Inhibition of HDAC3 Modulates Neuronal Memory for Vocal Formation in Zebra Finches

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Abstract

Taeniopygia guttata, otherwise known as the Zebra Finch, is a songbird, a member of one of the few animal groups, besides humans, that is capable of producing learned vocalizations. Similar to humans, male songbirds produce songs by copying adult caregivers during a critical period as juveniles, relying on auditory feedback to manipulate the song produced into one that is unique to each individual. We took advantage of the critical period necessary for the formation of birdsong by eliminating any exposure to adult song for the hatchlings during that period. In doing so, male songbirds grew up to produce an isolate song, having higher variance than that of a tutored song. In this experiment we studied adult male songbirds, who produced an isolate song as a result of no exposure to a tutor song during the critical period. We asked if we can use an epigenetic approach along with exposure to an adult song to reopen the critical period and to allow for modifications of the isolate song. We used an HDAC3-I (RGFP966) to enhance gene expression mechanisms that are biased for the learning and remembering of conspecific songs. Our results indicated that the birds injected with RGFP966 (n = 2) incorporated more features of the tutor song than vehicle injected subjects (n =2). Our data suggested that RGFP966 might have reopened the critical period thus allowing for the incorporation of new song elements.

Background

Work in the Vicario Laboratory, using adult male zebra finches, have shown that epigenetic mechanisms can enhance and extend the long term formation for species specific songs. *In my project, we asked if a similar mechanism can allow an adult male that is singing a crystallized, isolate song to incorporate new song elements.* In fact, by making zebra finches, which are close-ended learner, reopen their critical period and modify their song, they become open-ended learners like the canaries and starlings.

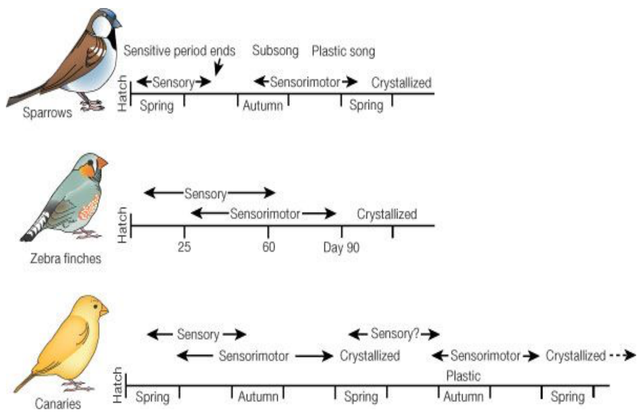


Figure 1: Songbird learning exemplifies the neuroethological approach of neuronal plasticity and the crucial role the critical period plays in vocalization. This study uses zebra finches, whose development of bird song has a relatively clear and stereotypic pattern (figure 1; Brainard, Doupe 2002).

Materials and Methods

In the typical procedure, both male and female Zebra Finches are raised with both parents in the general aviary until day ten. At day ten, hatchlings and their mother are separated from their father into isolation chambers where they continue to grow until day twenty-five. At day 25 when birds become independent, each male juvenile is placed in a separate isolation chamber, their mother is returned back to the general aviary, and tutoring of the males begins. At day 45, each bird is introduced to a standard conspecific song ("SAMBA", the tutor song) through a playback initiated by a key peck. The bird receives 20 exposures of the standardized tutor song per training day (10 in the morning and 10 in the afternoon). After, tutoring ends and song analysis begins, recordings of their final song are taken at approximately day 110 (**Figure 2;** Phan et al., 2006). This paradigm results in successful imitation of the tutor song in exposed birds. In this study, instead of males beginning tutoring with SAMBA at day 25, they remained with their siblings in the isolated chamber until day 80. At approximately 80 days after hatching, four adult male songbirds were selected at random from the chamber and then placed in separate cages found in the isolation chambers, which prevent any noise from being heard inside or outside of the cages, where baseline recordings took place of their isolate songs and tutoring began (**Figure 3**).

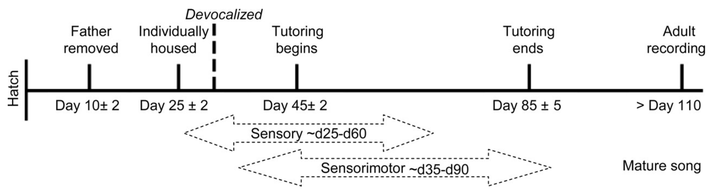


Figure 2: In a typical experiment, males begin tutoring with a standardized tutor song ("SAMBA") at day 25. This paradigm results in successful imitation of the tutor song in exposed birds. In this study, males are not exposed to SAMBA until they are adults.

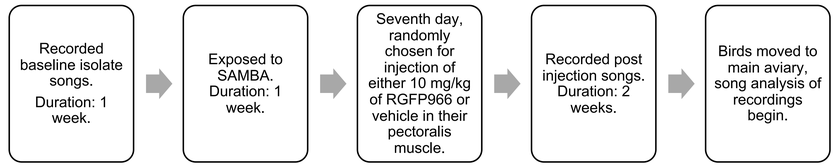
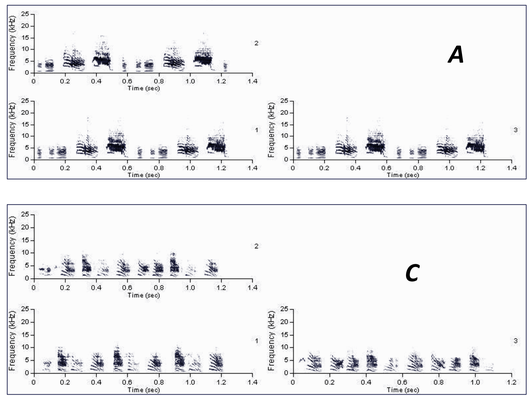


Figure 3: In this study, tutoring with SAMBA began when males were adults (~80 phd) and they had already established a crystalized isolate song.

Results and Discussion

- We propose that HDAC3-i may have led to the effective re-opening of the sensitive period to allow for modification of the isolate song to occur. Without the inhibitor, the sensitive period remains closed and modification of the isolate song occurs at a slower rate.
- It's unclear, however, whether male songbirds were influenced in the production of their song. During the normal critical period, this study's birds grew up with their siblings so it may be possible that the isolate songs produced by their brothers influenced their own song. To determine whether this is the case, a future experiment can be done in which males are raised with only females to determine the effect siblings can have on the production of their isolate song.
- Understanding songbird learning is critical in understanding the production of human language. If we are able to re-open the sensitive period to allow modification of the isolate song into a more structured one, it may prove to be quite useful in aiding children with learning disabilities and their language production.



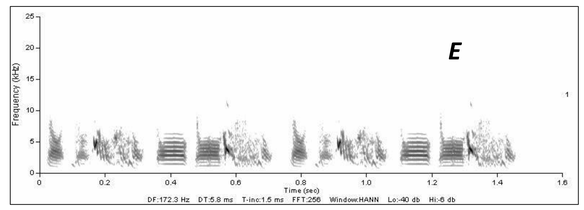


Figure 4: Spectrogram of three songs of the vehicle bird at the start of the BASELINE period (*A*) and at the end of the POST period (*B*) as well as spectrograms of the songs of the drug bird at the start of the BASELINE period (*C*) and at the end of the POST period (*D*). Each song was compared to a standardized tutor song (SAMBA) *(E)*.

	Vehicle	Drug
Similarity to SAMBA BASELINE period	38.3%	31.5%
Similarity to SAMBA POST period	39.1%	50.1%

Figure 5: Mean comparison between songbird injected with vehicle (n=1) and drug RGFP966 (n=1). There is an increase in similarity percentage from the BASELINE period to the POST period and those injected with vehicle had a small rise in similarity percentage throughout the study.

References and Acknowledgements

[1] Phan, M. L., Pytte, C. L. & Vicario, D. S. Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. *Proc. Natl Acad. Sci.* USA 103, 1088–1093 (2006).
[2] Brainard, M. S. & Doupe A.J. What songbirds teach us about learning. *Nature* 417, 351-358 (2002).
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