Supplementary Material, Methods

Methods

Avian husbandry

We obtained adult male and female zebra finches (*Taeniopygia guttata*) from commercial suppliers across the Tampa Bay area of Florida (2012), and an active breeding colony was maintained at the University of South Florida, College of Medicine. Finches were housed in 15 - 18 free-flight cages in separate sex groups of ~8-10 individuals until experiments began. Finches were later randomly selected from these groups for experiments to minimize biasing treatments with cage-mates. For experimental procedures, birds were individually housed in small cages. Birds were acclimated to these cages for 3 days prior to surgeries and were allowed to recover from surgery for 2 days at the College of Medicine, and were then moved in the same-style cages into the University of South Florida Biosafety Level 3 facility where they acclimated for another 24 h before being exposed to WNV. For the duration of the study, finches were fed a standard seed diet (ABBA 1900 exotic finch food, ABBA Products Corp., Hillside, NJ). Photoperiod was maintained at 13h of light and 11h of dark for the study duration (on at 0600 and off at 1900), and finches were maintained at an average temperature of ~70-72°F and ~50% relative humidity. All experimental procedures were carried out in compliance with approved institutional animal care and biosafety protocols at the University of South Florida.

Corticosterone Implant Surgery and Validation

All implants were sealed with multi-purpose silicone sealant (Dow Corning, Midland, MI, product #732), and just before implantation, a 0.5 mm hole was made through both sides of the implant to facilitate release of the hormone [17]. Implants were administered subcutaneously on the flank of each bird while birds were under light isoflurane anesthesia. After implantation, the < 3 mm wound was sealed with a surgical adhesive (Vetbond, 3M, St. Paul, MN, product #1469). All birds returned to normal activity (perching and feeding) within ~5 minutes of surgery completion. Finches were allowed to recover from surgery for 2 days prior to baseline blood sample collection and were then moved to the ABSL-3 laboratory, where they were acclimated for an additional 24 hours before blood was taken to assess circulating hormone levels. This blood sampling/CORT validation took place immediately prior to inoculation of finches with the virus. We measured CORT concentrations in finch plasma with enzyme immunoassay (EIA) kits (Arbor Assays, Ann Arbor, MI, product # K014-H5). Our surgical implantation approach affected CORT as expected: control birds had significantly lower CORT than CORT-implanted birds, and CORT+ in CORT++ implanted birds had circulating CORT concentrations that varied in a dose-dependent manner (Fig. S1).

WNV quantification

Collected serum samples from finches were immediately frozen at -40°C and viremia was later quantified with real-time qRT-PCR [33]. We extracted viral RNA from serum samples with the QIAmp Viral RNA kit (Qiagen Cat. No. 52906) using 10 ul of serum diluted in 130 ul sterile PBS and according to the kit protocol. We used a one-step Taq-based qRT-PCR kit to quantify the amount of viral RNA in samples (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No.

1725141). Forward and reverse primer sets for WNV 1999 were obtained from the Unnasch lab. Our forward primer sequence was, 5' CAGACCACGCTACGGCG 3'; reverse sequence was, 5' CTAGGGCCGCGTGGG 3'; and our WNV probe sequence was, 5' [6~FAM] CTGCGGAGAGTGCAGTCTGCGAT [BHQ1a~6FAM]. All samples were run in triplicate in qPCR, and a negative control and a known WNV-positive control were run alongside experimental samples in plates. A standard curve was generated from serial dilutions of the stock virus used in finch inoculations, which was tested for viral titer by Vero cell plaque assay [33]. All samples fell within the range of our standard curve.

Cytokine expression quantification

On 2 and 4dpi, 5 - 10µL of whole blood was extracted from the brachial vein of each bird and stored in 500µl of RNAlater at -40°C (i.e., separate aliquots of blood were taken from one sample/bird). Some was used to estimate viremia only, as above, and some to estimate cytokine expression only, described below). RNA extractions were performed using the Fisher BioReagents™ SurePrep™ Leukocyte RNA Purification kit (Fischer Catalog No. BP280750). The extracted RNA was used as the template for SYBR Green qRT-PCR [iTaq™ Universal SYBR® Green One-Step kit (Bio-Rad Catalog No. 1725151)]. For more information about cytokine expression quantification, see electronic supplementary material, Methods.

Forward and reverse primers (see supplementary materials) were developed using Primer3 Plus and Primer-BLAST from predicted mRNA sequences for zebra finch interleukin-10 (NCBI Reference Sequence: XM_002194605.1) and interferon gamma (NCBI Reference Sequence: XM_002188923.1). The qRT-PCR set-up consisted of a standard curve (derived from

zebra finch liver RNA) run in triplicate and the samples and negative controls (no RNA template) run in duplicate.