**Persistent vocal learning in an aging open-ended learner reflected in neural FoxP2 expression**

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**Abstract**

Most vocal learning species exhibit an early critical period during which their vocal control neural circuity facilitates the acquisition of new vocalizations. Some taxa, most notably humans and parrots, retain some degree of neurobehavioral plasticity throughout adulthood, but both the extent of this plasticity and the neurogenetic mechanisms underlying it remain unclear. Downregulation of the transcription factor FoxP2 in both songbird and parrot vocal control nuclei has been identified previously as a key expression pattern facilitating vocal learning. We hypothesize that open-ended vocal learning is resistant to cognitive decline, and that this resilience will be reflected in an absence of age-related changes in neural FoxP2 expression. We tested this hypothesis in the budgerigar (*Melopsittacus undulatus*), a small gregarious parrot in which adults converge on shared call types in response to shifts in group membership. We formed novel flocks of 4 previously unfamiliar males belonging to the same age class, either “young adult” (6 mo - 1 yr) or “older adult” (≥ 3 yr), and then collected audio-recordings over a 20-day learning period to assess vocal learning ability. Following behavioral recording, whole brains were extracted and immunohistochemistry was performed to measure FoxP2 protein expression in a parrot vocal learning center, the magnocellular nucleus of the medial striatum (MMSt), and its adjacent striatum. We find similarly downregulated FoxP2 expression and equivalent vocal plasticity and vocal convergence in young and older adults suggesting the maintenance of two components of open-ended learning into old age in the budgerigar. No relationship between individual variation in vocal learning measures and FoxP2 expression was detected.

**Keywords**

Aging, budgerigar, cognitive senescence, FoxP2, open-ended learning, vocal learning, vocal plasticity

**Introduction**

Aging is commonly associated with progressive deterioration in many aspects of organismal function, including cognitive domains such as learning and memory (Wright, 2016; Yankner et al., 2008). One complex behavior at the nexus of these two cognitive processes and thus vulnerable to age-related senescence, is language proficiency (Wright, 2016). Vocal production learning, in which individuals produce new vocalizations based on the encoding of an auditory template and sensorimotor experience, is a key substrate for language development (Jarvis, 2019). While language itself is considered unique to humans, vocal production learning is found in a handful of distantly related mammalian and avian taxa, most notably in songbirds and parrots. These avian vocal learners share convergent forebrain pathways with humans that contain analogous cerebral circuits for the detection, production and learning of vocalizations, and similar gene expression patterns within these circuits, which are absent in non-vocal learning taxa (Petkov & Jarvis, 2012). Such anatomical and molecular similarities make these avian taxa valuable models for studying learned vocal communication (Brainard & Doupe, 2002; Jarvis, 2004).

The first gene to be definitively linked to human speech and language was the transcription factor forkhead box P2 (FOXP2) (Lai et al., 2001). Individuals possessing a mutation in this gene suffer from impaired orofacial fine motor control and deficits in language processing, as well as abnormal morphology and dysfunction in motor and language related brain regions such as the basal ganglia and Broca’s area (Belton et al., 2003; Liégeois et al., 2003). Following this discovery, FoxP2 has been extensively studied in avian vocal learning pathways, wherein its differential expression has been found to facilitate vocal flexibility in a variety of developmental and social contexts (Chen et al., 2013; Haesler et al., 2004; Hara et al., 2015; Miller et al., 2008; Whitney et al., 2015). In male zebra finches (*Taeniopygia guttata*), *FoxP2* mRNA expression increases in the striatal vocal control nucleus Area X during the juvenile sensorimotor learning period when song is nearing adult “crystallized” song (Haesler et al., 2004) and is downregulated in Area X when adult males sing in the absence of a female, producing more variable song syllables—during what is thought to be vocal “practice”—compared to when singing is directed towards a potential mate (Teramitsu & White, 2006).

This link between FoxP2 expression patterns and vocal learning is further supported by the persistent downregulation of this gene in a taxon capable of life-long vocal learning, the parrots (Hara et al., 2015; Whitney et al., 2015). While the zebra finch, and many other songbirds, are closed-ended learners, in which the ability to produce new vocalizations is restricted to an early developmental critical period after which adult songs stabilize (Brainard & Doupe, 2002), parrots are open-ended learners and in captivity will exhibit extraordinary vocal mimicry that appears to persists throughout their adult lives (Bradbury, 2016). Adult male budgerigars (*Melopsittacus undulatus*) exhibit consistently downregulated *FoxP2* mRNA and protein expression in the parrot analogue of Area X, the magnocellular nucleus of the medial striatum (MMSt), regardless of vocal state (Hara et al., 2015; Whitney et al., 2015).

This persistent low level MMSt FoxP2 expression is consistent with the vocal plasticity that has been commonly observed in previous experimental studies with captive budgerigars. In captivity, adult male budgerigars readily imitate the contact calls of female mates (Hile et al., 2000), and adult budgerigars of both sexes rapidly converge on shared contact calls in response to joining new social groups via a combination of imitation, improvisation, and recombination of frequency modulation patterns (Christine et al., 2014; Dahlin et al., 2014; Farabaugh et al., 1994; Hile & Striedter, 2000). In a recent study investigating whether aging budgerigars exhibit a decline in vocal learning ability, we find that many components of vocal learning are maintained in this open-ended learner (Moussaoui et al., 2023). While it is assumed that this apparent resilience to senescence in open-ended vocal learners would be reflected in a correspondingly persistent FoxP2 downregulation pattern in aging adults, this relationship remains unconfirmed as FoxP2 expression has not yet been characterized in different adult age groups of an open-ended vocal learner. Additionally, a correlation between behavioral vocal learning measures (such as the degree of acoustic similarity between social associates) and FoxP2 striatal expression has not been established.

In this study, we tested whether aging affects adult vocal learning and its neural underpinnings in an open-ended learner. To do this, we examined whole brains of birds from our previously published study (Moussaoui et al., 2023) in which we conducted a vocal learning assay of male budgerigars of two different adult ages (young adult: 6 mo. – 1 yr.; older adult ≥ 3 yr.). Immunohistochemistry was performed on collected neural tissue to measure FoxP2 protein expression in the MMSt and the surrounding striatum (VSP) to determine if expression profiles differ between young and older adults. We hypothesized that open-ended vocal learning is resilient to senescence and that a persistence of vocal learning is related to patterns of FoxP2 expression characteristic of vocal flexibility. We predict that older birds will not exhibit diminished vocal learning ability and thus will have a similarly low FoxP2 MMSt/VSP expression ratio compared to young adults. We also predict that individual variation in vocal learning can be explained by FoxP2 MMSt/VSP expression such that individuals demonstrating greater vocal learning will exhibit greater downregulation of FoxP2.

**Methods**

***Behavioral Vocal Learning Assay***

To assess the effect of adult age on vocal learning ability, we formed novel flocks of 4 previously unfamiliar male budgerigars belonging to the same age class, either “young adult” (6 mo-1 yr) or “older adult” (≥ 3 yr), close to or exceeding the mean life expectancy of 4.57 years for this species in captivity (Smeele et al., 2022), and then collected audio-recordings from all individuals over a 20-day learning period to measure changes in contact call repertoires over time (figure 1). Full details of this behavioral vocal learning assay are described in Moussaoui et al. (2023), as a subset of birds from that main experiment were used in this study focused on the neural underpinnings of age-related differences in vocal learning ability. In brief, 24 birds of each adult age class were acquired from a commercial breeder (McDonald Bird Farms, Kerrville, TX) and from our own research colony at the New Mexico State University Animal Care Facility. The commercial breeder provided young birds and old birds housed in four separately built aviaries and three separately built aviaries, respectively. This, in combination with a set of old males from our colony, generated four independent source populations for each age class such that birds originating from different populations were socially and acoustically unfamiliar and could thus be combined to form flocks of novel membership. Prior to novel flock formation, baseline contact call repertoires were collected for each individual during a 4-day audio-recording block (block 1 in figure 1). Upon being placed with novel flockmates, birds were audio-recorded daily across four 4-day blocks (blocks 2-5 in figure 1). At the end of this vocal recording period, neural tissue was collected for a randomly selected subset of individuals to measure expression of a key vocal learning related gene.

Contact calls were isolated from these audio-recordings using a semi-automated signal detection procedure in the R package *ohun* (*version 1.0.0;* Araya‐Salas et al., 2023) in R version 4.0.5 (R Core Team, 2021). This involved applying optimized amplitude, frequency, and duration thresholds, the use of supervised random forests to classify detections as “signal” (contact calls) or “noise” (other vocalization types, feather ruffling, cage rattling, background flockmates), and lastly a manual quality control step in which we visually confirmed detections classified as contact calls. Seventeen standard acoustic features were then measured from contact call spectrograms, including various frequency parameters, duration, entropy, skew, and kurtosis, using the R package *warbleR* (*version 1.1.27;* Araya‐Salas et al., 2017). The dimensionality of these multiple extracted acoustic measures was reduced using t-Distributed Stochastic Neighbor Embedding (t-SNE) (van der Maaten & Hinton, 2008) implemented in the R package *Rtsne* (*version 0.15*). We mapped contact calls in an acoustic trait space, hereafter “acoustic space”, generated by projecting the first two dimensions such that acoustically similar calls appear closer together in space. We then quantified the kernel density area of each individual’s acoustic space subset by recording block using the R package *PhenotypeSpace* (*version 0.1.0*; Araya-Salas & Odom, 2022)to assess changes in contact call repertoires over time. All acoustic spaces were generated using the same number of contact calls (180) as randomly selected using a rarefaction subsampling procedure.

From these acoustic space areas, three vocal learning measures were computed for each bird. Firstly, we defined *vocal diversity* as the change in acoustic space area of an individual’s contact call repertoire from the beginning of the vocal learning assay (audio-recording block 1) to the end (audio-recording block 5). Secondly, we defined *vocal plasticity* as 1 minus the intersection over union of an individual’s beginning and ending acoustic space areas, where higher values indicate less acoustic similarity between initial and final contact calls, and thus greater vocal plasticity. Thirdly, we defined *vocal convergence* as the intersection over union of an individual’s acoustic space area and the combined acoustic space area of its flockmates at the end of the vocal learning assay, where higher values indicate greater matching of ones’ contact call repertoire to that of its social group.

Timeline

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**Figure 1.** Experimental timeline outlining the formation of novel flocks, audio-recording of vocalizations, and neural tissue collection.

***Tissue Preparation & Immunohistochemistry***

Following the vocal learning assay (figure 1), two birds from each flock of 4 birds (N = 12 individuals per age class) were randomly selected for sacrifice to collect whole brains for neural analysis of the *FoxP2* gene. On day 28 of the experimental timeline, selected birds were euthanized via an overdose inhalation of isoflurane and whole brains were extracted and flash frozen within 5 minutes using liquid nitrogen and then stored at -80 °C until later use. All collected brains were coronally cryosectioned using a Leica CM1850 cryostat microtome (Leica Microsystems) at -20 °C. The left or right hemisphere of each brain was randomly selected for extraction of 1 mm deep punches with an 18 gauge Luer stub from both MMSt and VSP for future RNA isolation work. The non-punched hemisphere (young adults: N = 6 RH, 3 LH; older adults: N = 5 RH, N = 4 LH) was used in this study for immunohistochemical staining for FoxP2 protein expression. Sections of 20 μm thickness were thaw-mounted onto positively charged slides (Fisher Scientific) in 7 replicate series and stored at -80 °C. One series was Nissl stained for visualization of cytoarchitectural boundaries to enable identification of the key brain regions of interest, MMSt and its adjacent striatum. With reference to the budgerigar brain atlas (<http://www.brauthlab.umd.edu/atlas.htm>), adjacent slides were selected for immunohistochemical staining.

Brain sections were first fixed with 4% paraformaldehyde (titrated with NaOH and HCl to achieve a pH of 7) for 5 mins, dip-rinsed twice with 1X phosphate buffered saline (PBS), then rinsed three times with 1X PBS with 0.4% Triton X-100 (PBST) for 5 minutes each. Slides were then blocked with 5% sheep serum (Sigma-Aldrich) in PBST for 1 hour at room temperature to prevent nonspecific binding followed by overnight incubation at 4 °C in the FoxP2 primary antibody (Mouse, 1:500, Thermo Fisher Scientific) solution. Slides were then rinsed in 1X PBST three times at 5 minutes each prior to incubation in the Alexa Fluor 594 secondary antibody (Goat anti-mouse, 1:200, Thermo Fisher Scientific) for 2 hours at room temperature. Sections were then rinsed four times at 5 minutes each in 1X PBS, once in ddH20, and finally coverslipped using Vectashield with 405 nm excitable DAPI (Vector Laboratories). Negative controls were performed identically as above except for the omission of primary antibody.

Following immunohistochemistry, tissue slides were imaged using a TCS SP5 II Confocal microscope (Leica Microsystems) to capture fluorescent images for quantification of FoxP2 protein expression. Images were taken within the MMSt and VSP regions from each of two sections per bird at 40X magnification. Images of each region were taken sequentially between frames for each channel (405 nm for DAPI, 594 nm for FoxP2, and their overlay) and saved as TIFF image files (figure 2). Images were imported into Image J 1.53e (NIH), converted to an 8-bit grayscale and auto-thresholded. DAPI and FoxP2 labeled cells were then manually counted using the multi-point tool while referencing cell morphology in original images. To avoid counting noise arising from secondary antibody background staining, FoxP2 labeled cells were only counted if they overlaid atop a DAPI counted cell. For each image, FoxP2 cell counts were divided by the total number of cells (DAPI counted cells) yielding a percentage of neuronal cells that were expressing FoxP2 in the MMSt and the VSP, which was then used to calculate a MMSt/VSP FoxP2 expression ratio per section. This MMSt/VSP ratio was averaged for the two imaged sections per bird. Cells were counted by two trained observers and inter-observer reliability was assessed at “good” to “excellent” (ICC = 0.965; 95% CI = [0.769, 0.995]) by employing a single-measurement, absolute-agreement two-way mixed-effects model using the package *irr* (*version 0.84.1*; Gamer et al., 2019). **Background pattern

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**Figure 2.** Immunohistochemical staining of FoxP2 protein in the magnocellular nucleus of the medial striatum (MMSt) and the adjacent striatal non-vocal learning region (VSP). Images were taken at 40X magnification using a confocal microscope. These example images are from a single older adult male budgerigar. Blue signal (a and d) indicates DAPI stained cell nuclei, representing the total number of cells present in the area imaged. Red signal (b and e) indicates FoxP2 labeled cell nuclei. DAPI and FoxP2 labeled images are overlaid (c and f) to identify the percentage of total cells that express FoxP2.

***Statistical Analysis***

All statistical analyses were carried out in R version 4.2.1 (R Core Team, 2021) using the *stats* (*version 4.2.1*)and *car* (*version 3.1-1*) packages. To test whether FoxP2 expression differs by adult age, we conducted independent samples t-tests for FoxP2 expression in MMSt, VSP, and the MMSt/VSP expression ratio, given that each of these measures passed the Shapiro-Wilk test for normality and Levene’s test for homogeneity of variances among age classes. To determine whether neural FoxP2 expression predicts vocal learning, and whether this relationship differs between young and older adults, we conducted analysis of covariance (ANCOVA) with each of the three vocal learning measures (vocal diversity, vocal plasticity, and vocal convergence) as a response variable in three separate models. For these ANCOVA models, we included adult age as a categorical explanatory variable and FoxP2 MMSt/VSP expression as a continuous covariate. Although, vocal learning measures were computed for each recording block, we chose to only include vocal learning measures computed during the last audio-recording block, as this was closest in time to neural tissue collection, and FoxP2 expression levels more accurately reflect more recent vocal behavior (Miller et al., 2008). We extracted this vocal data for each of the 12 young adult and 12 older adult birds for which we had measured FoxP2 MMSt/VSP expression, the key measure that has been linked to persistent vocal learning ability (Hara et al., 2015; Whitney et al., 2015). Four of the older adults, however, had produced fewer than 6 contact calls during the last audio-recording block (2 birds produced 4 calls, and 2 birds did not call), failing to meet our minimum threshold for accurate measurements and comparisons of acoustic space and thus could not be included in this analysis, leaving a sample size of 8 older adults for analyses of call learning.

**Results**

***Neural FoxP2 Protein Expression in Young and Older Adults***

Young adult budgerigars exhibited a significantly lower proportion of MMSt cells expressing FoxP2 compared to older adults (*t* = -2.12, *df* = 22, *p* = 0.045) but the two age classes did not significantly differ in either the proportion of non-vocal learning adjacent striatum (VSP) cells expressing FoxP2 (*t* = -0.95, *df* = 22, *p* = 0.35) or the MMSt/VSP expression ratio (*t* = -0.86, *df* = 22, *p* = 0.40) (figure 3a-c). Both young and older adult age classes budgerigars exhibited a mean downregulation of FoxP2 in the MMSt vocal learning nucleus compared to the non-vocal learning adjacent striatum (figure 3c). All individuals displayed FoxP2 downregulation except for two older adults who had MMSt/VSP expression ratios of 1.00 and 1.25 and one young adult with a ratio of 1.03 with young adults exhibiting slightly more downregulation, having a mean expression ratio of 0.74 ± 0.05 (mean ± SE) compared to 0.81 ± 0.06 in older adults.

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**Figure 3.** Boxplots display FoxP2 protein expression levels in the striatal vocal learning nucleus, magnocellular nucleus of the medial striatum (MMSt), and the adjacent striatum in young (N = 12) and older adults (N = 12). (a) Proportion of MMSt cells expressing FoxP2. (b) Proportion of non-vocal learning adjacent striatum, VSP, cells expressing FoxP2. (c) Ratio of expression percentages (MMSt/VSP). A ratio of 1, as marked by the horizontal dashed line, indicates equal expression levels in MMSt and VSP. A ratio below 1 indicates downregulation of FoxP2 in the MMSt (characteristic of more plasticity) and a ratio above 1 indicates upregulation of FoxP2 in the MMSt (characteristic of less plasticity). Boxplot horizontal bar indicates the median, boxes represent the IQR, and whiskers represent minimum and maximum values. \*P < 0.05; ns = not significant.

***Individual Variation in Vocal Learning Ability and Neural FoxP2 Expression***

For our three learning measures, adult age was only found to significantly explain the difference in vocal diversity between an individual’s beginning and ending contact call repertoires (*F* = 6.71, *p* = 0.020) (table 1). Young adults exhibited a greater change in acoustic area (more positive) compared to older adults (more negative), suggesting that older adults displayed a loss in vocal diversity over the course of the vocal learning assay (figure 4a). Young and older adults did not significantly differ with respect to vocal plasticity or vocal convergence (table 1).

Neural FoxP2 expression was not found to significantly explain individual variation in vocal learning ability (figure 4; table 1). Some trends, however, support a role of FoxP2 downregulation in MMSt as a facilitator of vocal learning. For instance, the negative relationship between vocal diversity and FoxP2 expression observed for both age classes, indicates birds that exhibited larger increases in acoustic area had greater downregulation of FoxP2 in the MMSt (figure 4a). Additionally, the negative relationship between vocal plasticity and FoxP2 expression for both age classes matches expectations of lower FoxP2 levels in individuals exhibiting higher vocal plasticity (figure 4b).

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**Figure 4.** Scatterplots of the relationship between vocal learning measures and FoxP2 protein expression ratio (MMSt/VSP), fit linearly for each adult age class. FoxP2 expression ratios below 1 indicate downregulation of FoxP2 in the vocal learning striatal nucleus, MMSt. (a) Change in the area of an individual’s acoustic space from the beginning of the vocal learning assay (audio-recording block 1) to the end (audio-recording block 5), where 0 indicates no change in vocal diversity, positive values indicate an increase in vocal diversity, and negative values indicate a decrease in vocal diversity. (b) 1 minus the intersection over union of an individual’s beginning and ending acoustic spaces, where higher values on the y-axis indicate greater vocal plasticity. (c) Intersection over union of an individual’s acoustic space and the combined acoustic space of its flockmates, where higher values indicate greater vocal convergence.

**Table 1.** Analysis of Covariance results from models assessing the explanatory power of FoxP2 MMSt/VSP expression ratio, adult age, and their interaction, in predicting each of the three vocal learning measures computed for audio-recording block 5.

|  |  |  |  |
| --- | --- | --- | --- |
| Response | Fixed effect | F-value | p-value |
| Vocal diversity | FoxP2 | 1.381 | 0.257 |
| **Age** | **6.706** | **0.020** |
|  | FoxP2 x Age | 0.122 | 0.732 |
| Vocal plasticity | FoxP2 | 2.425 | 0.139 |
| Age | 0.000 | 0.997 |
| FoxP2 x Age | 0.189 | 0.670 |
| Vocal convergence | FoxP2 | 0.078 | 0.784 |
| Age | 2.706 | 0.120 |
| FoxP2 x Age | 0.354 | 0.560 |

**Discussion**

Downregulation of FoxP2 in vocal learning brain nuclei (Area X in songbirds and MMSt in parrots) relative to adjacent non-vocal learning regions is associated with both social context-dependent periods of vocal variability as well as active periods of vocal learning (Teramitsu & White, 2006; Whitney et al., 2015). A key factor differentiating taxa as closed-ended learners (vocal learning restricted to an early life sensitive period) or open-ended vocal learners (vocal learning continuing into adulthood) is thought to be the persistent downregulation of FoxP2 in the vocal learning nuclei of adult learners (Hara et al., 2015). Studies of FoxP2 expression patterns in open-ended learners, however, have defined adults as a single age class, leaving it unclear whether this vocal learning-related FoxP2 downregulation pattern is similar for adults of different ages, as is generally assumed. In this study, we sought to better understand age-related changes in vocal flexibility in an open-ended learner by conducting a vocal learning assay in which we formed novel flocks of either young adult budgerigars or older adult budgerigars, tracked contact call production over time, and measured neural FoxP2 expression levels from a subset of individuals. Findings of similar neural FoxP2 expression and vocal learning ability between young and older adults suggest that these open-ended learners largely maintain vocal flexibility into later adulthood, with little to no deterioration in this learning program.

***Persistent FoxP2 Downregulation Maintained in Old Age***

Although older adult budgerigars exhibited a significantly higher percentage of MMSt cells expressing FoxP2 compared to young adults, they exhibited a similar level of downregulation of FoxP2 in MMSt relative to the non-vocal learning adjacent striatum. Previous studies in adult budgerigars reported similar FoxP2 MMSt/VSP expression ratios as we found, although adults in these studies were broadly defined as being at least 120 days old, which is roughly at least 0.3 years old (Hara et al., 2015; Whitney et al., 2015). Given that budgerigars have a mean life expectancy of 4.57 years (Smeele et al., 2022; Young et al., 2012), our findings of downregulation in older adults in at least their 3rd year of age, suggest that neural plasticity in vocal learning circuits, as regulated by FoxP2, persists close to the end of the average life span of these open-ended learners. The older adult individual exhibiting the highest MMSt/VSP expression ratio of 1.25 was a bird obtained directly from our research colony and thus had a known hatch date of August 29, 2016 and could reliably be aged at 4.6 years old. Other older adults of comparable ages to this individual (and for whom hatch date is also known), ranging from 4.3 to 4.8 years old, had much lower expression ratios, ranging from 0.66 to 0.73, eliminating the possibility that the outlying high expression in this individual is due to a much older age than the rest of his cohort. Additionally, the lowest FoxP2 MMSt/VSP expression ratio, 0.46 was observed in an older adult, further supporting the maintenance of the neural underpinnings of vocal flexibility into old age in an open-ended learner.

***Role of FoxP2 Expression in Facilitating Vocal Learning***

Vocal learning measures were generally similar between age classes. While we did see a difference in vocal diversity (amount of acoustic space covered by an individual’s calls) between the two age classes, we saw no significant differences in vocal plasticity (acoustic dissimilarity between individuals’ starting and ending contact call repertoires) or vocal convergence (acoustic similarity between individuals’ contact calls to those of their flockmates at the end of the vocal learning assay). These findings generally support our hypothesis that older budgerigars are resilient to aging with respect to altering their calls and matching the calls of their flockmates. These results parallel findings from our larger experiment (N = 24 young adults; N = 24 older adults) from which this subset of bird was randomly selected for neural analysis (Moussaoui et al., 2023). Given that in the larger study, reduced vocal diversity in older birds coincided with fewer and weaker affiliative social bonds, it may be that this component of vocal learning is constrained more by social context than an age-related cognitive decline (Moussaoui et al., 2023). A previous study of age-related changes in song traits in female European starlings (*Sturnus vulgaris*), which are considered open-ended vocal learners, similarly reported a reduction of repertoire size in older adults in both cross-sectional and longitudinal analyses (Pavlova et al., 2010). Other studies, however, have found the opposite trend, such as an investigation of age-dependent song variation in open-ended learning collared flycatchers (*Ficedula albicollis),* which found that repertoire size increased with adult age as sampled from adult males with known ages ranging from 2-7 years old (Eriksen et al., 2011; Garamszegi et al., 2007).

Although young and older adults largely did not differ in two out of the three measures of vocal learning, we expected individual vocal variation to be explained by individual variation in neural FoxP2 protein expression levels. MMSt/VSP expression ratios of this gene, however, were not found to significantly predict any vocal learning characteristic we measured. This result might be explained by our sample sizes not capturing a wide enough range of individual variability in each of these measures necessary to establish such a relationship should it exist. It is worth noting that the relationship between FoxP2 and two of the vocal learning measures, vocal plasticity and vocal convergence, did trend in the directions that would be predicted based on FoxP2’s role in facilitating vocal learning.

Assessing both neural expression of FoxP2 in the parrot vocal learning nucleus, MMSt, and contact call learning in naturalistic flocks of captive budgerigars of two different adult age classes, we find support for continued vocal learning into late adulthood with largely the same fidelity as in 1st year adults, with respect to vocal plasticity and vocal convergence to flockmates. This is the first experimental study to confirm that the persistent downregulation of FoxP2 in MMSt relative to the adjacent striatum identified in parrots as a key contributing factor to their apparent life-long ability to learn new vocalizations is maintained during late adulthood and at the same levels as is observed in early adulthood.

**Data & software availability**

Repository: Neural FoxP2 expression in an aging open-ended vocal learner.

<https://doi.org/10.5061/dryad.gb5mkkwvj> (private for peer review)

<https://datadryad.org/stash/share/qKRObOqgUtSaVgcZ0nJfNFYjryWoTaJPLXTigmysaRw>

This project contains the following underlying data:

* CellCounts\_03\_09\_2023\_Complete\_Final.xlsx (Dataframe containing MMST/VSP expression ratios for each bird averaged across two imaged and counted brain sections.)
* Vocal\_Brain\_Correlation\_03\_09\_2023\_Final.r (R script consisting of code used for data analysis and statistics.)
* AgeLearn\_Treatments\_Master.csv (Dataframe containing experiment metadata such as bird identities and corresponding age class treatment.)
* agg\_dat.csv (Dataframe containing vocal learning measures for each bird and each recording block. Column names “acoustic.area”, “acoustic.overlap”, and “acoustic.overlap.to.group” correspond to vocal diversity, vocal plasticity, and vocal convergence, respectively.)
* Vocal\_Brain\_Corr\_03\_09\_2023\_Final.csv (Dataframe of FoxP2 MMSt/VSP expression ratios and vocal learning measures taken during the 5th recording block.)
* Vocal\_Brain\_Corr\_03\_09\_2023\_Final\_WithAdditionalMMSTVSP.csv (Dataframe of FoxP2 % expression in MMSt, FoxP2 % expression in VSP, FoxP2 MMSt/VSP expression ratio, and vocal learning measures taken during the 5th recording block.)

**Competing interests**

The authors declare they have no competing interests.

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