An Avian Basal-Ganglia Forebrain Circuit Modulates the Reversal of Externally Reinforced Changes to Adult Zebra Finch Song

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Abstract:

Songbirds learn their songs through a trial and error process that shows remarkable similarities to human language learning, making them an ideal model for studying the neural substrates of vocal learning. Although adult zebra finch song is generally highly stable, a recent white noise aversive reinforcement learning paradigm has made it possible to shift the pitch of targeted song syllables. When aversive reinforcement is stopped, syllable pitch recovers to its stable baseline value over the course of several days. This recovery provides evidence that zebra finches are intrinsically motivated to match song performance to a previously memorized target version of the song. In this study, I tested the hypothesis that the lateral magnocellular nucleus of the anterior nidopallium (LMAN), a cortico-basal ganglia outflow nucleus implicated in both juvenile and externally reinforced adult learning, is necessary for intrinsically motivated pitch recovery. I drove down the fundamental frequency of targeted song syllables using white noise aversive reinforcement. I then performed bilateral electrolytic lesions of LMAN to determine whether normal pitch recovery would take place without LMAN activity. All three birds lesioned demonstrated significantly reduced recovery rates, providing convincing preliminary evidence that LMAN is implicated in song recovery. However, these results were not conclusive due to small sample size and the lack of histological data to verify lesion efficacy. Further characterization of the role of LMAN in pitch recovery could provide a valuable context for explaining phenomena associated with human language re-learning, such as how stroke victims might have difficulty recovering speech, or how adults are able access and easily re-learn elements of languages to which they were exposed during early childhood.
Introduction:

Humans are remarkable in their capacity to learn one or more languages. Of particular interest in the study of human language learning is the means by which the adult brain is able to recover a forgotten language. For example, childhood experiences with a language result in improved pronunciation and grammar in those who re-learn the language as adults as compared to novice speakers, indicating that early language experience may have a lasting impact on memory networks which are accessed during re-learning (Oh et al. 2002; Au et al. 2008). An understanding of this process is also medically important, as it could help to explain why language recovery is often impaired in victims of stroke-induced aphasia (Sinanovic et al. 2011). The detailed neural mechanisms underlying the reacquisition of a previously learned vocal pattern remain unknown. A major limitation is that the circuits underlying language learning in humans are not well mapped (Doupe and Kuhl 1999). Another is that experiments that seek to test causal relationships between neural circuits and vocal learning are simply impractical in humans (Doupe and Kuhl 1999). Songbirds provide a great opportunity to understand neural circuit mechanisms important to the reacquisition of a vocal pattern that was previously learned and then overwritten.

Songbirds undergo a process of song learning that has many parallels to human language learning (Marler 1970a) and is mediated by a specialized cortical-basal ganglia circuit called the Anterior Forebrain Pathway (AFP) (Doupe and Kuhl 1999). The study of this specialized circuit could allow for insights into the neural mechanisms involved in human language learning which have been difficult to fully characterize due to the experimental intractability of the human brain (Doupe and Kuhl 1999). Although the AFP is unique to songbirds, its basic patterns of connectivity are similar to those found in mammalian basal-ganglia circuits with nuclei situated
in the striatum, thalamus, and frontal cortex (Doupe et al. 2005). In addition to structural similarities, songbirds and humans also exhibit parallel patterns of song and speech learning. For example, both undergo vocal learning during a sensitive period early in life through exposure to and imitation of adult vocalizations, and through sensorimotor practice become capable of producing mature vocalizations that closely resemble the external model (Marler 1970a; Doupe and Kuhl 1999). Due to the structural and behavioral parallels between language and song learning, the study of the songbird AFP could provide insight into the specific functions of basal-ganglia circuits in human language learning. In this study, we assessed the role of the lateral magnocellular nucleus of the anterior nidopallium (LMAN), a frontal cortical analogue that serves as the output nucleus of the AFP, in the recovery of syllable pitch after aversive reinforcement learning in adult zebra finches.

In songbirds, song learning occurs in two distinct phases (Mooney 2009; Roberts et al. 2012). In the sensory learning phase, exposure to the song of a male tutor early in development results in the formation of a tutor engram, or a memorized version of the tutor song (Marler 1970b; Funabiki and Konishi 2003; Mooney et al. 2008; Roberts et al. 2012). During sensorimotor learning, auditory feedback generated from song performance is continuously compared to this memorized template (Marler 1970b; Mooney et al. 2008). This comparison is thought to generate an instructive signal that drives changes in song toward the stored target (Brainard and Doupe 2000; Andalman and Fee 2009). These learning processes result in the acquisition of a “crystallized” song that is characterized by a stereotyped and highly stable yet complex spectrotemporal structure (Roberts and Mooney 2013).

Although mature birdsong is generally highly stable, a recently developed aversive reinforcement learning paradigm has made it possible to condition adult birds to modify the
frequency of previously stable syllables. This paradigm, developed by Tumer and Brainard (2007), takes advantage of the very slight natural rendition-by-rendition variation in the fundamental frequency (i.e. the pitch) of crystallized adult song. A computer can be programmed to detect real time variations of pitch in a target syllable and play white noise when the syllable is sung above or below a specified pitch threshold (Tumer and Brainard 2007). Over many hours, exposure to the white noise causes the bird to shift the pitch of the syllable to avoid the noise (Tumer and Brainard 2007). Thus, learned changes can be achieved in adult song by using external reinforcement to exploit the bird’s natural vocal variations (Tumer and Brainard 2007).

When performed over many hours and days with periodic threshold adjustments, average syllable pitch can be shifted significantly away from the baseline pitch value (Tumer and Brainard 2007). When the aversive treatment is discontinued, syllable pitch recovers back to its baseline level through a process that mirrors learning under the aversive reinforcement learning paradigm, but does not depend on external reinforcement with aversive or appetitive stimuli (Tumer and Brainard 2007). Identifying the neural mechanisms that enable this process of song recovery may help to provide insight into the neural processes that underlie our capacity to recover skills that are learned early in life but fall into disuse, including those that result from early multilingual experience.

Two separate basal-ganglia circuits drive these processes of song performance and learning. The song motor pathway (SMP) is essential to song timing and production, whereas the aforementioned AFP is necessary for the expression of vocal plasticity in both juvenile and adult songbirds (Roberts and Mooney 2013). Lesions of the AFP in adult birds generally have little effect on normal song performance which, in crystallized song, is primarily mediated by the SMP (Roberts and Mooney 2013). However, because aversive reinforcement learning allows for
the expression of plasticity in adult song, it has become possible to determine the role of the AFP in adult pitch learning and recovery.

The AFP is necessary to juvenile song learning: LMAN lesions in juvenile birds result in the crystallization of immature song, indicating that AFP output is necessary for sensorimotor learning (Bottjer et al. 1984). AFP activity is also necessary for the expression of plasticity in adult learning, as inactivation of LMAN in adult birds prior to aversive reinforcement learning prevents plastic changes in song driven by external cues (Charlesworth et al. 2012). Additionally, LMAN inactivation during aversive reinforcement learning has been shown to result in an immediate regression of learned changes in song (Andalman and Fee 2009). These results suggest that the AFP is the source of an error-reducing adaptive motor bias signal that drives learning in adult birds in response to external stimuli (Andalman and Fee 2009).

Although the AFP is necessary to adult pitch learning in response to external reinforcement using white noise, its role in internally motivated song recovery has yet to be fully characterized. There is evidence that output from the AFP could play a substantial role in the ability to recover song after aversive reinforcement learning. Recovery to baseline pitch after white noise is discontinued is thought to occur in a manner analogous to sensorimotor learning wherein the comparison between singing-related auditory feedback and the stored tutor engram generates an error signal to drive adaptive changes to song production (Brainard and Doupe 2000). Consistent with this model, Warren et al. (2011) found that transient inactivation of LMAN reverses recent pitch recovery. Although these findings suggest that AFP activity is necessary for recovery, it has not yet been shown whether recovery can take place in the absence of AFP output altogether.

We hypothesized that AFP activity is necessary for recovery following aversive
reinforcement learning. To test this hypothesis, we used targeted white noise to shift the fundamental frequency of a specified syllable away from its baseline level. After this shift, we lesioned LMAN to determine whether normal recovery of baseline pitch is able to proceed in the absence of AFP output.

**Methods:**

All experiments were performed on adult (>90 days-post hatch) male zebra finches (*Taeniopygia guttata*). Pitch learning was driven using white noise aversive reinforcement according to procedures described by Tumer and Brainard (2007) and Warren et al. (2011). During pitch learning, birds were housed in individual soundproof acoustic chambers. LabView software (EvTaf) was used to record song and deliver white noise feedback. Songs were recorded for 2-3 days prior to learning to measure the fundamental frequency distribution of a target syllable, typically a harmonic stack or tonal syllable in the bird’s motif (fig. 1B). A single target syllable was chosen and a template based on a power spectrum of 5% of baseline renditions of this syllable was created. In all cases this template was able to recognize >90% of labeled syllables. A contingency time was specified for the chosen syllable at which point the EvTaf software is designed to record the time and calculate the fundamental frequency of the previous 4 ms of song. If the monitored frequency value fell above the designated threshold (the 25th percentile of previously measured rendition frequencies) white noise was played to deliver disruptive feedback. If the fundamental frequency of the rendition was below the threshold, white noise was withheld. Additionally, to allow for post-training frequency analysis, EvTaf conditions were specified so that no noise was played in a random 5% of song renditions, creating a ‘catch’ trial.
The fundamental frequency of all catch trials was calculated each day and a new threshold for white noise was set at the 25th percentile of these pitch measurements. Because learned changes in the syllable accrue over time and cause the template to become less well matched to the current syllable conditions, it was necessary to create a novel template every 1-2 days so that > 90% of syllables could be recognized. Training times varied from 5 to 7 days due to differences in learning rates and responses to aversive reinforcement of individual subjects.

In all birds, learning was driven until a highly significant (>2 SD) change in the fundamental frequency of the target syllable from its baseline level was achieved. After this shift, the threshold was held at the same level for 2-7 full days to allow for pitch consolidation to occur (Fig. 1C). Allowing time for consolidation was necessary because it has been shown that in adult Bengalese finches, learned changes in pitch remain dependent on LMAN activity for several days after a learned shift in fundamental frequency is first expressed (Warren et al. 2011). Thus, it is likely that learned changes to zebra finch song also remain LMAN-dependent for a substantial duration after the initial expression of learning, although results from Andalman and Fee (2009) suggest that consolidation occurs more quickly in zebra finches than in Bengalese finches. The extended consolidation period controlled for the possibility of the reversion of learned changes toward baseline due to the lesion, which could be a potential confound for active pitch recovery.

After aversive reinforcement learning and pitch consolidation were complete, white noise was turned off and recovery was allowed to proceed in order to establish a within-subject baseline recovery rate. This baseline recovery rate was measured in only two of the three birds in this study (unfortunately, the procedure was modified to include within-subject controls only after the first subject had been subjected to LMAN lesions). Upon establishment of baseline
recovery rate, the pitch learning procedure was repeated, and after consolidation had occurred, LMAN was lesioned. Electrolytic lesions were placed in LMAN according to established stereotaxic coordinates (4.9mm anterior and 1.85 lateral from the Y-sinus at a depth of 2000-2100 µm) and the accuracy of the targeting was verified by recording and observation of the bursts of neural activity that characterize the region. Electrolytic lesions were performed bilaterally using monopolar electrodes to pass a current of 100[µ]A into the LMAN for 60 seconds at five different locations surrounding the nucleus. After the lesion was made, the subjects were placed into a sound chamber and songs were recorded for 9-11 days without any white noise. Although the timeline of this thesis did not allow sufficient time for histological measurements of the lesion sites, counts of remaining LMAN neurons in the ablated region will be performed to verify the efficacy of the lesions.
**Fig. 1:** Experimental design. (A) Shows targeting of electrolytic lesion in LMAN. Adapted from “Neurobiology of Song Learning,” by R. M. Mooney, 2009, *Curr Opin Neurobiol*, 19, pp. 654-660. (B) Song syllables targeted by white noise learning are outlined in black. (C) Example schematic of white noise learning paradigm.

**Results:**

I subjected three adult male zebra finches to an aversive reinforcement pitch learning paradigm developed by Tumer and Brainard (2007) and refined by Andalman and Fee (2009) and Warren et al. (2011) in which white noise was used to drive the pitch of a target syllable below its baseline level. In all cases, pitch of the target syllable was driven more than three standard deviations from its baseline median, resulting in a >5% decrease in pitch relative to the baseline value. LMAN lesions were shown to impede recovery in all birds as compared to pre-lesion controls (Fig. 2). Average percent recovery per rendition was significantly lower post-lesion than pre-lesion across birds (p < .01, student’s t-test) (Fig. 3). Additionally, within bird comparisons all showed significantly reduced recovery rates post lesion (p < .01 in all within bird comparisons, paired t-test) (Fig. 3). Furthermore, the standard deviation of pitch, representing rendition-to-rendition pitch variation, was significantly reduced in all subjects following LMAN ablation (p < .001, two sample t-test) (Fig. 3), consistent with prior studies and suggestive that our electrolytic lesions encompassed much or all of LMAN (Andalman and Fee 2009; Brainard and Doupe 2000). Notably, in two birds subjected to longer consolidation times, LMAN lesions rapidly displaced the pitch of the target syllables further away from the baseline value, while immediate slight reversion occurred in the other subject, in which the consolidation period was limited to only two days (Fig. 4). These immediate changes were not observed to occur during the pre-lesion recovery.
Fig. 2: LMAN lesions slow pitch recovery. In all three birds, restoration of baseline pitch occurred at a significantly reduced rate post-lesion (red lines) as compared to pre-lesion (blue lines) \((p < .01, \text{ student’s t-test})\). Pitch trajectory is normalized so that \(-100\) represents the total learned shift, while \(0\) corresponds to baseline pitch (grey dashed line). Intermediate values represent the amount of shift maintained. Rendition number was highly variable between birds, but was normalized within birds to be able to compare pre and post-recovery rate per rendition.
**Fig. 3:** LMAN lesions impair pitch recovery to baseline and result in a decrease in rendition-to-rendition variation as compared to baseline control. (A-B) Show pre-lesion recovery trajectory (blue) compared to post-lesion trajectory (red). Recovery is normalized to percent difference from baseline pitch. Black lines represent a moving mean of percent change in pitch throughout the recovery period, while red and blue patches show a moving standard deviation. In (C), a pre-lesion recovery was not recorded, and so post-lesion data is compared to pre-lesion trajectories of the two other experimental subjects. In (A-C) the grey dashed line represents full recovery at 0% change from baseline. (A-C) Show that standard deviation in performed pitch was significantly decreased post-lesion in all subjects as compared to pre-lesion controls (p < .001, two sample t-test). (D) Shows average percent recovery per rendition pre- and post-lesion. Pre-lesion recovery rates were significantly higher than post-lesion rates (p < .01, student’s t-test).
Fig. 4: Lesions result in immediate changes in pitch either toward or away from baseline. (A-B) Show that an immediate shift in fundamental frequency away from baseline occurred in subjects blk79 (p < .001, paired t-test) and org73 (p < .001, paired t-test) post-lesion. Immediate reversion toward baseline was observed post-lesion in subject sil67 (p < .001, paired t-test). (C) An example aggregate heat-map of spectrograms of the last 100 renditions pre-lesion (left) and the first 100 renditions post-lesion (right) in subject blk79 shows a pronounced downward shift in fundamental frequency, but no change in overall song structure.

Discussion:

In this study I performed bilateral lesions of LMAN after aversive reinforcement learning in zebra finches to test the hypothesis that LMAN ablation impedes pitch recovery. The preliminary data I obtained support the idea that LMAN lesions impair normal pitch recovery in adult zebra finches. I found that recovery rate was significantly diminished post-lesion as compared to pre-lesion controls in all experimental subjects (n = 3) (Figs. 2-3).
Because varying degrees of recovery occurred in all three lesioned birds, my results were only somewhat consistent with those of Warren et al. (2011) who showed that LMAN activity is necessary for pitch recovery. It is possible, however, that the observed recovery was due to incomplete lesions which reduced but did not fully impede LMAN functionality. Thus, it will be necessary to collect histological data to determine the number of LMAN cells that survived the lesion. A second possibility is that recovery occurred due to an unidentified mechanism that also contributes to the expression of learned changes in song. This seems unlikely, however, as LMAN lesions in juvenile birds undergoing song learning have been shown to result in the permanent loss of ability to express plasticity in song (Bottjer et al. 1984).

The data showed several trends characteristic of song in LMAN lesioned birds that have been previously observed in the literature. First, consistent with a trend first observed in adult birds by Kao and Brainard (2006), LMAN lesions resulted in a decrease in rendition-to-rendition variation in pitch (Fig. 3). This observation supports previous findings that LMAN plays an active role in inducing variability in song (Mooney, 2009). It has been proposed that this reduction in variability could play a causal role in LMAN’s mediation of vocal plasticity, as acute variability in song produced by LMAN activity could be acted upon to produce more substantial changes in fundamental frequency over time (Mooney, 2009). Although this study cannot address whether or how reduced variability in pitch affects recovery rate, the connection between pitch variation and recovery merits further study.

A second notable observation is that the expression of learned changes in pitch became increasingly consolidated, or independent of LMAN activity, over time, a result consistent with findings reported by Warren et al. (2011) (Fig. 4). Consolidation of learned changes in pitch was achieved by holding the threshold for aversive reinforcement at a stable level over a series of
days. Allowing for consolidation minimizes the acute contributions that LMAN makes to the learned pitch, the loss of which following LMAN lesions could potentially be interpreted as recovery. In fact, in the one bird that only underwent pitch consolidation for two days, a significant reversion in pitch toward the baseline level was observed immediately post-lesion (Fig. 3). This result indicates that the most recent learning was still LMAN dependent at the time of the lesion. In the two birds that underwent pitch consolidation for seven and nine days respectively, a significant further decrease in pitch away from the baseline level was observed post-lesion, demonstrating that learned changes in pitch had been fully consolidated and were independent of LMAN activity (Fig. 3). One drawback of this study is that pitch consolidation time was not consistent for within bird comparison of recovery rate pre and post-lesion. It is possible that consolidation could somehow play a role in slowing recovery, as it can be imagined that LMAN is better able to modulate plastic changes in pitch that are dependent, rather than independent of its function. Thus, it would be worthwhile to establish whether consolidation time affects recovery rate.

The occurrence of both a reduction in variation and immediate changes in pitch post-lesion (either reversion toward baseline or away from baseline depending on consolidation time) provides evidence that LMAN was at least partially ablated in all three birds. The significant effect of LMAN lesions on normal pitch recovery rate in all three birds suggests that the AFP drives intrinsically motivated pitch recovery. Thus, in addition to mediating song learning, and external reinforcement learning, my results suggest that the AFP also facilitates the recovery of previously learned behaviors. This provides two valuable insights into vocal learning mechanisms. First, the AFP continues to play an active and primary role in the maintenance of
song structure well into adulthood. Second, externally reinforced and intrinsically motivated learning both occur via similar processes that require an intact LMAN.

Although my results show that juvenile song learning, external reinforcement learning, and recovery all rely on AFP activity, they do not provide insight into whether or how inputs into the AFP, or even AFP activity itself might vary during these different forms of learning. For example, recovery is thought to occur via an instructive signal that is produced by the mismatch between the performed song and the target engram. This is supported by the Canopoli et al. (2014) finding that the caudal medial nidopallium (NCM), a region of the auditory cortex which is thought to contain a central representation of tutor song memory, is necessary for pitch recovery. NCM activity, however, is not required for externally reinforced learning (Canopoli et al. 2014). Thus, it is likely that the process of error recognition differs between externally reinforced and intrinsically motivated learning. Further characterization of AFP inputs as well as the higher order AFP nuclei, DLM and Area X, is necessary for distinguishing whether and how the mechanisms of juvenile learning, external reinforcement learning, and recovery differ.

As suggested by Roberts and Mooney (2013), a possible means of further characterizing the role of the AFP and higher order neurobiological substrates in song learning could involve the pairing of online pitch learning programs such as the one used in this study paired with optogenetic manipulation. This would allow for spatially and temporally precise targeting of the various inputs or nuclei of the AFP that could be programmed to coincide with specific aspects of song.

The identification of the AFP as the primary mediator of both juvenile and adult, as well as externally and internally driven forms of song plasticity is an important step in understanding how and by what means different forms of vocal learning occur. Still, there is much work to be
done in characterizing the specific differences in the neural processes that underlie the distinct types of learning and recovery. Although birdsong typically is a simpler vocal behavior than human speech, a possible contribution of this study is to provide insights into the brain mechanisms that enable the reacquisition of previously dormant speech skills.
References


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