



THE IMPACTS OF STRESS AND AGING ON VOCAL PRODUCTION LEARNING IN ADULT BUDGERIGARS

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ABSTRACT

While death and taxes are widely regarded as inevitable, stress and aging are nearly as ubiquitous. We have used the budgerigar (*Melopsittacus undulatus*), an open-ended vocal learner, to investigate the effects of chronic stress and aging on vocal production and learning in adults. We assayed vocal learning by monitoring changes in acoustic space overlap of contact calls in newly-formed flocks of unfamiliar individuals. In our stress study we contrasted these patterns between flocks subjected to minor randomized disturbances in the captive environment versus baseline controls; in our aging study we contrasted them between flocks of young versus older adults. We found that our stress protocol impacted physiological markers of stress and some measures of vocal learning, with stressed birds producing more contact calls and showing less vocal plasticity in calls than controls, but similar levels of vocal diversity and vocal convergence. For aging, we found that older birds had lower vocal diversity than younger birds. Older birds also had fewer social interactions than younger ones, and social networks based on affiliative interactions were sparser, with weaker ties, in flocks of older individuals. These results suggest that vocal learning, even in an adept open-ended learner, is not immune to the stresses of life and the ravages of time.

Keywords: *aging, budgerigar, stress, vocal production learning*

1. INTRODUCTION

Humans rely on vocal production learning to acquire and modify spoken language, our primary communication system [1]. While initial language learning typically occurs early in life during a critical juvenile period, language learning also occurs in adult immigrants, and in people recovering from post-stroke aphasia, post-traumatic stress disorder, and other traumatic brain injuries, albeit often with less facility [1-4]. Both aging and stress are ubiquitous and there is evidence that each of them negatively impacts the ability to learn new vocal signals [5,6]. Understanding these impacts, and the neuroendocrine mechanisms that underly them, could help mitigate their negative effects on social interactions and well-being in aging and stressed populations.

The budgerigar (*Melopsittacus undulatus*) is a gregarious small parrot that is commonly bred in captivity. Like many other parrots, it is capable of learning new vocalizations into adulthood [7]. Its primary vocalization is a contact call used by both males and females within flock contexts, where it appears to mediate social associations [8]. Individuals will develop a repertoire of 3-6 distinct contact call variants that are differentially shared with other members of a flock, and individuals introduced to a new flock will quickly learn to produce some call variants used by other members of that flock [9,10]. This rapid vocal production learning, coupled with rich social dynamics, provides an opportunity to investigate the impacts of stress and aging on vocal learning and social interactions.

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2. METHODS

To investigate these questions, we used a general approach of placing four mutually-unfamiliar individuals into a novel flock and then monitoring social interactions and changes in vocal repertoires due to learning over the subsequent three-week period. We recorded the vocalizations of individuals daily and used automated measures of acoustic variables to characterize the multivariate acoustic space occupied by the calls of each individual and each flock on a weekly basis [11]. We used these acoustic space characterizations to derive three measures of vocal learning: *vocal diversity*, the amount of acoustic space occupied by an individual's calls, *vocal plasticity*, the degree of acoustic space overlap between an individual's calls in the first week versus subsequent weeks (with higher overlap indicating less plasticity), and *vocal convergence*, the amount of acoustic space overlap between an individual's calls and those of its flock mates [11]. Physical proximity, affiliative interactions and agonistic interactions were scored from video recordings and used to create social networks illustrating the strength of social interactions and the degree of social connectedness within flocks. We investigated potential underlying neuroendocrine mechanisms through weekly measurement of circulating corticosterone (CORT) and other physiological markers of stress, and measurement of expression of a gene with known involvement in vocal learning, *FoxP2*, in a budgerigar brain region devoted to vocal learning, the magnocellular nucleus of the medial striatum (MMSt).

We used these measures to assess the effects of chronic stress and aging on learning and sociality in two separate experiments. To study the effects of chronic stress, we subjected flocks of budgerigars either to a protocol of randomized disturbances in the captive environment (at either high or moderate levels of disturbance) or to the baseline stress of daily caretaking as a control (4 birds per flock, 3 flocks per treatment, 36 birds total). We then compared vocal learning behavior, physiological stress measures, and FoxP2 protein expression in MMSt between the three treatments. To study the effects of aging, we constructed flocks of either young adults or of older adults who were nearing the mean life expectancy of captive budgerigars (4 birds per flock, 6 flocks per age class, 48 birds total). We then compared sociality, vocal learning and FoxP2 protein expression in MMSt between the two age treatments.

3. RESULTS

3.1 Effects of chronic stress on physiological stress markers, vocal learning and neural gene expression

The chronic stress protocol resulted in significantly higher levels of baseline CORT in the high stress treatment than in flocks subjected to moderate disturbance or baseline controls (Fig. 1). We found no consistent effects of the stress protocol on absolute values of acoustic measures of calls, but birds in the high stress treatment had a higher vocal output (i.e. called more) than those in the moderate treatment or baseline controls. Birds in the high stress treatment showed lower vocal plasticity (i.e. had more overlap with their original repertoires) than birds in the other treatments (Fig. 2). The three treatments did not differ in vocal diversity or vocal convergence. There were no differences among the three treatments in expression levels of FoxP2 protein in MMSt relative to expression levels in the neighboring ventral striatal-pallial (VSP) region not involved in vocal learning.

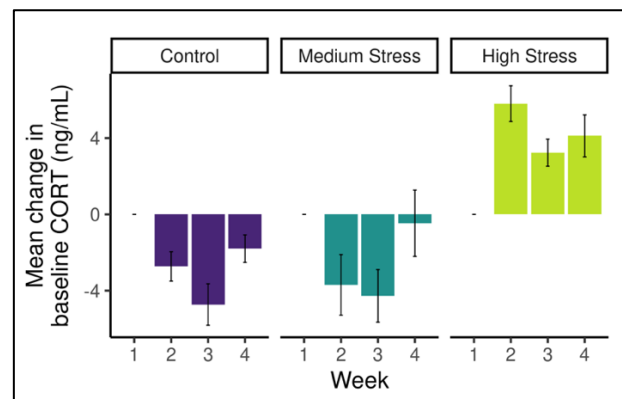


Figure 1. Baseline corticosterone (CORT) was higher in budgerigars subjected to the high stress treatment with higher levels of randomized disturbances in their captive environment.

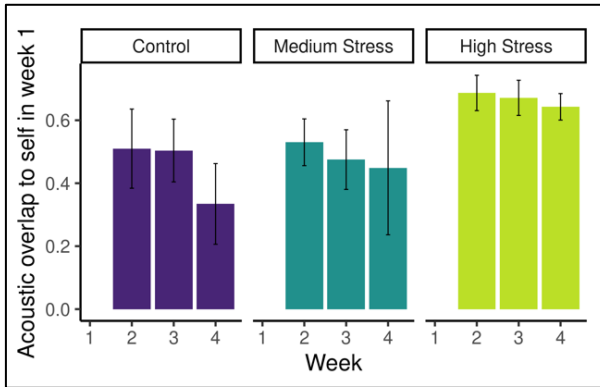


Figure 2. Vocal plasticity, as measured by the degree of overlap in acoustic space of each individual to its initial repertoire, was lower in budgerigars subjected to the high stress treatment.

3.2 Effects of aging on vocal learning and sociality

In our comparisons of flocks composed of either young or older adults, older adults showed lower vocal diversity (i.e. occupied a smaller amount of acoustic space) than did younger adults (Fig. 3). There were no differences between the two age classes in vocal output, vocal plasticity, or vocal convergence during the three-week experiment. Social networks constructed from affiliative interactions were denser and had stronger ties in flocks of young adults than in older adults (Fig. 4). There were no differences between the two age groups in networks constructed from proximity measures or agonistic interactions. There were no differences between age groups in expression levels of FoxP2 protein in MMSt relative to expression levels in the neighboring VSP.

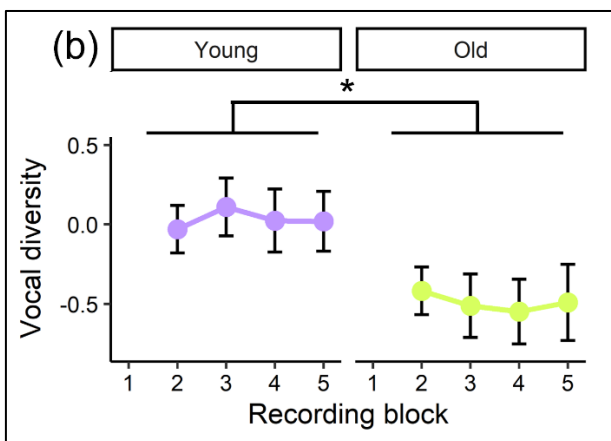


Figure 3. Vocal diversity, as measured by the amount of acoustic space occupied by an individual's calls relative to week 1, was higher in young than in older adult budgerigars.

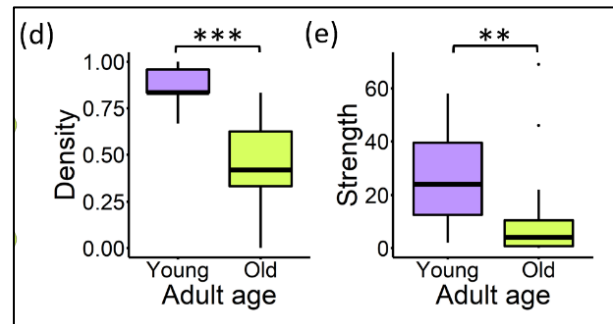
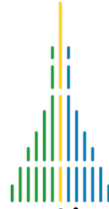


Figure 4. Flocks of young adults showed higher density and strength than did flocks of older adults in networks constructed from affiliative interactions.

4. DISCUSSION

Our experiments with flocks of captive budgerigars indicate that both chronic stress and aging impact some dimensions of vocal production learning. Our chronic stress protocol resulted in measurable impacts on physiological measures of stress and in turn resulted in higher vocal output and lower vocal plasticity in birds in the high stress treatment. That vocal convergence, or the degree of vocal matching, did not differ between the three stress treatments, may be due to the fact that we did not start recording vocalizations until after flocks had formed and the stress protocol had begun. This aspect of our design allowed for the possibility that all flocks underwent rapid learning-based convergence in the initial few days of the protocol, before chronic stress began to have an impact. Our aging experiment described here, and other ongoing experiments, are sampling vocal repertoires before flock formation to provide a better baseline for assessing vocal learning. We are also developing backpack microphones to allow more comprehensive sampling of the vocal repertoire and finer-scale mapping of the learning process.

We found measurable impacts of age on vocal learning and social interactions in the budgerigar. Older adults had lower vocal diversity than young adults. They also engaged in fewer affiliative interactions, with fewer individuals, than did young adults. It is possible there is a direct connection between the two measures; if vocal learning functions to



produce shared contact call repertoires with close social associates, then older individuals with fewer close social associates may learn fewer shared calls. Conversely, if aging diminishes vocal learning ability, then this senescence might impact both vocal diversity and the ability to engage in affiliative interactions with flock mates. The absence of measurable differences in vocal plasticity and vocal convergence suggest that age-related sociality is driving differences in learning, rather than vice versa, but additional experiments would be required to truly distinguish between these alternatives.

Neither experiment showed strong impacts on expression levels of the language-associated gene *FoxP2*. Previous experiments in both zebra finches and budgerigars have suggested that this gene contributes to the regulation of vocal plasticity [12,13], so our lack of differences is somewhat surprising given the effects of stress and aging on some aspects of vocal learning observed here. Studies of differential gene expression in neural regions associated with learning in both the zebra finch and the budgerigar have implicated a broad array of genes involved in vocal learning [14] and we are currently using these approaches to investigate specific changes with stress and aging. Such studies will further enhance our understanding of how vocal learning might be susceptible, or resilient, to the timeless tolls of stress and aging.

8. REFERENCES

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