the GRHR1 gene

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CRHR1** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | -2.52 | -2.97 – -2.08 | **<0.001** |
| Treatment [Dulled] | 0.01 | -0.60 – 0.61 | 0.980 |
| Capture [3] | 0.13 | 0.02 – 0.24 | **0.022** |
| BibB1 | 0.00 | -0.01 – 0.01 | 0.739 |
| Treatment [Dulled] × Capture [3] | 0.11 | -0.06 – 0.29 | 0.209 |
| Treatment [Dulled] × BibB1 | 0.00 | -0.01 – 0.02 | 0.795 |
| Capture [3] × BibB1 | 0.00 | -0.00 – 0.00 | 0.795 |
| (Treatment [Dulled] × Capture [3]) × BibB1 | -0.01 | -0.01 – -0.00 | **0.020** |
| **Random Effects** | | | |
| σ2 | 0.04 | | |
| τ00 Band | 0.08 | | |
| τ00 cpg | 0.25 | | |
| ICC | 0.88 | | |
| N Band | 68 | | |
| N cpg | 19 | | |
| Observations | 2116 | | |
| Marginal R2 / Conditional R2 | 0.010 / 0.884 | | |
| Variance partitioning (repeatability): | |  | |
|  | Band [CI] | 0.212 [0.124, 0.35] | |
|  | CpG [CI] | 0.67 [0.479, 0.797] | |
|  | Fixed effects [CI] | 0.01 [0.007, 0.05] | |

Table S6: Effects of treatment, capture number, and initial brightness on baseline corticosterone

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Baseline cort** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | 3.31 | -0.29 – 6.92 | 0.071 |
| Capture [3] | -0.68 | -2.05 – 0.69 | 0.327 |
| Treatment [Dulled] | -0.27 | -1.88 – 1.33 | 0.737 |
| Initial brightness | 0.03 | -0.05 – 0.12 | 0.453 |
| **Random Effects** | | | |
| σ2 | 13.40 | | |
| τ00 Band | 3.32 | | |
| ICC | 0.20 | | |
| N Band | 69 | | |
| Observations | 119 | | |
| Marginal R2 / Conditional R2 | 0.013 / 0.208 | | |

Table S7: LMM output modeling the relationship between baseline corticosterone and methylation in CRHR1.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CRHR1** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | -2.34 | -2.57 – -2.10 | **<0.001** |
| Baseline cort | -0.01 | -0.01 – -0.00 | **<0.001** |
| **Random Effects** | | | |
| σ2 | 0.05 | | |
| τ00 Band | 0.08 | | |
| τ00 cpg | 0.25 | | |
| ICC | 0.88 | | |
| N cpg | 19 | | |
| N Band | 69 | | |
| Observations | 2129 | | |
| Marginal R2 / Conditional R2 | 0.003 / 0.877 | | |

Table S8: LMM output modeling the relationship between baseline corticosterone and methylation in CRH.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CRH** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | -1.52 | -1.81 – -1.22 | **<0.001** |
| Baseline cort | 0.00 | -0.00 – 0.00 | 0.967 |
| **Random Effects** | | | |
| σ2 | 0.04 | | |
| τ00 Band | 0.05 | | |
| τ00 cpg | 0.26 | | |
| ICC | 0.88 | | |
| N cpg | 12 | | |
| N Band | 69 | | |
| Observations | 1412 | | |
| Marginal R2 / Conditional R2 | 0.000 / 0.882 | | |

Table S9: LMM output modeling the relationship between baseline corticosterone and methylation in FKBP5.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **FKBP5** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | 0.46 | 0.19 – 0.74 | **0.001** |
| Baseline cort | 0.00 | -0.00 – 0.01 | 0.251 |
| **Random Effects** | | | |
| σ2 | 0.13 | | |
| τ00 Band | 0.27 | | |
| τ00 cpg | 0.17 | | |
| ICC | 0.78 | | |
| N cpg | 11 | | |
| N Band | 70 | | |
| Observations | 1283 | | |
| Marginal R2 / Conditional R2 | 0.001 / 0.775 | | |

Table S10: LMM output modeling the relationship between baseline corticosterone and methylation in GR.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **GR** | | |
| *Predictors* | *Estimates* | *CI* | *p* |
| (Intercept) | -0.89 | -1.49 – -0.28 | **0.004** |
| Baseline cort | -0.00 | -0.01 – 0.01 | 0.893 |
| **Random Effects** | | | |
| σ2 | 0.19 | | |
| τ00 Band | 0.05 | | |
| τ00 cpg | 1.32 | | |
| ICC | 0.88 | | |
| N cpg | 14 | | |
| N Band | 69 | | |
| Observations | 1551 | | |
| Marginal R2 / Conditional R2 | 0.000 / 0.877 | | |

Figure : A) Mean methylation across all interrogated CpG sites before and after treatment