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Chronic stress raises baseline circulating corticosterone and reduces vocal plasticity in male budgerigars, an avian model for adult vocal learning

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ABSTRACT

Chronic stress affects cognitive function across many domains, including memory, decision making and learning. While the effects of early-life stress on vocal learning in juveniles are well-demonstrated in both humans and songbirds, less is known about how stress experienced by adults affects their ability to learn new vocalizations or the neural substrates that underlie this behavior. We investigated the effects of chronic stress on the production and learning of contact calls, and on the expression of a key learning related gene, FoxP2, in the vocal learning circuit in adult budgerigars (*Melopsittacus undulatus*), a small parrot with open-ended vocal learning. We induced chronic stress via unpredictable disturbances in the captive environments of nine newly-formed replicate flocks of 4 adult male budgerigars who were previously unfamiliar to each other. We then recorded calling behavior daily and measured weight, breath rate, and baseline and stress response levels of circulating corticosterone weekly. At the end of the experiment brains were collected to examine mRNA and protein levels of the gene FoxP2 in the vocal learning region magnocellular nucleus of the medial striatum (MMSt) using qPCR and immunohistochemistry. Physiological measures of stress consistently showed stronger responses in birds subjected to the highest level of disturbance than those in the medium or baseline control treatments, although only differences in baseline corticosterone were detected among treatments. We used machine learning approaches to map calls onto a shared acoustic space to assess four measures of vocal behavior and learning: vocal output (the number of contact calls produced), vocal diversity (the amount of acoustic space occupied by the calls of an individual), vocal plasticity (the amount of change in acoustic space over time) and vocal convergence (the degree of overlap between an individual's calls and the calls of its group). Birds in the high stress treatment showed higher vocal output and lower vocal plasticity than those in medium stress or baseline control groups, but there were no differences among treatments in vocal diversity or vocal convergence. There were no differences detected among treatments in expression levels of either FoxP2 mRNA or protein, perhaps due to the timing of neural sampling relative to the behavioral measures. These results suggest that, as seen in juvenile learning, chronic stress can negatively impact vocal learning in adults via changes in patterns of circulating corticosterone.

Stress, broadly defined as the physiological impacts of perturbations to homeostasis, is common in the lives of wild organisms. Stressors such as extreme weather, infection, predation threat, anthropogenic impacts and social competition can disrupt homeostasis such that organisms experience altered energetic or other physiological demands (Romero and Wingfield, 2015). In vertebrate animals, physiological responses to such stressors are regulated in part by the hypothalamic-pituitary-adrenal (HPA) axis via the release of glucocorticoid hormones (Sapolsky et al., 2000). These chemical messengers help regulate internal energy use and external behavior by binding to mineralcorticoid (MR) and glucocorticoid (GR) receptors expressed on the cell membranes of target tissues. These receptors, when activated, will initiate

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both rapid nongenomic effects and, when translocated to the nucleus, act as transcription factors that bind directly to glucocorticoid response elements on DNA and alter cellular gene expression (de Kloet, 2022; Joëls et al., 2012; Koning et al., 2019). In the short term, during responses to short-lived stressors, these physiological responses are typically beneficial as they help animals reallocate energy and other resources in order to regain homeostasis. In the longer term, in the face of chronic stressors, however, these responses can have negative effects on the organism (de Kloet, 2022), a situation sometimes termed "homeostatic overload" (See Fig. 1 in Romero et al., 2009).

One arena in which the detrimental effects of chronic stress can be seen is in cognitive function. The brain is central to the perception and integration of information about stressors in the external environment, and for directing behavioral responses to these stressors (Romero and Wingfield, 2015). Consequently, the vertebrate brain is one of the primary targets of the glucocorticoid hormones and shows wide expression of MR and GR, including in the hippocampus, amygdala and pre-frontal cortex (Deppermann et al., 2014; Koning et al., 2019; McEwen, 2008). The brain is also a major consumer of energy within the organism and provides regulation of the HPA axis via the hippocampus (Lupien et al., 2009). These multiple demands create the potential for conflicts and tradeoffs between different functions within the brain, particularly when an organism experiences chronic stress. There is considerable evidence from rodents and humans that sustained elevation of glucocorticoids can have negative effects on learning and memory (de Kloet et al., 1999; Deppermann et al., 2014; Dumas et al., 2010)(Het et al., 2005). Interestingly, there is evidence from both rodents and humans that, in some cases, these effects are non-linear, such that intermediate levels of stress can have beneficial effects on learning and memory, while lower or higher levels are detrimental. This phenomenon, which was first observed by Yerkes and Dodson in 1908, is sometimes termed the "inverted U-shaped effect" or the "Yerkes and Dodson Law" (Lupien et al., 2007; Yerkes and Dodson, 1908). This pattern may arise from the differential effects on gene expression of the high affinity MR, which are occupied first by glucocorticoids, compared to the low affinity GR, which become occupied at higher glucocorticoid levels after the MR become saturated (de Kloet et al., 1999; Deppermann et al., 2014; Joëls, 2018). If these two types of glucocorticoid receptors have opposing effects on learning-related genes, then non-linear responses in learning could result with increasing glucocorticoid levels (de Kloet, 2022; Dumas et al., 2010). For example, if MR activation generally promotes the expression of genes that enhance learning but GR activation generally represses those same genes, then low to moderate levels of circulating glucocorticoids that primarily bind MR would promote learning while higher levels of circulating glucocorticoids that increasingly bind GR would diminish learning, resulting in an inverted U-shaped effect on learning measures.

One cognitive domain that shows distinctive effects of chronic stress in humans is language learning and comprehension. Children who experience childhood stress via poverty or trauma typically show elevated levels of cortisol and diminished verbal abilities and language learning than non-stressed peers (Blair et al., 2011a; Blair et al., 2011b; Malarbi et al., 2017; Pierce et al., 2021). Similar effects on vocal learning and production are observed in bird species that learn their vocalizations early in life. Work in songbird models for vocal learning has demonstrated that various stressors, including nutritional deprivation, parasitic infection, increased clutch size, and application of exogenous corticosterone (the primary glucocorticoid in birds) can have effects on vocal learning and its neural substrates in juveniles that can persist into adulthood. These effects vary between species and stressor type, but include changes in learning, memory, and production of adult song (Bell et al., 2018; Buchanan et al., 2004; Buchanan et al., 2003; Buyannemekh et al., 2020; Nowicki et al., 2002; Schmidt et al., 2012; Schmidt et al., 2014; Schmidt et al., 2013; Sewall et al., 2018; Spencer et al., 2003, 2004; Spencer et al., 2005). These effects are correlated with changes in the size and density of dedicated vocal learning nuclei and patterns of neural gene expression (Buchanan et al., 2004; Honarmand et al., 2016; Nowicki et al., 2002; Schmidt et al., 2014; Schmidt et al., 2013; Sewall et al., 2018). Importantly, both MR and GR have been found to be widely expressed in the song control system of songbirds, providing a mechanistic link between stress, hormones, and the observed changes in vocal learning and associate brain regions in juveniles (Shahbazi et al., 2011; Suzuki et al., 2011).

It is less clear what effects chronic stress might have on vocal learning during the adult stage in humans. While primary language learning occurs during childhood, adult language learning does occur in some populations, including immigrants (Pandey et al., 2021; Söndergaard and Theorell, 2004) and recipients of cochlear implants (Pisoni, 2014; Tomblin et al., 2007). There is some evidence that chronic stress is associated with acculturation and rate of second language learning, although effects are varied and the directionality of causality can be difficult to determine (Scholaske et al., 2021). However, it remains unclear whether chronic stress has the same long-term impacts on language learning in adults as it has in children, or if, alternatively, adult systems are more resilient to this stress. This gap arises in part because the predominant songbird models for vocal learning are closed-ended learners that learn new vocal signals as juveniles but not as adults (Bolhuis et al., 2010; Doupe and Kuhl, 1999).

Parrots provide a useful animal model for the study of adult vocal learning. Like humans, parrots of both sexes can learn new vocalizations throughout their adult life and often do so in response to joining new social groups (Dahlin et al., 2014; Farabaugh et al., 1994; Salinas-Melgoza and Wright, 2012). One parrot, the budgerigar, *Melopsittacus undulatus*, is a particularly tractable laboratory subject due to its small size (~35 g) and facile breeding. It has become the favored parrot model for studies of hearing, vocal development and neurobiology (Brittan-Powell et al., 1997; Dahlin et al., 2014; Farabaugh and Dooling, 1996; Haesler et al., 2004; Hile and Striedter, 2000; Matsunaga et al., 2011; Striedter, 1994). Contact calls are the most common call used by budgerigars; each individual will have a repertoire of several contact call

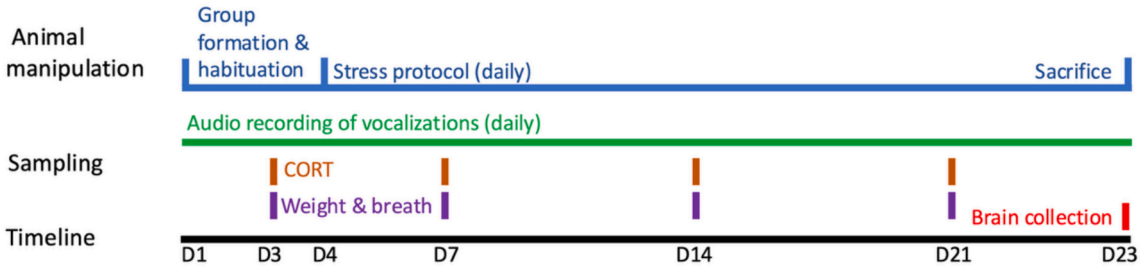

Fig. 1. Experimental timeline showing the timing of animal manipulations (including habituation and stress treatments periods) and sampling for vocalizations, social behavior, weight, corticosterone (CORT in the figure), and brain collection. Birds are introduced to cages on Day 1, and stress treatments start on Day 4. Birds are captured for corticosterone sampling and stress measures on Day 3, 7, 14 and 21, corresponding to sampling weeks 1–4. Birds are sacrificed for brain collection on Day 24.

types that are shared with others in their social group. Birds that change social groups quickly learn to match the calls of their new group via imitation, providing a strong assay for adult vocal learning (Bartlett and Slater, 1999; Dahlin et al., 2014; Farabaugh et al., 1994; Hile et al., 2000; Hile and Striedter, 2000). The neural circuitry underlying learning in parrots is well characterized and shares many similarities with both songbirds and humans (Feenders et al., 2008; Pfenning et al., 2014; Striedter, 1994). Work in nestling green-rumped parrotlets (*Forpus passerinus*) has demonstrated that this vocal learning circuit is responsive to changes in exogenously-administered corticosterone (Eggleston et al., 2022; McLean et al., 2025). Finally, there are similarities in patterns of gene expression in the neural pathways for vocal learning between parrots, songbirds and humans, including a prominent role for the language-related gene FoxP2 (Fisher and Scharff, 2009; Hara et al., 2015; Teramitsu et al., 2010; Whitney et al., 2014; Whitney et al., 2015). Budgerigars thus offer a novel route to improve our understanding of the effects of chronic stress on adult vocal learning and underlying gene expression.

In this study, we used budgerigars to explore the impacts of chronic stress on vocal learning in adults. We used an established protocol of unpredictable disturbances in the captive environment (Gormally et al., 2018) to create chronic stress at baseline control, medium and high levels in newly-formed flocks of budgerigars. We then measured a suite of physiological markers of stress on a weekly basis, including weight, breath rate, and baseline and stress-response circulating corticosterone, and recorded changes in the vocal repertoire of individuals over the three-week stressor protocols to assess several dimensions of vocal learning. At the conclusion of the experiment, we measured levels of FoxP2 protein and mRNA in a primary vocal learning center, the magnocellular nucleus of the medial striatum (MMSt), a striatal region thought to be functionally similar and potentially homologous to the songbird Area X (Striedter, 1994). These data were used to test whether chronic stress negatively impacts vocal learning in adults, and whether these effects are linear or show an inverted U-shaped effect.

1. Methods

1.1. Experimental subjects

Subjects for this experiment were 36 adult male budgerigars, 27 of which were acquired from a commercial breeder, McDonald Bird Farms (Kerrville, Texas) and 9 of which were bred in our research colony from parents acquired from the same breeder. We sourced birds from the commercial breeder from 3 aviaries housed in different buildings; in concert with our research colony, these represented 4 independent source populations, each of whose members were unfamiliar to birds from the other 3 populations. We housed these 4 populations in separate rooms at the New Mexico State University Animal Care Facility (NMSU-ACF) and maintained on a 12:12 h light:dark cycle and provided water and commercial pellets ad libitum. Although the precise hatching date was not known for all individuals, we morphologically confirmed all birds to be full adults based on plumage characteristics and iris color. We confirmed sex from blood samples via PCR using the P0-P2-P8 avian sexing primers (Han et al., 2009). Female budgerigars are capable of vocal learning but typically have smaller contact call repertoires and may learn more slowly than males (Hile et al., 2000; Hile and Striedter, 2000). Although sex differences in learning are of considerable interest, here we focused on learning in single-sex male groups to avoid potential confounds of different learning capacities and of mating relationships between the sexes. All care and procedures were approved by the NMSU Institutional Animal Care and Use Committee (protocols 2019-011 and 2021-008) and adhere to the National Institutes of Health's standards as detailed in the *Guide for the Care and Use of Laboratory Animals*.

1.2. Experimental design

We used our 4 independent populations to create 9 replicate novel social groups of 4 individuals, each consisting of 1 individual from each source population (36 birds in total). Members of each group were thus unfamiliar with each other, and with each other's vocal repertoire, prior to group formation. This design created a situation where the 4 members of each group were motivated to learn each other's contact calls in order to establish the patterns of contact call sharing typically seen in groups of budgerigars (Dahlin et al., 2014; Farabaugh et al., 1994; Hile et al., 2000; Hile and Striedter, 2000). We formed these groups on Day 1 of our experimental timeline (Fig. 1) and randomly assigned to one of three chronic stress treatments: high, medium, and baseline control stress, as detailed below. We moved each group to its own holding room within the NMSU-ACF where it was housed in a standard holding cage (78 cm wide by 52 cm deep by 135 cm tall) and groups were allowed to habituate to new individuals for 3 days prior to initiating chronic stress exposure treatments on Day 4. Vocal repertoires of birds were recorded daily following procedures described below starting on Day 1 and continuing throughout the 23-day timeline except on days where physiological stress measures were collected. On Day 3, we captured all birds for the first collection of physiological stress measures (baseline measurements) as detailed below; subsequent collections occurred on Days 7, 14 and 21. On Day 4 the experimental stress protocol began and ran daily through the remainder of the experiment until birds were collected on Day 23 to quantify neural gene expression (below). We repeated this timeline for 3 rounds, with each round consisting of 1 group in each of the 3 chronic stress treatments (9 groups total, 3 in each treatment).

1.3. Chronic stress protocol

We followed an established protocol that uses unpredictable minor disruptions in the captive environment to induce chronic stress (Gormally et al., 2018; Lattin and Romero, 2014). These disruptions consisted of lab members i) walking around the room and looming over the bird cage, ii) rolling the cage around the holding room, iii) tapping on the cage, iv) placing a hand in the cage, v) playing a clip of predator sounds from the internet (https://www.youtube.com/watch?v=xneiSfKk0Lo&t=21s), vi) playing radio music, or vii) reading to the birds. Disruptions occurred daily from Day 4 to Day 22 in a randomized order for 30 minute blocks, with blocks randomly scheduled between 10:30 and 18:00. Groups assigned to the high stress treatments received 5 sessions per day, groups in the medium stress treatment received 3 sessions per day, and groups in the control baseline treatment received no disruptions beyond daily care and recording sessions and weekly physiological measures. Investigators wore lab coats and animal face masks during stress sessions to avoid birds becoming sensitized to their presence during recording sessions and routine care.

1.4. Physiological measures of stress

We captured all birds on Days 3, 7, 14, and 21 to collect physiological measures of stress. We captured birds by hand between 06:30 and 07:00 (0.5–1 h after lights on) and collected 50–100 μl of whole blood from the brachial vein into heparinized capillary tubes (Fisherbrand, Fisher Scientific) within 5 min of entry to the room to assess baseline levels of corticosterone. Our median time to baseline bleed was 95 s, and over 90 % of our baseline bleeds were collected in under 3 min. We then measured weight and breath rate over 1 min of manual confinement, and placed birds individually into a dark cloth bag until 30 min had elapsed from initial blood collection. We then collected a second sample of 50–100 μl of whole blood to measure stress response corticosterone and returned birds to their group cages.

1.5. Corticosterone analysis

Plasma was separated from whole blood by centrifuging the capillary tubes for 5 min at 1200 RPM and then extracting with a Hamilton syringe before storing at −20 °C until corticosterone analysis. We conducted analyses using Arbor Assays Corticosterone ELISA kits (K014-H5, Arbor Assays,) and following methods described in Duckworth and Jawor (Duckworth and Jawor, 2018) and Ramos-Güivas et al. (Ramos-Güivas et al., 2021). We extracted corticosterone using kit-supplied steroid displacement buffer following manufacturer instructions; 5 μl of plasma added to 5 μl of disassociation reagent and diluted 1:100 with the kit-supplied assay buffer immediately prior to performing the assay. In the ELISA assay, 50 μl replicates from each extracted sample were incubated with kit-supplied capture and detection antibodies, this was followed by four rounds of wash using kit-supplied wash buffer (300 μl per round). Following a second incubation with TMB substrate, plates were read at 450 nm on a plate reader (BioTek Epoch2; Agilent Technologies) and corticosterone content estimated by comparison to a standard curve using kit-supplied materials and directions. All samples from an individual were analyzed on the same assay plate while individuals and treatments were randomized across plates. We determined intra and inter-assay variation using a plasma pool obtained from a northern cardinal (*Cardinalis cardinalis*) population with aliquots from this plasma pool treated similarly to experimental samples. Intra-assay averaged 20.2 %, with values ranging from 13.0 % to 31.3 % across plates. Inter-assay was 34.6 % and was calculated as the variation in intra-assay CVs across plates (n = 9).

1.6. Vocal recording and acoustic analysis

Each bird was individually recorded for 50 min daily using an "odd bird out" protocol in which the bird was removed from its group cage and placed in a small wire cage (16 cm by 19 cm by 11 cm) inside of an acoustic isolation chamber (50 cm by 28 cm by 22 cm) located next to the group cage. These isolation chambers were constructed from commercial coolers (Igloo) lined with acoustic foam and with a clear plexiglass door that allowed the isolated bird visual contact with their remaining 3 flockmates but attenuated any calls made by those flockmates during recording sessions sufficiently to allow automated identification and segmentation of the target bird's calls. Birds were recorded in a randomized order within a 4-hour block between 6:30 and 10:30 on 6 days of the week, with stress measures taken on the seventh day. The calls were recorded using Audio-Technica Pro 37 microphones powered by a Focusrite SaffirePro 40 pre-amplifier connected to a PC running the Sound Analysis Recorder module in Sound Analysis Pro (Tchernichovski et al., 2000). We recorded continuously at a sampling rate of 44.1 kHz with the recording stream partitioned into serial 5-minute sections and saved in separate files to the PC hard disk. After each recording session we uploaded all files from the session to a Synology Rack Station network attached storage device.

Calls were detected using automated amplitude-based detection in package ohun (Araya-Salas et al., 2022) in R (R Core Team, 2022) and optimizing detection parameters based on a manually annotated subset of acoustic data. We then trained a supervised random forest classification model to distinguish contact calls from other call types and cage noises, in order to mitigate incorrect detections. We used the random forest implementation from the R package ranger (Wright and Ziegler, 2017) to identify contact calls based on spectro-temporal features and statistical descriptors of Mel-frequency cepstral coefficients (MFCCs). The acoustic features were measured with the R package warbleR (Araya-Salas and Smith-Vidaurre, 2017). Detections were then exported into Raven Pro 1.6 (Cornell Lab of Ornithology, 2022) using the R package Rraven (Araya-Salas, 2020) for visual screening of spectrograms, manual annotation of missed calls and removal of incorrect detections. Most incorrect detections consisted of budgerigar calls of types other than contact calls, which share some acoustic similarity to contact calls. Detections were then imported back into R for further analysis using the package Rraven.

We measured 17 acoustic features related to the distribution of energy in the frequency and time domains and the variation in dominant frequency contours to characterize the structure of contact calls (Fig. S1). Acoustic features were obtained using the function *spectro_analysis* in the R package warbleR (Araya-Salas and Smith-Vidaurre, 2017). We applied the dimensionality reduction algorithm t-SNE (Van der Maaten and Hinton, 2008) on the z-transformed acoustic parameters of calls to estimate a bi-dimensional latent space representing variation in the structure of contact calls (hereafter 'acoustic space'). Latent acoustic spaces are useful tools for quantifying structural diversity of vocal repertoires (Keen et al., 2021); we used tSNE to create this latent space because its improved ability to map local relationships over linear scaling approaches like classical scaling or Principle Components Analysis (Van der Maaten and Hinton, 2008). In the latent acoustic space, each observation (i.e. each point) represents a call and the distance between observations indicates their acoustic similarity. t-SNE was run with the R package Rtsne (Krijthe, 2015) with a maximum number of iterations set to 5000 and a perplexity value of 30. The bi-dimensional acoustic space was then used to calculate 3 parameters related to features of the individual's vocal repertoire: 1) *vocal diversity*: acoustic space area (weekly from week 2 to 4, normalized by week 1), 2) *vocal plasticity*: the change in acoustic space overlap of an individual's current repertoire compared to its starting repertoire across time (weekly from week 2 to 4, each compared to week 1); and 3) *vocal convergence*: acoustic space overlap of the individual to its group acoustic space over time (weekly from week 1 to 4). We also calculated *vocal output* as the number of calls produced by each individual during recording sessions in Weeks 1 through 4. We measured acoustic space area as the 95 % probability density area estimated with the bivariate normal kernel method (Silverman, 1986). Acoustic space overlap was quantified as the mean of the proportions of the space areas that overlap (i.e. the mean of the proportion of A overlapping B and B overlapping A). The degree of overlap was weighted by the density of the overlapping regions, such that overlap was higher when it included denser areas. The three acoustic space features were rarified to have the same number of observations across individuals. The number of observations was set to the smallest sample size of any combination of individual-week. This procedure was iterated 30 times, selecting random data subsets on each iteration. The final acoustic space features were calculated as the mean value across iterations. Acoustic space features were measured with the functions *rarefact_space_similarity* (space overlap) and *rarefact_space_size* (space area) from the R package PhenotypeSpace (Araya-Salas and Odom, 2022).

1.7. Measurement of neural FoxP2 protein and mRNA

On Day 23 of the experimental timeline, we euthanized birds via an overdose of inhaled isoflurane followed by decapitation. Their brains were extracted and flash frozen on liquid nitrogen within 5 min and then stored at −80 °C until processing. Brains from six randomly selected individuals from each of the three treatments were selected for examination of neural expression of FoxP2 protein, while *FoxP2* mRNA expression was examined in four individuals in each of the high stress and control treatments (note FoxP2 refers to non-human form of the protein, while *FoxP2* refers to the non-human form of the gene). These samples were sectioned on a cryostat (Leica CM1850. Leica Microsystems) at −20 °C in 20 μm sections in the coronal plane in a series of 5 slides. Sections were thaw-mounted in series onto positively charged slides (Superfrost Plus, Fisher Scientific) and stored at −80 °C. For the mRNA samples, tissue punches were collected during slicing directly from the exposed portions of MMSt and adjacent ventral striatal and pallial layers (VSP) of the unsliced brain using a 26 gauge Luer stub inserted to a depth of 2 mm (Burkett et al., 2018). One series from each individual was Nissl stained with thionin dye to locate target brain

regions on adjacent sections.

We conducted immunohistochemistry to assess levels of FoxP2 protein in the MMSt and VSP of 18 randomly selected individuals from the three treatments following validated protocols for this species (Hara et al., 2015; Whitney et al., 2015). Briefly, selected tissue sections were first fixed on the slide in 4 % paraformaldehyde. Tissues were then washed and incubated for 1 h in 5 % sheep serum solution with phosphate buffered saline plus 0.3 % Triton-X, then incubated overnight at 4 °C with FoxP2 primary antibodies (Mouse, 1:500, 5C11A2, ThermoFisher Scientific). Slides were then washed and incubated for 2 h with Alexa Fluor 594 secondary antibody (Donkey Anti-Mouse, 1:200) at room temperature before a final wash. Sections were then stained with Vectashield DAPI (Vector Labs). The same procedure without primary antibody was used as a negative control.

Sections were imaged using a confocal microscope (Leica TCS SP5 237 II, Leica Microsystems) with images taken of at least four sample sections per bird of both MMSt and the neighboring VSP region not thought to be involved in vocal learning. Cells were quantified using ImageJ (NIH) and counts performed by hand using the cell counter plugin. The number of FoxP2 expressing cells was divided by the number of DAPI stained cells for both MMSt and VSP, and the mean ratio of MMSt to VSP FoxP2 expression for each individual was calculated to assess the degree to which FoxP2 was differentially expressed in the striatal vocal learning center MMSt relative to surrounding striatal tissue not involved in vocal learning, as performed in previous studies of FoxP2 expression in vocal-learning birds (Hara et al., 2015; Teramitsu et al., 2010).

We used quantitative PCR to assess levels of *FoxP2* mRNA in the tissue punches from MMSt and VSP of 4 individuals each from the control and high stress treatments. mRNA was prepared from approximately 1 mg of brain tissue preserved in 50 μl of RNALater. Briefly, tissue was homogenized with Qiagen Tissue Lyzer II set at 20 Hz for two rounds of 2 min with a 5 mm stainless steel bead purchased from Qiagen in 350 μl of buffer RULT provided by the RNeasy UCP micro kit in an RNase-free 2 ml microcentrifuge tube. Extracts were then treated with Qiagen's RNase-Free DNase set following the manufacturer's protocol. RNA was quantified with Qubit RNA HS assay kit (Invitrogen) with Invitrogen's Qubit Flex fluorometer. We prepared cDNA from 200 ng of RNA following the manufacturer's protocols from the iScript cDNA Synthesis or the Reverse Transcription Supermix kits (BioRad) in a Bio Rad C1000™ thermocycler. qPCR was performed in a CFX Connect qPCR thermocycler (Biorad) using PowerUp™ SYBR™ Green Master Mix following the manufacturer's protocol for a 25 μl reaction volume. We used primers previously designed to quantify *FoxP2* levels in avian brain tissue: forward 5′-CCTGGCTGTGAAAGCGTTTG-3′ and reverse 5′ATTTGCACCCGACACTGAGC-3′ (Burkett et al., 2018; Olias et al., 2014). We used *TFRC* (transferrin receptor protein 1) as a reference gene with primers: forward 5′- GGAACTTGCCCGTGTGATC-3′ and reverse 5′-GTAGCACCCACAGCTCCGT-3′ (Olias et al., 2014). Cycling conditions were 50 °C for 2 min, 95 °C for 2 min, then 40 cycles of 95 °C for 15 s and 6 °C for 1 min. After 40 cycles a final melt curve was performed starting at 65 °C ramping to 95 °C for 30 s per degree. All reactions were run in triplicate and *FoxP2* expression was quantified relative to *TFRC* and normalized to controls using the $2^{-\Delta\,\Delta CT}$ method (Livak and Schmittgen, 2001). Samples representing different individuals, brain regions, and treatment status were randomly distributed across plates to minimize batch effects. As with protein expression, we calculated the differential expression of FoxP2 in MMSt by taking the ratio of expression in MMSt to VSP.

1.8. Statistical analysis

We used Bayesian general linear mixed models to assess the effect of stress treatments on the four physiological measures of stress (weight, breath rate, baseline corticosterone and stress response corticosterone), the four parameters characterizing vocal behavior (vocal output, vocal diversity, vocal change and vocal convergence), and the two measures of FoxP2 expression (FoxP2 protein positive cells and *FoxP2* mRNA expression). All models were run in Stan (Stan Development Team, 2021) via the brms R package (Bürkner, 2017). For model estimation, we used four Hamiltonian Monte Carlo chains, each with 50,000 warm-up iterations, which were discarded to allow the chains to stabilize, followed by 50,000 sampling iterations used for inference. This extensive sampling ensured reliable convergence to the posterior distribution. We applied weakly informative priors—normal(0, 5) for coefficients, normal(0, 10) for intercepts, Student-t(3, 0, 10) for variances, and gamma/inverse-gamma for shapes—to regularize estimates and improve model stability without strongly influencing the results. Physiological measures and vocal diversity were analyzed as z-transformed change scores to standardize the scales and focus the analysis on change from baseline. Separate models were run for each response variable. The models for physiological and vocal parameters included treatment and z-transformed week (as a continuous predictor) as fixed effects, with individual as a random intercept (varying intercept) to account for repeated measures. The FoxP2 models included only treatment as a fixed effect. To appropriately model the different types of data, we selected specific likelihood functions: physiological data were fitted with a student-t distribution for robustness to outliers; vocal parameters used a beta distribution (suitable for proportional data) except for vocal output, which used a negative binomial for overdispersed count data; and FoxP2 data used a gamma distribution for positive, continuous measurements. Model convergence and performance were rigorously checked. The potential scale reduction factor ($\hat{R}$) was kept below 1.01 for all parameters, indicating successful convergence. The effective sample size was above 100 per chain for all parameters, ensuring precise estimates, and the number of divergent transitions was below 1 %, confirming the sampler explored the posterior distribution effectively. We present effect sizes as median posterior estimates with 95 % Highest Posterior Density Intervals. Parameters in which uncertainty intervals did not include zero were regarded as having an effect on the response variable. We examined the pairwise correlations between each of the four physiological variables and the four vocal behavior variables measured for each individual at Week 4, and the values for FoxP2 protein expression for each individual, using Pearson correlations.

2. Results

2.1. Physiological measures of stress

Birds in the high stress treatment showed higher baseline corticosterone levels relative to pre-experimental levels over the course of the three-week stress protocol than did birds in the medium stress or baseline control treatments (Fig. 2c, note 95 % uncertainty intervals of effect of high stress treatment do not overlap zero). Although not statistically significant, similar patterns were seen in the other three physiological measures, with birds in the high stress treatment trending towards higher breath rates, lower mass, and lower stress response corticosterone relative to pre-experimental levels than the baseline controls (Fig. 2a, b, d). There was also a detectable effect of week on breath rate but not the other three variables (Fig. 2). Raw values for the four physiological measures of stress (i.e. not corrected for values in Week 1) are illustrated in the Supplementary Materials (Fig. S2).

2.2. Measures of vocal output and vocal learning

Automated detection identified 86,344 putative contact calls. After random forest filtering and manual validation, we retained 13,409 contact calls from our 36 birds. The number of calls per individual ranged from 6 to 1693, with a mean ± SE of 372.5 ± 69.3 calls per individual. Preliminary data analysis suggested that sample sizes smaller than 30 were inadequate to characterize change in the vocal repertoire of an individual over the course of the experiment; for this reason we

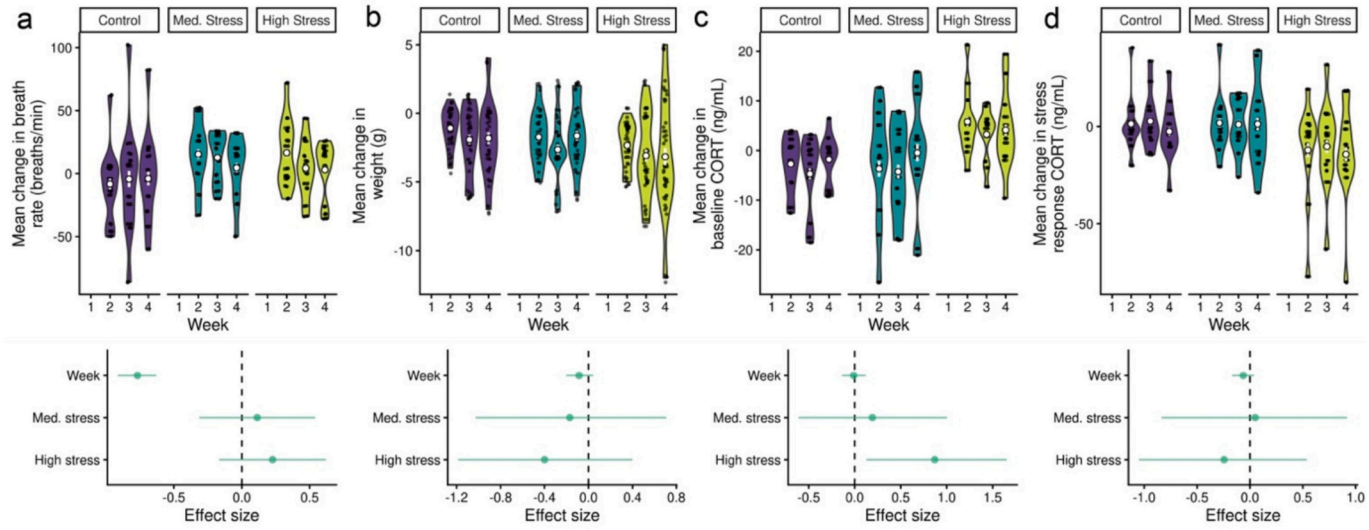

Fig. 2. Changes in physiological measures by week in the three stress treatments (purple = baseline control, blue = medium stress, green = high stress treatment). a) breath rate (breaths/min), b) weight (g), c) baseline corticosterone (abbreviated as CORT in axes labels) (ng/ml), and d) stress-response corticosterone (ng/ml). The top row shows the raw values relative to levels in Week 1, prior to the onset of the stress protocol, illustrated as violin plots with mean ± 1 SE in white and individual points in black. The bottom row shows effect sizes for week, medium stress treatment compared to control, and high stress treatment compared to control (mean and 95 % uncertainty interval). Effects for which the uncertainty interval does not overlap zero are considered to have an effect on the response variable.

dropped five individuals from further vocal analysis (4 in the control and 1 in the medium stress treatments) leaving a sample of 13,329 calls from 31 individuals (mean ± SE 430 ± 75.6 calls per individual) for automated measurement using warbleR. Ordination of these calls in two-dimensions using the 17 acoustic parameters (Fig. S1) and t-SNE resulted in a single global acoustic space that represented the calls for all 31 individuals over the three weeks of the experiment (Fig. 3). The distribution of calls from birds in each of the three stress treatments exhibited no differences in the portion of acoustic space occupied (i.e. no consistent acoustic differences between treatments) with an average density-weighted overlap of 93 % (range: 90–97 %) between treatments. There was, however, a striking difference in the number of calls produced by birds in the different treatments, with birds in the high stress treatment producing significantly more calls than the control birds, and birds in the medium stress treatment producing an intermediate level of calls (Fig. 4a).

From this global acoustic space map, we then extracted the regions of acoustic space occupied by each individual for each week. These regions were used to characterize the individual vocal repertoire for each week of the experiment and used to calculate our three measures of individual vocal learning: *vocal plasticity, vocal diversity,* and *vocal convergence* (Fig. 3b shows representative samples of individuals showing low and high plasticity). We found no difference among the three treatments in *vocal diversity*, measured as the area of acoustic space occupied by an individual's calls, relative to week 1 of the experiment (e.g. uncertainty intervals for treatment effects overlapped zero, Fig. 4b). We did find an effect of treatment on *vocal plasticity*, with birds in the high stress treatment showing greater overlap to their own acoustic space in week 1 (e.g. less plasticity) than birds in the control treatment; birds in the medium stress treatment did not differ in vocal plasticity from the control group (Fig. 4c). There was no difference among the three treatments in the degree of vocal *convergence*, as measured by the degree of overlap of an individual's acoustic space to that of its flock (Fig. 4d).

2.3. Measurements of FoxP2 in the vocal learning center MMSt

Levels of FoxP2 mRNA or protein expression in the striatal vocal learning region MMSt relative to the adjacent VSP did not differ between treatments. There was no difference between the high stress or medium stress and the control groups in the expression levels of the FoxP2 protein as measured by IHC (Fig. 5, all credible intervals for effect sizes include zero). Likewise, there was no difference between the high stress treatment and the controls in the level of FoxP2 mRNA expression measured by qPCR (Fig. 5). The raw values for FoxP2 protein and mRNA expression in MMSt are also illustrated (Fig. 5).

2.4. Individual correlations between physiological, behavioral and gene expression

Pairwise individual correlations between the physiological measures, the vocal behavior measures and FoxP2 protein expression showed a negative correlation between vocal convergence and vocal plasticity ($r = -0.77$), and a positive correlation between vocal convergence and FoxP2 expression ($r = 0.83$). All other correlation coefficients were less than 0.75 (Fig. S3).

3. Discussion

Chronic stress is known to impact multiple aspects of cognitive function, particularly when experienced early in life. In this study we examined the effects of chronic stress on vocal learning in adults using the budgerigar, a parrot model for adult-stage vocal learning. Our protocol of unpredictable disturbances in the captive environment produced demonstrable effects on some physiological measures of stress and on some measures of vocal output and learning, with birds in the high stress treatment showing higher levels of baseline circulating corticosterone, higher vocal output, and lower vocal plasticity than birds in the control group. Both physiological measures and vocal learning measures were generally intermediate for the medium stress group. We did not detect an effect of stress treatment on expression of either protein or mRNA of FoxP2, a gene thought to influence neural plasticity in vocal learning birds. Below we discuss each of these results in more detail and compare to prior results in budgerigars and other avian systems.

3.1. Effects of disturbance regime on stress physiology

Our stress protocol featured a menu of minor stressors to which birds were subjected in a randomized order and at randomized times of the day. Our treatments differed only in the number of stressors they

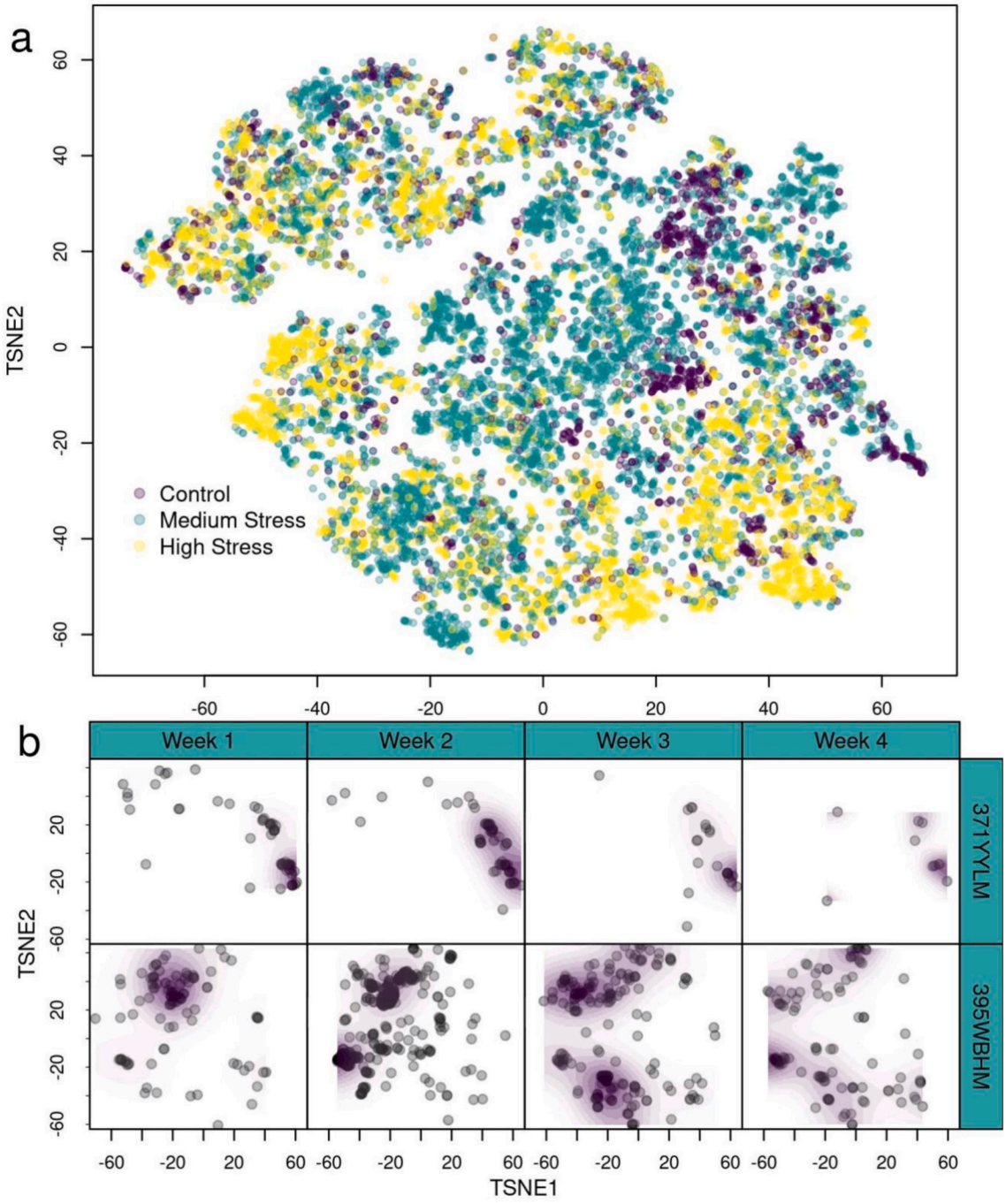

Fig. 3. a) Acoustic space for all 13,329 calls recorded in the study, coded by the stress treatment: purple = control, blue = medium stress, green = high stress. b) Representative illustration of the change in acoustic space for two individuals. Bird 371YYLM (top row) from the high stress treatment remained stable in acoustic space over the 4 weeks of the experiment, while bird 395WBHM from the medium stress treatment showed plasticity in acoustic structure over the experiment.

received each day, with our high stress bird receiving five stressors, the medium stress treatment receiving three, and the baseline controls receiving none. This protocol impacted measures of the physiological stress response in subject birds across the four-week experiment. This impact was most apparent in baseline levels of corticosterone, which were higher in the high stress treatment than in the medium stress treatment or the baseline controls. Although no treatment effect was detected for our other three measures, breath, weight, and stress response corticosterone, similar patterns were seen in each, with the high stress treatment showing the greatest difference from baseline controls. Absolute levels of both baseline and stress response corticosterone in our baseline controls (Fig. S2) were similar to those seen in budgerigars housed under similar conditions and not subjected to chronic stressors (Medina-Garcia et al., 2017).

These patterns indicate that our protocol of unpredictable disturbances, which was adapted from a similar protocol developed by Romero and colleagues (Gormally et al., 2018; Lattin and Romero, 2014), is effective in creating a condition of chronic stress in captive budgerigars. Variants of this protocol have now been applied in a number of avian species and have generally resulted in measurable changes in physiological markers of stress, health and immune function. Wild-caught starlings (*Sturnis vulgaris*) subjected to this protocol over a 20-day period showed decreased levels of baseline, stress responses and post-stress recovery levels of corticosterone (Rich and Romero, 2005).

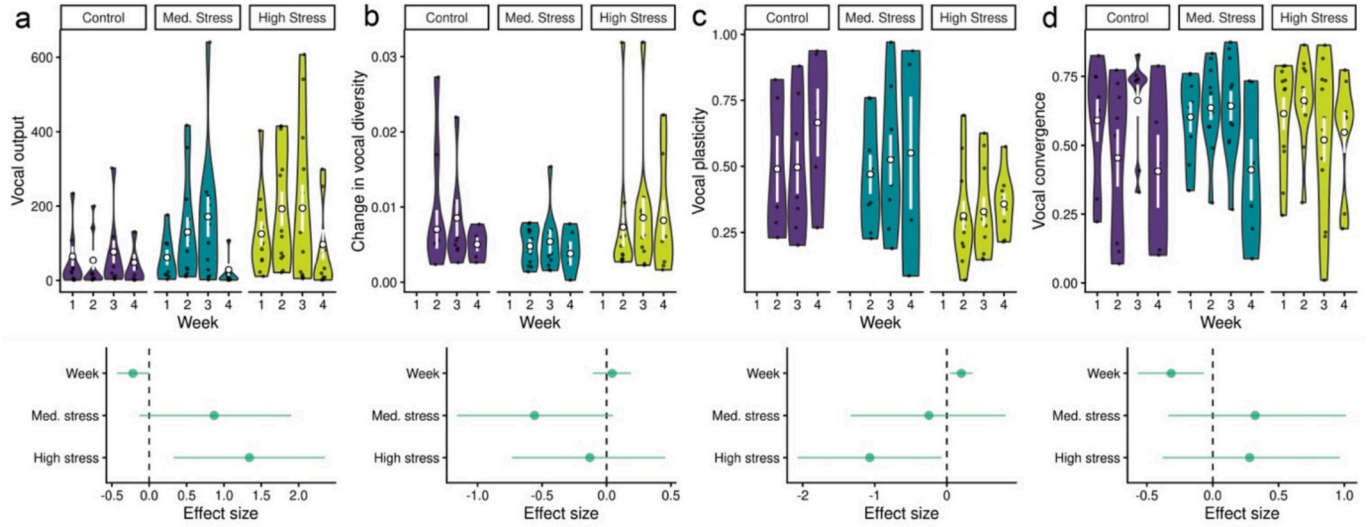

Fig. 4. Changes in measures of vocal output and vocal learning for individuals from the three stress treatments, by week of the experiment. a) *Vocal output*, as measured by total number of calls produced by individuals from each treatment. b) *Vocal diversity*: the area of acoustic space occupied by the calls of a bird, normalized by the area in week 1. c) *Vocal plasticity*, measured as the change in acoustic space overlap of an individual's current repertoire to its starting repertoire in week 1. d) *Vocal convergence*, the degree of acoustic space overlap of the individual to its group acoustic space over each week. The top row of figures illustrates raw values as violin plots, with mean ± 1 SE in white and individual points in black. The bottom row shows effect sizes for week, medium stress treatment compared to control, and high stress treatment compared to control (mean and 95 % uncertainty interval). Effects for which the uncertainty interval does not overlap zero are considered to have an effect on the response variable.

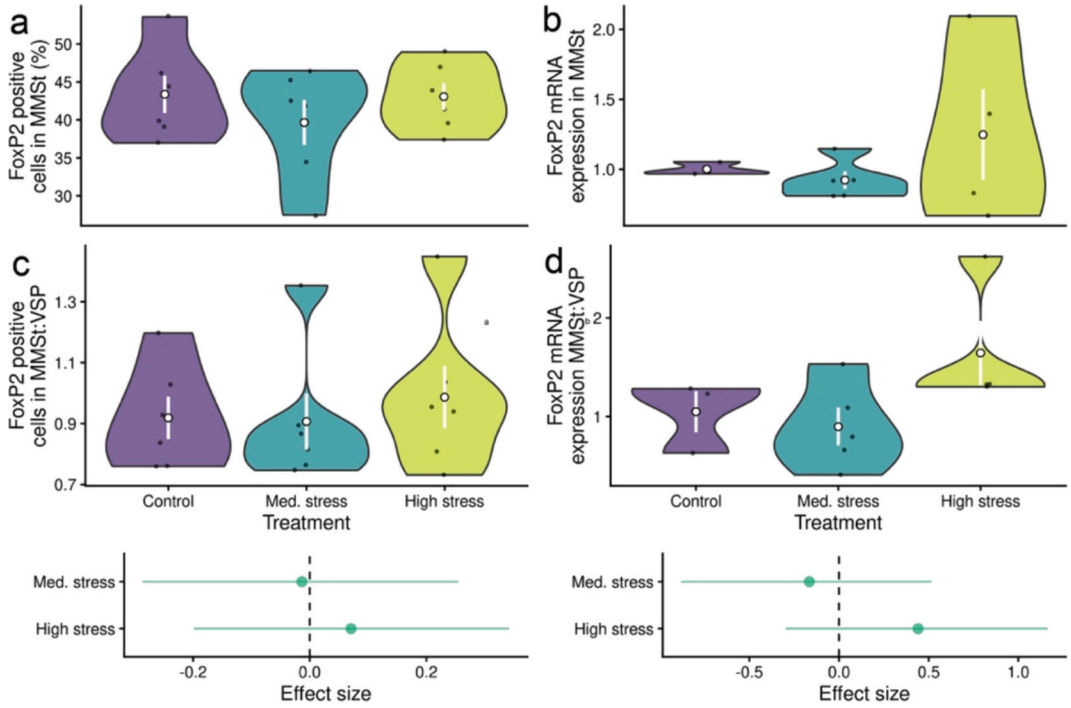

Fig. 5. Levels of FoxP2 expression in the budgerigar striatal vocal learning center MMSt. The top row illustrates absolute levels of FoxP2 in MMSt with mean ± SE in white and individual points: a) FoxP2 protein as measured by percentage of immuno-positive neural cells and b) FoxP2 mRNA as measured by differential expression of FoxP2 mRNA. The middle row shows the ratio of FoxP2 expression in MMSt relative to expression in the adjacent striatal VSP for c) FoxP2 protein and d) FoxP2 mRNA, with effect sizes for each comparison directly below. Effect sizes are shown for the medium stress treatment compared to control, and the high stress treatment compared to control (mean and 95 % uncertainty interval). Effects for which the uncertainty interval does not overlap zero are considered to have an effect on the response variable.

Wild-caught house sparrows (*Passer domesticus*) subjected to a six-day protocol showed no measurable changes in levels of baseline, stress response or post-stress recovery corticosterone, but did show changes in some measures of immune function and physiological stress including uric acid levels and DNA damage (Gormally et al., 2018). In contrast, wild caught house sparrows subjected to a 21-day protocol showed significant decreases in stress response corticosterone but not baseline corticosterone (Lattin and Romero, 2014). It is important to note that none of the individual stressors in this protocol are particularly noxious in isolation nor do they cause visible distress to the birds (Wright et al.

pers obs). Rather it is the unpredictable nature of the protocol with its randomized order of presentation, simulating unpredictable changes in the environment, that elicit a chronic stress response. Notably, the rise in baseline corticosterone we observed coincides with a decline in the ability of individuals in the high stress treatment to mount a stress response, suggesting this protocol is inducing long-term changes in the ability of individuals to cope with sustained stress. These results mirror general patterns seen with implants of exogenous corticosterone in birds: a review of 50 studies in 22 species showed that implants increased baseline corticosterone in 72 % of studies and decreased stress response corticosterone in 78 % of studies (Torres-Medina et al., 2018). In budgerigars, and more generally, this long-term change may correspond to "homeostatic overload" in the terminology of the reactive scope model (Romero et al., 2009) in which levels of the physiological mediator (i.e. hormone) exceeds those typically seen during maintenance of homeostasis (i.e. reactive scope) and may begin to cause wear and tear on the organism (Romero and Wingfield, 2015). Further work is needed to explore whether these effects persist past the duration of the stressor protocol, and whether they drive compensatory changes in the HPA axis or in molecular targets of corticosterone in the brain or elsewhere.

3.2. Effects of chronic stress on vocal behavior and learning

The chronic stress induced by our stress protocol produced changes in vocal behavior and learning in adult male budgerigars. We measured vocal output during recording sessions and characterized vocal learning using three dimensions: vocal diversity (the amount of acoustic space occupied by a bird's calls), vocal plasticity (the amount of change over time in acoustic space occupied by a bird's calls), and vocal convergence (the amount of overlap in acoustic space between a bird's calls and those of its flockmates). Birds in the high stress treatment produced more contact calls during recording sessions than did birds in either the medium stress treatment or baseline controls. Importantly, the high stress treatment also showed reduced vocal plasticity relative to controls. Similar patterns were seen for vocal diversity and vocal convergence, although in both cases these patterns were not statistically significant. The effect of week was significant for both vocal plasticity and vocal convergence, perhaps reflecting either habituation to the stressors or familiarization with the initially novel members of the experimental flocks. There was a negative correlation at the individual level between vocal plasticity and vocal convergence. Birds in the medium stress treatment were generally intermediate in vocal learning measures to the control and high stress treatments, suggesting that the "inverted U-shaped effect" proposed by the Yerkes and Dodson Law is not seen in the domain of vocal learning, at least at the levels of stress produced by our treatments (Lupien et al., 2007; Yerkes and Dodson, 1908).

Although studies of the effect of chronic stress experienced by adults on their vocal learning behavior are limited, our results do show some similarities to a number of studies examining the impacts of various early-life stressors on singing behavior in adult birds. For example, starlings subjected to an unpredictable food supply as juveniles sang less, had shorter song bouts, and had smaller vocal repertories as adults than did control birds (Buchanan et al., 2003; Spencer et al., 2004), canaries (*Serinus canaria*) infected with avian malaria as juveniles sang less complex songs as adults than did uninfected controls (Spencer et al., 2005), juvenile zebra finches exposed to elevated endogenous corticosterone levels copied their fathers less accurately (Boogert et al., 2018), and juvenile male song sparrows (*Melospiza melodia*) administered either endogenous corticosterone or food restriction as juveniles had a reduced song repertoire size as adults (Schmidt et al., 2013). In contrast, some other studies have not demonstrated changes in singing behavior with early life stress (e.g. Gil et al., 2006).

Several studies have also documented impacts on the neural substrates underlying vocal learning and production. For example, the food-stressed juvenile starlings that developed smaller song repertoires as adults also had smaller volumes of HVC, a songbird vocal control area (Buchanan et al., 2003), juvenile zebra finches fed on a lower quality diet showed reduced recruitment of new neurons to HVC (Honarmand et al., 2016), and juvenile song sparrows raised under food restriction showed a reduced size of HVC in one study (MacDonald et al., 2006), and of the song control nuclei RA in another (Schmidt et al., 2013). In contrast, some other studies in starlings (Buyannemekh et al., 2020) and zebra finches (Sewall et al., 2018) have detected impacts of juvenile-stage stressors on learning measures without detecting comparable impacts on the neural regions that permit learning. To date, few studies have examined the impacts of stress on the expression of genes thought to be related to vocal learning (but see Kraft et al., 2024; Moehn et al., 2025).

3.3. Effects of chronic stress on FoxP2 expression and relationship to vocal learning

We examined effects of chronic stress on levels of FoxP2 in the budgerigar vocal learning region MMST, a striatal region thought to be homologous to the songbird Area X. There is a robust body of work demonstrating that changes in the expression levels of FoxP2 in MMST relative to the surrounding striatal tissue are associated with changes in expression of learning-related genes (Burkett et al., 2018; Gedman et al., 2025; Hilliard et al., 2012) and vocal behavior (Chen et al., 2013; Miller et al., 2010; Teramitsu and White, 2006), with zebra finch males with higher expression in Area X showing reduced acoustic plasticity in the production of their undirected or practice song (Miller et al., 2010). Our own work in budgerigars, which show considerable learning-based plasticity as adults, has demonstrated that FoxP2 levels are consistently lower in MMST than in the surrounding VSP (Hara et al., 2013; Whitney et al., 2015), even in older adults (Moussaoui et al., 2024). To our knowledge, the impacts of chronic stress on the expression of FoxP2 have not previously been investigated, despite demonstrations that corticosterone impacts gene expression in cultured zebra finch brain (Rensel and Schlinger, 2020) and that the mineralcorticoid and glucocorticoid receptors that bind corticosterone are expressed in at least some of the vocal learning regions of the Bengalese finch (*Lonchura striata* var. *domestica*) (Suzuki et al., 2011) and budgerigar (T.F. Wright et al., unpub. res., Matsunaga et al., 2011). While we did not detect an effect of stress treatment on the expression of either FoxP2 protein or mRNA, in both cases there was a modest, albeit nonsignificant, trend towards higher expression of FoxP2 in the high stress treatment, consistent with the reduced vocal plasticity seen in the high stress treatment. At the individual level, however, vocal plasticity in Week 4 showed a positive correlation with FoxP2 protein expression, in the opposite direction predicted by the hypothesis that vocal plasticity is enhanced by reduced levels of FoxP2 in the MMSt relative to the surrounding striatum. It is worth noting that habituation to the stressors may have reduced their effect on neural FoxP2 expression, which was only measured at the conclusion of the experimental timeline when brains were collected. Future work will examine whether these stress-related changes in circulating corticosterone drive changes in expression of the many other learning- and language-related genes that are known to interact with FoxP2 in the avian brain. This work should further elucidate the links between chronic stress, circulating corticosterone, gene expression, and vocal learning behavior in an animal model for adult vocal learning.

CRediT authorship contribution statement

Timothy F. Wright: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marcelo Araya-Salas:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Alondra Villalba:**

Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Amelia M.-F. Clayshulte Abraham:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Carlos I. Campos:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Amanda L. Schmidt:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Connor Draney:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Jodie M. Jawor:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2026.105884.

Data availability

Data are publicly available in the Dryad repository at https://doi.org/10.5061/dryad.zcrjdfnrk

Chronic stress raises baseline circulating corticosterone and reduces vocal plasticity in male budgerigars, an avian model for adult vocal learning (Original data) (Dryad)

References

Araya-Salas, M., 2020. Rraven: Connecting R and Raven Bioacoustic Software, R Package v1.0.9. Ed.
Araya-Salas, M., Odom, K., 2022. PhenotypeSpace: and R Package to Quantify and Compare Phenotypic Trait Spaces, R Package v 0.1.0. Ed.
Araya-Salas, M., Smith-Vidaurre, G., 2017. warbleR: an R package to streamline analysis of animal acoustic signals. Methods Ecol. Evol. 8.
Araya-Salas, M., Smith-Vidaurre, G., Chaverri, G., Brenes, J.C., Chirino, F., Elizondo-Calvo, J., Rico-Guevara, A., 2022. ohun: an R package for diagnosing and optimizing automatic sound event detection. In: bioRxiv, 2022.2012.2013.520253.
Bartlett, P., Slater, P.J.B., 1999. The effect of new recruits on the flock specific call of budgerigars (*Melopsittacus undulatus*). Ethol. Ecol. Evol. 11, 139–147.
Bell, B.A., Phan, M.L., Meillère, A., Evans, J.K., Leitner, S., Vicario, D.S., Buchanan, K.L., 2018. Influence of early-life nutritional stress on songbird memory formation. Proc. Biol. Sci. 285.
Blair, C., Granger, D.A., Willoughby, M., Mills-Koonce, R., Cox, M., Greenberg, M.T., Kivlighan, K.T., Fortunato, C.K., 2011a. Salivary cortisol mediates effects of poverty and parenting on executive functions in early childhood. Child Dev. 82, 1970–1984.
Blair, C., Raver, C.C., Granger, D., Mills-Koonce, R., Hibel, L., 2011b. Allostasis and allostatic load in the context of poverty in early childhood. Dev. Psychopathol. 23, 845–857.
Bolhuis, J.J., Okanoya, K., Scharff, C., 2010. Twitter evolution: converging mechanisms in birdsong and human speech. Nat. Rev. Neurosci. 11, 747–759.
Boogert, N.J., Lachlan, R.F., Spencer, K.A., Templeton, C.N., Farine, D.R., 2018. Stress hormones, social associations and song learning in zebra finches. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 373.
Brittan-Powell, E.F., Dooling, R.J., Farabaugh, S.M., 1997. Vocal development in budgerigars (*Melopsittacus undulatus*): contact calls. J. Comp. Psychol. 111, 226–241.
Buchanan, K.L., Spencer, K.A., Goldsmith, A.R., Catchpole, C.K., 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). Proc. Biol. Sci. 270, 1149–1156.
Buchanan, K.L., Leitner, S., Spencer, K.A., Goldsmith, A.R., Catchpole, C.K., 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Proc. Biol. Sci. 271, 2381–2386.
Burkett, Z.D., Day, N.F., Kimball, T.H., Aamodt, C.M., Heston, J.B., Hilliard, A.T., Xiao, X., White, S.A., 2018. FoxP2 isoforms delineate spatiotemporal transcriptional networks for vocal learning in the zebra finch. eLife 7, e30649.
Bürkner, P.-C., 2017. Brms: an R package for Bayesian multilevel models using Stan. J. Stat. Softw. 80, 1–18.
Buyannemekh, K., Zito, J.B., Tomaszycki, M.L., 2020. Early life nutritional stress affects song learning but not underlying neural circuitry in zebra finches. Behav. Neurosci. 134, 222–232.
Chen, Q., Heston, J.B., Burkett, Z.D., White, S.A., 2013. Expression analysis of the speech-related genes FoxP1 and FoxP2 and their relation to singing behavior in two songbird species. J. Exp. Biol. 216, 3682–3692.
Cornell Lab of Ornithology, 2022. Raven Pro: Interactive Sound Analysis Software (Version 1.6.3). The Cornell Lab of Ornithology, Ithaca, NY.
Dahlin, C.R., Young, A.M., Cordier, B., Mundry, R., Wright, T.F., 2014. A test of multiple hypotheses for the function of call sharing in female budgerigars, *Melopsittacus undulatus*. Behav. Ecol. Sociobiol. 68, 145–161.
de Kloet, E.R., 2022. Brain mineralocorticoid and glucocorticoid receptor balance in neuroendocrine regulation and stress-related psychiatric etiopathologies. Curr. Opin. Endocr. Metab. Res. 24, 100352.
de Kloet, E.R., Oitzl, M.S., Joels, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426.
Deppermann, S., Storchak, H., Fallgatter, A.J., Ehlis, A.C., 2014. Stress-induced neuroplasticity: (mal)adaptation to adverse life events in patients with PTSD - a critical overview. Neuroscience 283, 166–177.
Doupe, A.J., Kuhl, P.K., 1999. Birdsong and human speech: common themes and mechanisms. Annu. Rev. Neurosci. 22, 567–631.
Duckworth, B.M., Jawor, J.M., 2018. Corticosterone profiles in northern cardinals (Cardinalis cardinalis): do levels vary through life history stages? Gen. Comp. Endocrinol. 263, 1–6.
Dumas, T.C., Gillette, T., Ferguson, D., Hamilton, K., Sapolsky, R.M., 2010. Anti-glucocorticoid gene therapy reverses the impairing effects of elevated corticosterone on spatial memory, hippocampal neuronal excitability, and synaptic plasticity. J. Neurosci. 30, 1712–1720.
Eggleston, R., Viloria, N., Delgado, S., Mata, A., Guerrero, H.Y., Kline, R.J., Beissinger, S. R., Berg, K.S., 2022. Vocal babbling in a wild parrot shows life history and endocrine affinities with human infants. Proc. R. Soc. B Biol. Sci. 289.
Farabaugh, S.M., Dooling, R.J., 1996. Acoustic communication in parrots: laboratory and field studies of Budgerigars, *Melopsittacus undulatus*. In: Kroodsma, D.E., Miller, E.H. (Eds.), Ecology and Evolution of Acoustic Communication in Birds. Cornell University Press, Ithaca, New York, pp. 97–117.
Farabaugh, S.M., Linzenbold, A., Dooling, R.J., 1994. Vocal plasticity in budgerigars (*Melopsittacus undulatus*): evidence for social factors in the learning of contact calls. J. Comp. Psychol. 108, 81–92.
Feenders, G., Liedvogel, M., Rivas, M., Zapka, M., Horita, H., Hara, E., Wada, K., Mouritsen, H., Jarvis, E.D., 2008. Molecular mapping of movement-associated areas in the avian brain: a motor theory for vocal learning origin. PLoS ONE 3, e1768.
Fisher, S.E., Scharff, C., 2009. FOXP2 as a molecular window into speech and language. Trends Genet. 25, 166–177.
Gedman, G.L., Kimball, T.H., Atkinson, L.L., Factor, D., Vojtova, G., Farias-Virgens, M., Wright, T.F., White, S.A., 2025. CHIRP-Seq: FoxP2 transcriptional targets in zebra finch brain include numerous speech and language-related genes. BMC Neurosci. 26, 29.
Gil, D., Naguib, M., Riebel, K., Rutstein, A., Gahr, M., 2006. Early condition, song learning, and the volume of song brain nuclei in the zebra finch (*Taeniopygia guttata*). J. Neurobiol. 66, 1602–1612.
Gormally, B.M.G., Wright-Lichter, J., Reed, J.M., Romero, L.M., 2018. Physiological and behavioral responses of house sparrows to repeated stressors. PeerJ 6, e4961.
Haesler, S., Wada, K., Nshdejan, A., Morrisey, E.E., Lints, T., Jarvis, E.D., Scharff, C., 2004. FoxP2 expression in avian vocal learners and non-learners. J. Neurosci. 24, 3164–3175.
Han, J.-I., Kim, J.-H., Kim, S., Park, S.-R., Na, K.-J., 2009. A simple and improved DNA test for avian sex determination. Auk 126, 779–783.
Hara, E., Whitney, O., Lucero, E.M., Perez, J.M., Chen, Q., White, S.A., Wright, T.F., 2013. Neuronal FoxP2 Expression and Vocal Plasticity in Adult Budgerigars. Society for Neuroscience Annual Meeting, San Diego.
Hara, E., Perez, J.M., Whitney, O., Chen, Q., White, S.A., Wright, T.F., 2015. Neural FoxP2 and FoxP1 expression in the budgerigar, an avian species with adult vocal learning. Behav. Brain Res. 283, 22–29.
Het, S., Ramlow, G., Wolf, O.T., 2005. A meta-analytic review of the effects of acute cortisol administration on human memory. Psychoneuroendocrinology 30, 771–784.
Hile, A.G., Striedter, G.F., 2000. Call convergence within groups of female budgerigars (*Melopsittacus undulatus*). Ethology 106, 1105–1114.
Hile, A.G., Plummer, T.K., Striedter, G.F., 2000. Male vocal imitation produces call convergence during pair bonding in budgerigars, *Melopsittacus undulatus*. Anim. Behav. 59, 1209–1218.
Hilliard, A.T., Miller, J.E., Fraley, E.R., Horvath, S., White, S.A., 2012. Molecular microcircuitry underlies functional specification in a basal ganglia circuit dedicated to vocal learning. Neuron 73, 537–552.

Honarmand, M., Thompson, C.K., Schatton, A., Kipper, S., Scharff, C., 2016. Early developmental stress negatively affects neuronal recruitment to avian song system nucleus HVC. Dev. Neurobiol. 76, 107–118.
Joëls, M., 2018. Corticosteroids and the brain. J. Endocrinol. 238, R121–r130.
Joëls, M., Sarabdjitsingh, R.A., Karst, H., 2012. Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modess. Pharmacol. Rev. 64, 901–938.
Keen, S.C., Odom, K., Webster, M.S., Kohn, G.M., Wright, T.F., Araya-Salas, M., 2021. A machine learning approach for classifying and quantifying acoustic diversity. Methods Ecol. Evol. 00, 1–13.
Koning, A.-S.C.A.M., Buurstede, J.C., van Weert, L.T.C.M., Meijer, O.C., 2019. Glucocorticoid and mineralocorticoid receptors in the brain: a transcriptional perspective. J. Endocr. Soc. 3, 1917–1930.
Kraft, F.H., Crino, O.L., Adeniran-Obey, S.O., Moraney, R.A., Clayton, D.F., George, J.M., Buchanan, K.L., 2024. Parental developmental experience affects vocal learning in offspring. Sci. Rep. 14, 13787.
Krijthe, J., 2015. Rtsne: T-distributed Stochastic Neighbor Embedding Using Barnes-Hut Implementation, R Package v 0.16 Ed.
Lattin, C.R., Romero, L.M., 2014. Chronic stress alters concentrations of corticosterone receptors in a tissue-specific manner in wild house sparrows (Passer domesticus). J. Exp. Biol. 217, 2601–2608.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25, 402–408.
Lupien, S.J., Maheu, F., Tu, M., Fiocco, A., Schramek, T.E., 2007. The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. Brain Cogn. 65, 209–237.
Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445.
MacDonald, I.F., Kempster, B., Zanette, L., MacDougall-Shackleton, S.A., 2006. Early nutritional stress impairs development of a song-control brain region in both male and female juvenile song sparrows (*Melospiza melodia*) at the onset of song learning. Proc. Biol. Sci. 273, 2559–2564.
Malarbi, S., Abu-Rayya, H.M., Muscara, F., Stargatt, R., 2017. Neuropsychological functioning of childhood trauma and post-traumatic stress disorder: a meta-analysis. Neurosci. Biobehav. Rev. 72, 68–86.
Matsunaga, E., Suzuki, K., Kobayashi, T., Okanoya, K., 2011. Comparative analysis of mineralocorticoid receptor expression among vocal learners (Bengalese finch and budgerigar) and non-vocal learners (quail and ring dove) has implications for the evolution of avian vocal learning. Develop. Growth Differ. 53, 961–970.
McEwen, B.S., 2008. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur. J. Pharmacol. 583, 174–185.
McLean, C.R., Mata, A., Kline, R.J., Berg, K.S., 2025. Early corticosterone increases vocal complexity in a wild parrot: an organizational role of the hypothalamic-pituitary-adrenal axis in vocal learning? J. Neuroendocrinol. 37, e13365.
Medina-Garcia, A., Jawor, J.M., Wright, T.F., 2017. Cognition, personality, and stress in budgerigars, Melopsittacus undulatus. Behav. Ecol. 28, 1504–1516.
Miller, J.E., Hilliard, A.T., White, S.A., 2010. Song practice promotes acute vocal variability at a key stage of sensorimotor learning. PLoS ONE 5, e8592.
Moehn, K., Villalba, A., Clayshulte Abraham, A.M.-F., Ulmer, K., Jawor, J.M., Wright, T. F., 2025. Time the Avenger: Chronic Stress Induces Changes in Neural Expression of Glucocorticoid Receptors in the Vocal Learning Circuit of the Male Budgerigar. SSRN preprint server. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5615617.
Moussaoui, B., Ulmer, K., Araya-Salas, M., Wright, T.F., 2024. Persistent vocal learning in an aging open-ended learner reflected in neural FoxP2 expression. BMC Neurosci. 25, 12.
Nowicki, S., Searcy, W.A., Peters, S., 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 188, 1003–1014.
Olias, P., Adam, I., Meyer, A., Scharff, C., Gruber, A.D., 2014. Reference genes for quantitative gene expression studies in multiple avian species. PLoS ONE 9, e99678.
Pandey, M., Maina, R.G., Amoyaw, J., Li, Y., Kamrul, R., Michaels, C.R., Maroof, R., 2021. Impacts of English language proficiency on healthcare access, use, and outcomes among immigrants: a qualitative study. BMC Health Serv. Res. 21, 741.
Pfenning, A.R., Hara, E., Whitney, O., Rivas, M.V., Wang, R., Roulhac, P.L., Howard, J.T., Wirthlin, M., Lovell, P.V., Ganapathy, G., Mouncastle, J., Moseley, M.A., Thompson, J.W., Soderblom, E.J., Iriki, A., Kato, M., Gilbert, M.T.P., Zhang, G., Bakken, T., Bongaarts, A., Bernard, A., Lein, E., Mello, C.V., Hartemink, A.J., Jarvis, E.D., 2014. Convergent transcriptional specializations in the brains of humans and song-learning birds. Science 346, 1333-+.
Pierce, L.J., Reilly, E., Nelson, C.A., 2021. Associations between maternal stress, early language behaviors, and infant electroencephalography during the first year of life. J. Child Lang. 48, 737–764.
Pisoni, D.B., 2014. Some neuromyths concerning the effectiveness of cochlear implants: inferring function from dysfunction. J. Acoust. Soc. Am. 135, 2257.
R Core Team, 2022. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
Ramos-Güivas, B., Jawor, J.M., Wright, T.F., 2021. Seasonal variation in fecal glucocorticoid levels and their relationship to reproductive success in captive populations of an endangered parrot. Diversity 13, 617.
Rensel, M.A., Schlinger, B.A., 2020. The stressed brain: regional and stress-related corticosterone and stress-regulated gene expression in the adult zebra finch (Taeniopygia guttata). J. Neuroendocrinol. 32, 12.
Rich, E.L., Romero, L.M., 2005. Exposure to chronic stress downregulates corticosterone responses to acute stressors. Am. J. Phys. Regul. Integr. Comp. Phys. 288, R1628–R1636.
Romero, L.M., Wingfield, J.C., 2015. Tempests, Poxes, Predators, and People : Stress in Wild Animals and How They Cope. Oxford University Press, New York.
Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model — a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375–389.
Salinas-Melgoza, A., Wright, T.F., 2012. Evidence for vocal learning and limited dispersal as dual mechanisms for dialect maintenance in a parrot. PLoS ONE 7, e48667.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.
Schmidt, K.L., Furlonger, A.A., Lapierre, J.M., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A., 2012. Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. Horm. Behav. 61, 652–659.
Schmidt, K.L., Moore, S.D., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A., 2013. Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. Anim. Behav. 86, 25–35.
Schmidt, K.L., MacDougall-Shackleton, E.A., Kubli, S.P., MacDougall-Shackleton, S.A., 2014. Developmental stress, condition, and birdsong: a case study in song sparrows. Integr. Comp. Biol. 54, 568–577.
Scholaske, L., Wadhwa, P.D., Entringer, S., 2021. Acculturation and biological stress markers: a systematic review. Psychoneuroendocrinology 132, 105349.
Sewall, K.B., Anderson, R.C., Soha, J.A., Peters, S., Nowicki, S., 2018. Early life conditions that impact song learning in male zebra finches also impact neural and behavioral responses to song in females. Dev. Neurobiol. 78, 785–798.
Shahbazi, M., Schmidt, M., Carruth, L.L., 2011. Distribution and subcellular localization of glucocorticoid receptor-immunoreactive neurons in the developing and adult male zebra finch brain. Gen. Comp. Endocrinol. 174, 354–361.
Silverman, B.W., 1986. Density Estimation for Statistics and Data Analysis. Chapman & Hall, London.
Söndergaard, H.P., Theorell, T., 2004. Language acquisition in relation to cumulative posttraumatic stress disorder symptom load over time in a sample of resettled refugees. Psychother. Psychosom. 73, 320–323.
Spencer, K.A., Buchanan, K.L., Goldsmith, A.R., Catchpole, C.K., 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). Horm. Behav. 44, 132–139.
Spencer, K.A., Buchanan, K.L., Goldsmith, A.R., Catchpole, C.K., 2004. Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*). Proc. Biol. Sci. 271 (Suppl. 3), S121–S123.
Spencer, K.A., Buchanan, K.L., Leitner, S., Goldsmith, A.R., Catchpole, C.K., 2005. Parasites affect song complexity and neural development in a songbird. Proc. Biol. Sci. 272, 2037–2043.
Stan Development Team, 2021. Stan modeling language users guide and reference manual. Vers, p. 2.27.
Striedter, G.F., 1994. The vocal control pathways in budgerigars differ from those in songbirds. J. Comp. Neurol. 343, 35–56.
Suzuki, K., Matsunaga, E., Kobayashi, T., Okanoya, K., 2011. Expression patterns of mineralocorticoid and glucocorticoid receptors in Bengalese finch (*Lonchura striata* var. *domestica*) brain suggest a relationship between stress hormones and song-system development. Neuroscience 194, 72–83.
Tchernichovski, O., Nottebohm, F., Ho, C.E., Pesaran, B., Mitra, P.P., 2000. A procedure for an automated measurement of song similarity. Anim. Behav. 59, 1167–1176.
Teramitsu, I., White, S.A., 2006. FoxP2 regulation during undirected singing in adult songbirds. J. Neurosci. 26, 7390–7394.
Teramitsu, I., Poopatanapong, A., Torrisi, S., White, S.A., 2010. Striatal FoxP2 is actively regulated during songbird sensorimotor learning. PLoS ONE 5, e8548.
Tomblin, J.B., Barker, B.A., Hubbs, S., 2007. Developmental constraints on language development in children with cochlear implants. Int. J. Audiol. 46, 512–523.
Torres-Medina, F., Cabezas, S., Marchant, T.A., Wikelski, M., Romero, L.M., Hau, M., Carrete, M., Tella, J.L., Blas, J., 2018. Corticosterone implants make stress hyporesponsive birds. J. Exp. Biol. 221, jeb173864.
Van der Maaten, L., Hinton, G., 2008. Visualizing data using t-SNE. J. Mach. Learn. Res. 11.
Whitney, O., Pfenning, A.R., Howard, J.T., Blatti, C.A., Liu, F., Ward, J.M., Wang, R., Audet, J.N., Kellis, M., Mukherjee, S., Sinha, S., Hartemink, A.J., West, A.E., Jarvis, E.D., 2014. Core and region-enriched networks of behaviorally regulated genes and the singing genome. Science 346, 1334-+.
Whitney, O., Voyles, T., Hara, E., Chen, Q., White, S.A., Wright, T.F., 2015. Differential FoxP2 and FoxP1 expression in a vocal learning nucleus of the developing budgerigar. Dev. Neurobiol. 75, 778–790.
Wright, M.N., Ziegler, A., 2017. ranger: a fast implementation of random forests for high dimensional data in C++ and R. J. Stat. Softw. 77, 1–17.
Yerkes, R., Dodson, J., 1908. The relation of strength of stimulus to rapidity of habit-formation. J. Comp. Neurol. Psychol. 18, 459–482.