Goal-directed vocal planning in a songbird

Anja T. Zai[1,3], Anna E. Stepien[1,3], Sophie Cavé-Lopez[1], Nicolas Giret[2], Richard H.R. Hahnloser[1,3]

Author Affiliations:

[1] Institute of Neuroinformatics, University of Zurich and ETH Zurich, 8057 Zurich, Switzerland

[2] Institut des Neurosciences Paris Saclay, UMR 9197 CNRS, Université Paris Sud, Université Paris Saclay, 7 Orsay, France.

[3] Neuroscience Center Zurich (ZNZ), University of Zurich and ETH Zurich, 8057 Switzerland.

* Correspondence to R.H.R. Hahnloser: rich@ini.ethz.ch

Abstract

Songbirds' vocal mastery is impressive, but to what extent is it a result of practice? Can they, similar to humans, plan targeted changes to their songs in a practice-free manner without intermittently singing? In adult zebra finches, we drive the pitch of a song syllable away from its stable (baseline) variant acquired from a tutor, then we withdraw reinforcement and subsequently deprive them of song experience by muting or deafening. In this deprived state, birds do not recover their baseline song. However, they revert their songs towards the target by about one standard deviation of their recent practice, provided the latter signaled a pitch mismatch with the target. Thus, targeted vocal plasticity does not require immediate sensory experience, showing that zebra finches are capable of goal-directed vocal planning.

Significance Statement

Zebra finches are capable of making target-directed changes to their songs without requiring sensory feedback.

Main Text

Introduction

Speech planning is an important part of human communication and the inability to plan speech is manifest in disorders such as apraxia. But to what extent is targeted vocal planning an entirely human ability? Many animals are capable of volitional control of vocalizations (*1*, *2*), but are they also capable of planning to selectively adapt their vocalizations towards a target, such as when striving to reduce the pitch mismatch of a note in a song? Target-specific vocal planning is a cognitive ability that requires extracting or recalling a sensory target and forming or selecting the required motor actions to reach the target. Planning can be covert or overt. Evidence for covert planning is manifest when a targeted motor change is executed without intermittent practice, such as when we instantly imitate a word upon first hearing. Overt planning, by contrast, includes practice, but without access to the sensory experience from which target mismatch could be computed, such as when we practice a piano piece by tapping on a table.

1

The vocal planning abilities in animals and their dependence on sensory experience remain poorly explored. Motor learning has been mostly studied in tasks where a skilled behavioral response must be produced on the spot, such as when a visual target must be hit by a saccade or by an arm reaching movement (*3–6*). In this context, motor planning has been shown to enhance motor flexibility, as it allows separation of motor memories when there are conflicting perturbations (*7*). However, for developmental behaviors such as speech or birdsong that rely on hearing a target early in life (*8*, *9*), the roles of practice and of sensory feedback for flexible vocal control and for target-directed adaptation are unknown. The way in which vocal experience affects the maintenance of a learned vocal skill impacts the transmission and evolution of culture.

Recovery of a once-learned vocal skill could be instantaneous (covert), or it might require practice (overt). In support of the former, many motor memories are long-lasting (*10*), e.g., we can recall the happy-birthday song for years without practice. Some memories are even hard to get rid of such as accents in a foreign language. By contrast, practice-dependent, but feedback-independent recovery is argued for by arm reaching movements: following adaptation to biasing visual feedback, arm movements recover when the bias is either removed or the visual error is artificially clamped to zero (*4*, *5*). One explanation put forward is that motor adaptation is volatile and has forgetting built-in (*6*, *11*), leading to practice-dependent reappearance of the original motor program even without informative feedback (*11*). Given these possibilities, we set out to probe songbirds' skills of recovering their developmental song target when deprived of either practice or of sensory feedback.

Adult vocal performances in songbirds can be altered by applying external reinforcers such as aversive white-noise stimuli (*12*, *13*). When the reinforcer is withdrawn, birds recover their original song within hundreds of song attempts (*12*, *14–16*). We argued that these attempts may be unnecessary and birds could recover their original performance by recalling either 1) the original motor program (*17–19*), or 2) its sensory representation (*20*, *21*) plus the mapping required to hit it (*15*), or, 3) the sensory target (*22*) and the circuit for translating that into the original program (*14*), Fig 1A. These options might not need sensory feedback. Birds' large perceptual song memory capacity (*23*) would argue for such possibilities. That is, birds' song practice may be mainly expression of deliberate playfulness (*24*), conferring the skill of vocal flexibility rather than serving to reach a target, evidenced by young birds that explore vocal spaces close to orthogonal to the song-learning direction (*25*) and are already surprisingly capable of adult-like singing when appropriately stimulated (*26*).

Results

To test whether birds can recover a song syllable without practice, we first reinforced the pitch of a song syllable away from baseline and then we suppressed birds' singing capacity for a few days by muting their vocal output. We then tested whether the subsequently unmuted song has covertly reverted back to the original target. We used syllable pitch as the targeted song feature, because we found that birds did not reliably recover syllable duration in experiments in which we induced them to shorten or lengthen syllable duration, suppl. Fig. 1.

2

In these birds, we first drove pitch away by at least one standard deviation from baseline using an aversive white-noise (WN) stimulus (delivered whenever the pitch within a 16-ms time window locked to the targeted syllable was above or below a manually set threshold, see Methods), then we allowed them to sing a few hundreds of target syllables without reinforcement (birds did not recover a major fraction of the shifted pitch during this period, Fig. 1C). We assumed that unreinforced singing would initiate their song recovery process that we hypothesized birds would be able to accomplish during the muted period.

We muted these WNm (white-noise reinforced and muted) birds by implanting a bypass cannula into the abdominal air sac (see Methods). While muted, there is no buildup of high air pressure and there are no fast body vibrations from harmonic vocalizations, which essentially strips muted birds from all pitch experience. In some cases, the bypass cannula got clogged during the muted period and birds were spontaneously unmuted, allowing them to produce a few songs before we reopened the cannula, Fig. 1C-G. These spontaneous unmuting events were not detrimental to our experimental procedures, as they allowed us to inspect birds' current song motor program (Fig. 1C). After leaving birds several days in the muted state, we permanently unmuted them and kept them without further manipulations while recording their undisturbed songs.

After spending 5.1 ± 1.6 days (range 3-8 d, $N = 8$) in the muted state and upon unmuting, WNm birds displayed an average normalized residual pitch (NRP) of 89%, which was far from baseline (p=$6.2*10^{-8}$, tstat = -23.6, N=8 birds, two-sided t-test of H0: $NRP = 0\%$, songs analyzed in 2 h time window – early (E), see methods, Fig. 1), suggesting that in the muted state, birds are unable to recover their pre-reinforced songs. The average NRP in WNm birds was not significantly different from that in unmanipulated control birds (WNC) within the first 2 h after withdrawal of the reinforcer (p=0.87, tstat=-0.16, N=8 WNm and N=19 WNC birds, two-sided t-test, WNC: NRP = 91%, p=$7.9*10^{-12}$, tstat=15.5, two-sided t-test for NRP = 0%, N=19), revealing that 5 days without song practice did not lead to more recovery than is initially performed during normal song recovery. These findings did not sensitively depend on the size of the analysis window — we also tested windows of 4 and 24 h.

Subsequently, after 4 days of unmuted song experience (roughly 9 days after withdrawal of WN), WNm birds displayed an average NRP of 30%, which was significantly different from their NRP within the first 2 h after unmuting (p=$3*10^{-4}$, tstat=4.83, N=8 birds, two-tailed t-test early (E) vs. late (L) time window) but still significantly different from zero (p=0.04, tstat=2.59, N=8 birds, two-tailed t-test, late (L) time window). Possibly, birds did not fully recover pitch while permanently unmuted because of the preceding invasive treatments of muting and unmuting (see Suppl Fig. 2). Overall, these findings suggest that either motor practice, sensory feedback, or both, are necessary for recovery of baseline song.

Next, we therefore tested whether motor experience but not sensory experience is necessary for recovery, similar to arm reaching movements that can be restored without guiding feedback

3

(*4*, *27*). In a second group of WNd birds, we provided slightly more song experience (Fig. 2). Instead of muting, WNd birds were deafened through bilateral cochlea removal. This latter manipulation does not suppress the act of singing as does muting, but it eliminates auditory feedback from singing. Deaf birds could gain access to some pitch information via somatosensory stretch and vibration receptors and/or air pressure sensing. Our aim was to test whether such putative pitch correlates are sufficient for recovery of baseline pitch (Fig. 2A). However, in the deaf state, WNd birds did not recover baseline pitch even after 4 days of song practice: on the 5th day (late, L) after deafening, their average NRP was still 50%, which was different from zero (p=0.03, tstat=2.73, two-tailed t-test of H0: NRP=0%, N=10, Fig. 2D) and significantly larger than the average NRP of WNC birds on the 5th day since withdrawal of reinforcement (difference in $NRP = 48\%$, p=0.002, tstat=3.35, df=27, N=10 WNd and N=19 WNC birds, two-tailed t-test).

We speculated that the lack of pitch recovery in WNd birds could be attributable to the sudden deafening experience, which might be too overwhelming to uphold the plan to recover the original pitch target, even if animals are capable to do so. We thus inspected a third group of birds (dLO, Fig 3) taken from (*28*) that learned to shift pitch while deaf and that underwent no invasive treatment between the pitch reinforcing experience and the test period of song recovery.

dLO birds were first deafened, and after they produced stable baseline song for several days, their target syllable pitch was reinforced using pitch-contingent light-off (LO) stimuli, during which we briefly turned off the light in the sound recording chamber upon high- or low-pitch syllable renditions (*29*). dLO birds displayed an average NRP of 112% on the 5th day since release from LO, which was significantly different from zero (p=3.7*10^{-8}, tstat=25.4, N=8 birds, two-tailed t-test of H0: NRP=0) and was larger than the NRP in WNC birds on the 5th day since release (p=6.6*10^{-14}, tstat=14.8, df=25, N=8 dLO and N=19 WNC birds, two-sided t-test). Thus, dLO birds were unable to recover baseline pitch, suggesting that song recovery requires undiminished sensory experience, which includes auditory feedback.

That song practice and sensory experience are required for full recovery of song does not imply that without experience, birds are incapable of making any targeted changes to their songs at all. We therefore inspected birds' fine-grained vocal output and whether they changed their song in the direction of baseline when deprived of sensory experience. We hypothesized (Fig. 4A), that when birds experience a target mismatch during reinforcement (when they hear that their song deviates from the target), this mismatch will fuel their plan to recover the pitch target, and a portion of this plan they can execute without feedback. If, by contrast, they have no mismatch experience, they will make no corresponding plan. Hence, we predicted that WNd birds that experienced a pitch mismatch would slightly revert their song towards baseline even in the absence of auditory feedback. By contrast, dLO birds that did not experience a mismatch

because they did not hear their song while it was reinforced, would not revert towards the target, Fig. 4A.

Indeed, WNd birds, within 2 hours since release of reinforcement, already significantly changed their pitch towards baseline compared to the last 2 h of reinforcement (d'=-0.60, p=0.03, tstat=-2.19, df=9, N=10 birds, one-sided t-test of H0: d'=0) and a significant approach was still present after 4 days of practice, (d'= -1.27, p=0.02, tstat=-2.35, df=9, N=10 birds, one-sided t-test, Fig. 4B, E), showing that pitch reversion in deaf birds is reliable. Furthermore, in the initial 2 h time window of their deaf singing, their pitch reverted more than did the pitch in hearing control birds (WNC) in the same time window (difference in d' = -0.83, p=0.01, tstat=-2.67, df=27, two-tailed t-test, Fig. 4D, E), revealing that pitch reversion in deaf birds is initially faster than in hearing controls. Because the average pitch shift in WNd birds was on the order of one standard deviation ($d' \simeq 1$), we conclude that without song experience, birds are able to perform target-directed pitch shifts of about the same magnitude as their current exploratory range (i.e., the denominator of the d' measure).

In contrast, dLO birds showed no signs of reverting pitch, neither in the first 2 h since release of reinforcement (d'=-0.13, p=0.36, tstat=-0.37, df=7, N=8 birds, one-sided t-test), nor after 4 days of practice (d'=-0.08, p=0.43, tstat=-0.18, df=7, N=8 birds, one-tailed t-test, Fig. 4C, E). The NRP values suggested that their pitch showed a tendency to drift further away from baseline, Fig. 3C, D. But we found no support for the idea that dLO birds kept shifting pitch further away from baseline when looking at d' values (Fig. 4C, E). Namely, the pitch change in dLO birds was indistinguishable from that in deaf controls (dC) that were not pitch reinforced and had no plan to shift pitch in either direction (Fig. 4 A, F). To discount for the effect of time elapsed since deafening, we bootstrapped the difference in d' between dLO birds and dC birds in matched time windows (see methods). To discount for possible influences of circadian pitch trends, we assessed early and late pitch changes in reinforced birds and in dC birds also in 2 h time windows separated by multiples of 24 h: the result of all these analyses is that significant reversion towards baseline was only seen in WNd birds and very consistently so (Fig. F, Supplementary Table 1), showing that not current auditory feedback, but prior experience of a target mismatch is necessary for pitch reversion. Our results thus argue for a model of song maintenance in which birds extract from target mismatch experience a plan of reducing the mismatch (Fig. 5), and without sensory feedback, this plan is limited by the extent of current song practice.

Discussion
Our work shows that recent auditory experience can drive motor plasticity even while an individual is deprived of such experience, i.e. zebra finches are capable of overt vocal planning. But to reach a target beyond the range of recent experience necessitates auditory feedback, which sets a limit to their covert planning ability.

Birds' failure to recover baseline pitch without guiding sensory feedback agrees with reports that binary reinforcement (as we used) slows down or prevents forgetting of the adapted behavior (*5*). However, whereas forgetting is fast when sensory errors affect arm movements (*5*), the contrary applies to birdsong, where pitch learning from artificial sensory errors is slower and less forgotten (*30*) than is pitch learning from binary reinforcement (*12*, *14*). Hence, the commonality of short-term visuo-motor adaptation and of birdsong maintenance is that slow learning leads to slow forgetting, regardless of whether it is due to sensory errors or reinforcement. Such conclusion also agrees with observations that zebra finch song does not recover to pre-manipulated forms, both after restoring auditory feedback after long-term (>5 months) deprivation (*31*) and after restoring normal syrinx function after long-term (16 weeks) manipulation with beads (*32*), suggesting that song can spontaneously recover only within some time limit.

The overt planning ability suggests that recovery of a developmentally learned vocal target is controlled by two hierarchical processes, a highly flexible process with limited scope ($d' \simeq 1$, Fig. 4), and a dependent process enabled by experience of the former. Such motor learning based on separate processes for acquisition and retention is usually referred to as motor consolidation (*3*, *33*, *34*). Accordingly, the hierarchically lower process is independent of immediate sensory experience, but its consolidation requires experience. Perhaps then, it is the sensory experience itself that is consolidated, and therefore, consolidation of sensory experience may be a prerequisite for extensive planning.

Consolidation in motor learning generally emerges from anatomically separatated substrates for learning and retention (*4*). Such separation also applies to songbirds. Both reinforcement learning of pitch and recovery of the original pitch baseline depend on the anterior forebrain pathway and its output, the lateral magnocellular nucleus of the anterior nidopallium (LMAN) (*15*). LMAN generates a pitch bias that lets birds escape negative pitch reinforcers and recover baseline pitch when reinforcement is withdrawn (*13*), thus is likely involved in planning. This pitch bias is consolidated outside of LMAN (*15*, *35*) in a nonlinear process that is triggered when the bias exceeds a certain magnitude (*36*). This threshold magnitude is roughly identical to the planning limit we find ($d' \simeq 1$), suggesting that the consolidation of LMAN-mediated motor plasticity corresponds to birds' planning limit. Because LMAN seems capable of executing a motor plan without sensory feedback, our work provides a new perspective on the neural basis of birdsong learning and consolidation in and around LMAN.

The formation of a planned motor change may not require LMAN itself, because pharmacological suppression of LMAN sets the bias to zero, but upon removal of output suppression, the pitch of the song syllable that was targeted by reinforcement jumps by about 1% away from the reinforced pitch zone (*37*), which corresponds to about $d' = 1$ about the planning limit we find. Originally, this jump was interpreted as evidence of functional connectivity or an efference copy between the anterior forebrain pathway of which LMAN is part of and some other unspecified variability-generating motor area. However, in our view, a

6

simpler explanation is that LMAN is involved in putting a plan into action, which in that case is to produce syllable variants that are unaffected by WN.

Zebra finches' ability to plan directed song changes could hinge on song memories that feed into LMAN and that could drive neurons there to produce diverse perceptual song variants. LMAN neurons are selective for the bird's own song but not the target song (*20*, *21*), which makes them well suited for planning song in a manner congruent with experience. Furthermore, LMAN neurons show mirrored activity, i.e., similar activity when a zebra finch produces a vocal gesture and when it hears the same gesture played through a loudspeaker (*38*, *39*). This mirrored activity has been argued to be involved in translating an auditory target into the corresponding motor command, also known as an inverse model (*40*). Intriguingly, the extent of mirroring in LMAN corresponds to the extent of song variability (*40*), which is about the same as the planning limit we find. Zebra finches could thus transform a desired pitch change in the input into the corresponding motor plan via alignment of sensory and motor representations of recent vocal output.

Our observations in zebra finches could be relevant to other species including humans. The planning abilities we find bear resemblance to human motor imagery for movement learning, which is most effective when subjects already show some competence for the movements to be learned (*41*), in analogy of recall. Naively, human vocal flexibility seems superior to that of zebra finches, since we can flexibly change sound features such as loudness, pitch, and duration, to convey emotional state or to comply with the tonal and rhythmical requirements of a musical piece (*42*, *43*). Nevertheless, a limit of human vocal flexibility is revealed by non-native accents in foreign languages, which are nearly impossible to get rid of in adulthood. Thus, a seeming analogous task to re-pitching of zebra finch song, in humans, is to modify developmentally learned speech patterns.

Our findings inform the meaning of song signals in songbirds and the evolutionary pressures of singing. Because zebra finches seem incapable of large jumps in performance without practice, their current songs must be close to their recent songs, implying that song is an honest signal that they cannot adapt at will to deceive a receiver. Hence, if high or low pitch has either an attractive or repelling function on another bird, they must commit to their choice of singing either one or the other for some time. Thus, a limited vocal flexibility increases the level of commitment to a group and thereby might strengthen social cohesion.

Bibliography

1. K. F. Brecht, S. R. Hage, N. Gavrilov, A. Nieder, Volitional control of vocalizations in corvid songbirds. *PLoS Biol.* 17, e3000375 (2019).

2. L. Veit, L. Y. Tian, C. J. Monroy Hernandez, M. S. Brainard, Songbirds can learn flexible contextual control over syllable sequencing. *Elife*. 10 (2021), doi:10.7554/eLife.61610.

3. T. Brashers-Krug, R. Shadmehr, E. Bizzi, Consolidation in human motor memory. *Nature*. 382, 252–255 (1996).

4. J. M. Galea, A. Vazquez, N. Pasricha, J.-J. O. de Xivry, P. Celnik, Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cereb. Cortex*. 21, 1761–1770 (2011).

5. L. Shmuelof *et al.*, Overcoming motor "forgetting" through reinforcement of learned actions. *J. Neurosci.* 32, 14617–14621 (2012).

6. J. W. Krakauer, C. Ghez, M. F. Ghilardi, Adaptation to visuomotor transformations: consolidation, interference, and forgetting. *J. Neurosci.* 25, 473–478 (2005).

7. H. R. Sheahan, D. W. Franklin, D. M. Wolpert, Motor planning, not execution, separates motor memories. *Neuron*. 92, 773–779 (2016).

8. M. Konishi, Birdsong: from behavior to neuron. *Annu. Rev. Neurosci.* 8, 125–170 (1985).

9. K. Immelmann, Song development in the zebra finch and other estrildid finches. *Bird vocalizations* (1969).

10. S.-W. Park, T. M. H. Dijkstra, D. Sternad, Learning to never forget-time scales and specificity of long-term memory of a motor skill. *Front. Comput. Neurosci.* 7, 111 (2013).

11. M. A. Smith, A. Ghazizadeh, R. Shadmehr, Interacting adaptive processes with different timescales underlie short-term motor learning. *PLoS Biol.* 4, e179 (2006).

12. E. C. Tumer, M. S. Brainard, Performance variability enables adaptive plasticity of "crystallized" adult birdsong. *Nature*. 450, 1240–1244 (2007).

13. A. S. Andalman, M. S. Fee, A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors. *Proc. Natl. Acad. Sci. USA*. 106, 12518–12523 (2009).

14. A. Canopoli, J. A. Herbst, R. H. R. Hahnloser, A higher sensory brain region is involved in reversing reinforcement-induced vocal changes in a songbird. *J. Neurosci.* 34, 7018–7026 (2014).

15. T. L. Warren, E. C. Tumer, J. D. Charlesworth, M. S. Brainard, Mechanisms and time course of vocal learning and consolidation in the adult songbird. *J. Neurophysiol.* 106, 1806–1821 (2011).

16.   L. A. Hoffmann, V. Saravanan, A. N. Wood, L. He, S. J. Sober, Dopaminergic contributions to vocal learning. *J. Neurosci.* 36, 2176–2189 (2016).

17.   D. Aronov, A. S. Andalman, M. S. Fee, A specialized forebrain circuit for vocal babbling in the juvenile songbird. *Science*. 320, 630–634 (2008).

18.   F. Nottebohm, T. M. Stokes, C. M. Leonard, Central control of song in the canary, Serinus canarius. *J. Comp. Neurol.* 165, 457–486 (1976).

19.   J. F. Prather, S. Nowicki, R. C. Anderson, S. Peters, R. Mooney, Neural correlates of categorical perception in learned vocal communication. *Nat. Neurosci.* 12, 221–228 (2009).

20.   Y. Yazaki-Sugiyama, R. Mooney, Sequential learning from multiple tutors and serial retuning of auditory neurons in a brain area important to birdsong learning. *J. Neurophysiol.* 92, 2771–2788 (2004).

21.   S. Kojima, A. J. Doupe, Song selectivity in the pallial-basal ganglia song circuit of zebra finches raised without tutor song exposure. *J. Neurophysiol.* 98, 2099–2109 (2007).

22.   S. Yanagihara, Y. Yazaki-Sugiyama, Auditory experience-dependent cortical circuit shaping for memory formation in bird song learning. *Nat. Commun.* 7, 11946 (2016).

23.   K. Yu, W. E. Wood, F. E. Theunissen, High-capacity auditory memory for vocal communication in a social songbird. *Sci. Adv.* 6 (2020), doi:10.1126/sciadv.abe0440.

24.   L. V. Riters, J. A. Spool, D. P. Merullo, A. H. Hahn, Song practice as a rewarding form of play in songbirds. *Behav. Processes*. 163, 91–98 (2019).

25.   S. Kollmorgen, R. H. R. Hahnloser, V. Mante, Nearest neighbours reveal fast and slow components of motor learning. *Nature*. 577, 526–530 (2020).

26.   S. Kojima, A. J. Doupe, Social performance reveals unexpected vocal competency in young songbirds. *Proc. Natl. Acad. Sci. USA*. 108, 1687–1692 (2011).

27.   J. M. Galea, E. Mallia, J. Rothwell, J. Diedrichsen, The dissociable effects of punishment and reward on motor learning. *Nat. Neurosci.* 18, 597–602 (2015).

28.   A. T. Zai, S. Cavé-Lopez, M. Rolland, N. Giret, R. H. R. Hahnloser, Sensory substitution reveals a manipulation bias. *Nat. Commun.* 11, 5940 (2020).

29.   A. T. Zai, S. Cavé-Lopez, M. Rolland, N. Giret, R. H. R. Hahnloser, (ETH Research Collection, 2020).

30.   S. J. Sober, M. S. Brainard, Adult birdsong is actively maintained by error correction. *Nat. Neurosci.* 12, 927–931 (2009).

31.   J. D. Zevin, M. S. Seidenberg, S. W. Bottjer, Limits on reacquisition of song in adult zebra finches exposed to white noise. *J. Neurosci.* 24, 5849–5862 (2004).

32. G. E. Hough, S. F. Volman, Short-term and long-term effects of vocal distortion on song maintenance in zebra finches. *J. Neurosci.* 22, 1177–1186 (2002).

33. K. M. Fenn, H. C. Nusbaum, D. Margoliash, Consolidation during sleep of perceptual learning of spoken language. *Nature*. 425, 614–616 (2003).

34. A. Karni, D. Sagi, The time course of learning a visual skill. *Nature*. 365, 250–252 (1993).

35. L. Y. Tian, M. S. Brainard, Discrete Circuits Support Generalized versus Context-Specific Vocal Learning in the Songbird. *Neuron*. 96, 1168–1177.e5 (2017).

36. R. O. Tachibana, D. Lee, K. Kai, S. Kojima, Performance-Dependent Consolidation of Learned Vocal Changes in Adult Songbirds. *J. Neurosci.* 42, 1974–1986 (2022).

37. J. D. Charlesworth, T. L. Warren, M. S. Brainard, Covert skill learning in a cortical-basal ganglia circuit. *Nature*. 486, 251–255 (2012).

38. A. Hanuschkin, S. Ganguli, R. Hahnloser, A Hebbian learning rule gives rise to mirror neurons and links them to control theoretic inverse models. *frontiersin.org*.

39. E. Oztop, M. Kawato, M. A. Arbib, Mirror neurons: functions, mechanisms and models. *Neurosci. Lett.* 540, 43–55 (2013).

40. N. Giret, J. Kornfeld, S. Ganguli, R. H. R. Hahnloser, Evidence for a causal inverse model in an avian cortico-basal ganglia circuit. *Proc. Natl. Acad. Sci. USA*. 111, 6063–6068 (2014).

41. T. Mulder, S. Zijlstra, W. Zijlstra, J. Hochstenbach, The role of motor imagery in learning a totally novel movement. *Exp. Brain Res.* 154, 211–217 (2004).

42. B. K. Dichter, J. D. Breshears, M. K. Leonard, E. F. Chang, The control of vocal pitch in human laryngeal motor cortex. *Cell*. 174, 21–31.e9 (2018).

43. M. Belyk, S. Brown, Pitch underlies activation of the vocal system during affective vocalization. *Soc. Cogn. Affect. Neurosci.* 11, 1078–1088 (2016).

44. H. Yamahachi *et al.*, Undirected singing rate as a non-invasive tool for welfare monitoring in isolated male zebra finches. *PLoS One*. 15, e0236333 (2020).

45. P. C. Nilson, I. Teramitsu, S. A. White, Caudal thoracic air sac cannulation in zebra finches for isoflurane anesthesia. *J. Neurosci. Methods*. 143, 107–115 (2005).

Author Contributions

A.E. Stepien performed the muting experiments and analyzed the data.
A.T Zai performed the deafening experiments and analyzed the data.
S. Cave-Lopez performed some of the deafening surgeries.
A.T Zai and R.H.R Hahnloser performed statistical analysis.
A.T Zai, A.E. Stepien and R.H.R Hahnloser wrote the manuscript.

Materials and Methods

All experimental procedures were in accordance with the Veterinary Office of the Canton of Zurich (licenses 123/2010 and 207/2013) or by the French Ministry of Research and the ethical committee Paris-Sud and Centre (CEEA N°59, project 2017-12).

Subjects:

We used in total 81 birds. All birds were 100-300 days old (except one 853-day old control bird) and were raised in the animal facility of the University of Zurich or in Paris. During recording, birds were housed in single cages in custom-made sound-proof recording chambers equipped with a wall microphone (Audio-Technica Pro42), a loudspeaker, and a camera. The day/night cycle was set to 14/10 h except for one muted bird that was in constant light due to a technical problem.

Song Recordings:

Vocalizations were saved using custom song-recording software (Labview, National Instruments Inc.). Sounds were recorded with a wall microphone and digitized at 32 kHz. In all birds, we recorded baseline vocal activity for at least 3 days before doing any manipulation (deafening or pitch reinforcement).

Pitch Reinforcement:

We calculated pitch (fundamental frequency) as described in (14). To provide pitch reinforcement in real time, we used a two-layer neural network trained to detect a manually clustered syllable containing a harmonic stack (44). We evaluated the fundamental frequency of that syllable in a 16-24-ms time window following detection. For pitch reinforcement, we either broadcast a 50-60-ms long white noise (WN) stimulus through a loudspeaker or briefly switched off the light in the isolation chamber for 100-500 ms (LO) when pitch was below or above a manually set threshold. The WN/ LO stimulus onset occurred 6 ms after the pitch calculation offset. We performed cumulative pitch shifts across several days by adjusting the pitch threshold for WN/LO delivery each day, usually setting it close to the median value of the previous day. Sometimes the threshold was set more than once during a day, in this case we set

it close to the median of the pitch values measured so far during that day. All birds were shifted by at least 1 standard deviation ($d' > 1$, see Section Pitch Analysis).

Reported pitch values were collected as above, except in muted birds that directly after unmuting produced syllables of lower amplitude and with distorted spectral features (e.g. Fig. 1C), which resulted in frequent mis-detections by the neural network. In muted birds, we therefore performed semi-automatic (manually corrected) syllable detection and we computed pitch at a fixed (but matching) time lag after syllable onset.

Duration Reinforcement:

Duration reinforcement was performed similarly as pitch reinforcement but instead of measuring the pitch of a targeted syllable, we measured the duration of a targeted song element (either a syllable, a syllable plus the subsequent gap, or just a gap). Onsets and offsets of the targeted element were determined by thresholding of the root-mean square (RMS) sound amplitude.

Bird groups:

WN Control (WNC)*:* 18 birds (19 experiments, one bird was used twice) in the control group underwent WN pitch reinforcement (11/19 up-shifted, 8/19 down-shifted). Thereafter, the WN stimulus was withdrawn, and no further experimental manipulation took place.

WN muted (WNm)*:* in 8 birds, we first reinforced pitch using white noise (WN) auditory stimuli and then we reversibly muted the birds by performing an airsac cannulation.

Normally, when WN stimuli are contingent on low-pitch renditions, birds tend to shift the pitch up, and in 5/6 birds this was indeed the case. However, one bird shifted the pitch down, in an apparent appetitive response to WN, this bird responded appetitively also when the WN contingency was changed resulting in a net upward shift at the end of the WN period, see also (*44*). In 2 birds, we targeted high-pitch variants and these birds shifted the pitch down, as expected. Thus, in total, in 6/8 birds, we drove the pitch up and in 2/8 birds, we drove the pitch down.

After successful pitch adaptation and before the muting surgery, in 6/8 (4 up-shifted and 2 down-shifted) birds, we provided the opportunity to sing on average 649 song motifs (range 56 – 2400) without reinforcement (e.g., the example bird shown in Fig. 1C produced 56 song motifs within the 11 minutes it was allowed to sing without aversive reinforcement). Thereafter, we performed the muting surgery. The remaining 2 birds underwent the muting surgery directly after withdrawal of WN stimuli.

WN deaf (WNd): 10 birds were first pitch reinforced (5/10 were up-shifted and 5/10 down-shifted) with WN and then they were deafened by bilateral cochlea removal. WNd birds started singing on average 3±1 day after deafening (range 2 to 5) and were recorded for at least 15 days after the deafening surgery.

Deaf LO (dLO): 8/10 birds from (*28*) were recorded after the reinforcement period and we analyzed the associated data. These birds were first deafened by bilateral cochlea removal, then they underwent pitch reinforcement with light-off (LO) stimuli. The lamp in the recording chamber was switched off for 100-500 ms when the pitch was either above or below a manually set threshold (daily threshold adjustment followed the same procedure as for WNm birds). 3/8 birds received LO for low-pitched syllables and 5/8 birds for high-pitched syllables. One of the birds that received LO for high-pitched syllables changed its pitch away from LO instead of towards it, thus we ended up with a balanced data set with 4/8 birds shifting pitch up and 4/8 birds shifting down. dLO birds were recorded for at least 5 days after the deafening surgery. Details of light-induced pitch shifting are described in (*28*).

Deaf control (dC): we analyzed 26 syllables from 20 birds taken (12 from (*28*) and 8 additional ones) that were deafened and then recorded without any further manipulation. We used these birds to discount for pitch changes in WNd and dLO birds due to absence of auditory feedback, see bootstrapping.

WN duration (WNdur): 13 birds (14 experiments) underwent first WN duration reinforcement (1 bird used for WNsham before), in 11 cases (one bird was used twice) the target element was the target syllable duration, in 2 birds the target was the syllable-plus-gap duration, and in one bird the target was the gap duration. In 5 cases the duration was squeezed and in 9 cases the duration was stretched. As in WNC birds, we did no further experimental manipulation after

14

withdrawal of the WN stimulus. One bird changed its duration towards WN showed an apparent appetitive response to WN as for the one muted bird.

NCM sham (WNsham): 5 birds were first pitch reinforced with WN and then we injected saline into NCM as a control experiment (4 from (*14*) and 1 additional) before the WN stimulus was withdrawn and birds were recorded without any further manipulation.

Muting

We muted birds by inserting a by-pass cannula into the abdominal air sac (*45*) as follows.

Preparation of by-pass cannula: After incubation in 70% ethanol, we clogged a 7 mm long polyimide tube (diameter 1.2 mm) with sterile paper tissue. We created a suture loop around the cannula and fixed the thread to the cannula with a knot and a drop of tissue glue.

Cannula implantation: We anaesthetized the birds with Isoflurane (1.5-2%) and gave a single injection of Carprofen (4 mg/kg). Subsequently, we applied local analgesic to the skin (2% lidocaine) and removed the feathers covering the right abdomen. We applied Betadine solution on the exposed skin and made a small incision using sterilized scissors. We exposed the right abdominal air sac by shifting aside the fat tissue and punctured it to create an opening. Immediately, we closed the opening by inserting the cannula and by sealing the contact region with tissue glue. With the free end of the glued thread, we made one suture to the lowest rib. We closed the wound in the skin around the cannula with tissue glue and sutures using a new thread. Finally, we applied betadine solution on the wound and lidocaine gel around the injured site. Before releasing the bird to its cage, we removed the clog of the cannula with forceps and verified the air flow through the cannula.

We returned the birds to their home cage and monitored them for signs of suffering. We administered pain killers (Meloxicam 2 mg/kg or Carprofen 2-4 mg/kg) for 2 days after the surgery.

On the following days, we monitored the birds continuously for singing activity. If song was detected, the cannula was inspected for clogging and cleaned. 5 birds unmuted spontaneously, they produced at most 300 songs before the bypass cannula was inspected and the clog was removed to re-mute the bird. To unclog the bypass cannulas, we used sharp forceps and sterile

15

tissue dipped in saline. 6 of 8 birds produced quiet call-like vocalizations even on muted days on which no singing was detected.

Deafening

We bilaterally removed cochleas as described in (28).

Pitch Analysis

In individual birds, we studied the dynamics of pitch recovery during the test period. In WNm birds, the test period started with unmuting, and in all other reinforced birds it started with the end of reinforcement. We analyzed songs in early (E) time windows defined as the first 2 h window during the test period in which the bird produced at least 20 song motifs. We also assessed pitch recovery in late (L) windows defined exactly 4 days after the E window. We compared the pitch values in early and late windows to pitches produced in time-aligned windows during the last day of reinforcement (R) and during the last day of song on the last day of baseline (B) to make our insights robust to circadian fluctuations of pitch.

We used this time-of-day matched analysis to produce Fig. 1H, I, Fig. 2. C, D and Fig. 3. C, D. Exceptions where time alignment was not possible are listed in the following:

- One WNm bird started singing late on the last day of reinforcement (preventing us from time-aligning the R window with the E window), and therefore in this bird we defined R after end of WN but before muting (there is more than one day of song after WN before muting for this bird).
- In two birds (1 WNC and 1 dLO bird), we defined the L window one day earlier (on the 4th day, after 3 days of practice), because there was no data for these birds on the 5th day after reinforcement (our findings did not qualitatively change when we defined the L window on the 6th day instead of the 4th).
- One WNm bird was housed together with a female during the WN reinforcement; this bird did not sing during the time-match 2-h period on the 2nd, 3rd, and 4th day after reinforcement; therefore on those days we computed the mean pitch from all values produced on that day in Fig. 1G.

In early (E) and late (L) analysis windows, we computed the normalized residual pitch (NRP), which is the remaining fraction of pitch shift since release from WN, defined as $NRP(X) = (P_X - P_B)/(P_R - P_B)$, where P_X is either the mean pitch in the early $(X = E)$ or late $(X = L)$ window (Fig 1H, I, 2C, D, 3C, D). P_R and P_B are the mean pitches in the R and B windows, respectively. An NRP of 33% indicates that two-thirds of the reinforced pitch shift have been recovered and an NRP of 0% indicates full recovery of baseline pitch. Note that the NRP measure discounts for differences in the amount of initial pitch shift the birds displayed at the beginning of the test period.

We performed statistical testing of NRP to discount for this diversity in initial pitch. To test the hypothesis that WNm birds recovered their baseline pitch without practice or that WNd or dLO birds recovered baseline pitch without auditory feedback, we performed a two-tailed t-test for $NRP = 0$.

The results were qualitatively unchanged by different timings of the L window as long as there were at least 3 days between E and L because before that WNC birds have not recovered back to their baseline song $(p < 0.05)$. Thus, giving deaf birds more time did not allow them to recover their baseline pitch. Furthermore, we also tested 4 and 24 hours windows instead of 2 hours and found qualitatively similar results too.

We computed the pitch change (Fig. 4) as the difference in mean pitch between window X and the last 2 h of WN/LO reinforcement $\mathcal{R}$ in units of sensitivity $d' = (P_X - P_\mathcal{R})/S_\mathcal{R}$, where $S_\mathcal{R}$ is the standard deviation of pitch values in the $\mathcal{R}$ window. To test the hypothesis that WNd and dLO birds are able to make targeted pitch changes towards baseline, we performed a one-tailed t-test of the hypothesis that $d' < 0$. Normalizing the d' values differently (dividing by S_B, $\sqrt{(S_B^2 + S_\mathcal{R}^2)/2}$ or $\sqrt{(S_X^2 + S_\mathcal{R}^2)/2}$) did not qualitatively change the results in Fig. 4.

We verified that our results did not critically depend on circadian patterns by repeating the NRP tests with time-of-day matched B and R windows. Indeed, we found that all results in Fig. 1-3 were unchanged. Similarly, we repeated the d' tests in Fig. 4 with time-of-day matched R windows and found that the significant pitch difference between WNd or dLO birds remained,

17

although the d' values in both groups increased (in dLO birds d' was significantly positive in both early and late windows). The reason for the increases in d' likely is that birds further shifted their pitch away from baseline on the last day of reinforcement (after the R window).

Bootstrapping

To test whether deaf birds indeed make small pitch changes towards a target if and only if they experienced target-mismatch during reinforcement, we bootstrapped the difference in pitch changes between reinforced (WNd and dLO) and deaf control birds (dC). All dC birds were recorded for at least 5 days after they started singing while deaf.

In dC birds, we defined the $\mathcal{R}$, E and L windows either the same as for WNd (except that there was no WN before deafening) to control for WNd or such that they matched those of dLO birds both in terms of days since deafening and in terms of time-of-day to control for dLO. Thus, the $\mathcal{R}$ windows in dC birds either corresponded to the last 2 h before deafening (to control for WNd birds) or to the last 2 h of the day before E (to control for dLO birds for which LO always ended overnight).

For WNd birds, we obtained in total 26 control syllables (dCS) from 20 dC birds. For dLO birds, we obtain 17 dCS from 13 dC birds (some dC birds did not provide any useable data because they stopped singing or were not recorded for long enough).

In the bootstrapping procedure, we randomly paired the dCS (N=26 for WNd and N=17 for dLO) to one of the reinforced birds that can be matched (WNd or dLO) with replacement, took the corresponding windows to calculate $P_{\mathcal{R}}$, P_E, P_L and $S_{\mathcal{R}}$, multiplied the pitch changes (d' as above) by -1 if the reinforced bird was down-shifted to match it in direction and calculated the difference in average pitch change between manipulated (WNd and dLO) birds and dCS in E and L windows. We repeated this procedure 10'000 times and plotted the distribution of average pitch change differences between WNd and dCS (red) and between dLO and dCS (blue) in Figure 4F.

Our results were qualitatively unchanged (only WNd significantly reverted pitch towards baseline) when we aligned the R windows in WNd and dLO birds and dCS by the time-of-day of the corresponding E windows (Two dC birds started singing later on the day of the E window

18

than they stopped singing on the days before; in these two birds we used the $\mathcal{R}$ windows

instead), see supplementary Table 1. Also, results were robust when we analyzed pitch changes

after release from reinforcement in units NRP: without practice, WNd birds made small and

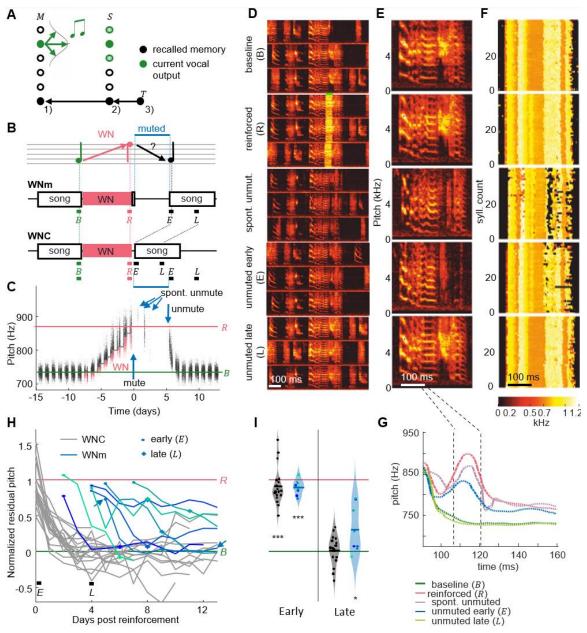significant pitch changes towards baseline, and dLO birds stayed at $NRP \geq 1$.

Figure 1. Recovery of pitch target requires practice. A) Three hypotheses on birds' ability to recover a song target away from their current vocal output (green): 1) they could recall its motor program (M), or 2) its sensory representation (S) plus the mapping (left black arrow) required to hit it, or, 3) the sensory target (T) and the circuit for translating that into the original motor program (arrows). B) WNm birds were first pitch-reinforced using white noise (WN), then muted, and subsequently unmuted. Pitch recovery from the reinforced (R) state towards the baseline (B) target is evaluated in early (E, no practice) and late (L, with practice) analysis windows and

compared to recovery in unmuted control birds (WNC). **C)** Syllable pitches (dots, red=reinforced syllables) of an example bird that while muted recovered only about 27% of pitch difference to baseline despite three spontaneous unmuting events (arrows). **D)** Same bird, spectrograms of example song motifs from 5 epochs: during baseline (B), reinforcement (R) with WN (green bar), spontaneous unmuting (spont. unmut), and during permanent unmuting (early $- E$ and late - L). **E)** Example syllables from same 5 epochs. **F)** Stack plot of pitch traces (pitch indicated by color, see color scale) of the first 40 targeted syllables in each epoch ('reinforced': only traces without WN are shown). **G)** Average pitch traces from F), revealing a pitch increase during the pitch-measurement window (dashed black lines) and pitch recovery late after unmuting. **(H)** WNm birds (blue lines, N=8) showed a normalized residual pitch (NRP) far from zero several days after reinforcement (circles indicate unmuting events, arrow shows bird from C) unlike WNC birds (gray lines, N=19). **I)** Violin plots of same data restricted to early and late analysis windows (***p<0.001, *p<0.05, two-tailed t-test of $NRP = 0$).
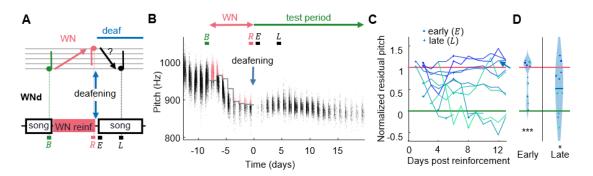

Figure 2. Recovery of pitch target is impaired after deafening. A) WNd birds were first pitch-reinforced using white noise (WN) and then deafened by bilateral cochlea removal. Analysis windows (letters) as in Fig. 1. **B)** Syllable pitches (dots, red=reinforced syllables) of example WNd bird that shifted pitch down by $d' = -2.7$ during WN reinforcement and subsequently did not recover baseline pitch during the test period. **C)** WNd birds (N=10) do not recover baseline pitch without auditory feedback (circles=deafening events), same control birds as in Fig. 1G,H. **D)** Violin plots of same data restricted to early and late analysis windows (***p<0.001, *p<0.05, two-tailed t-test of $NRP = 0$).
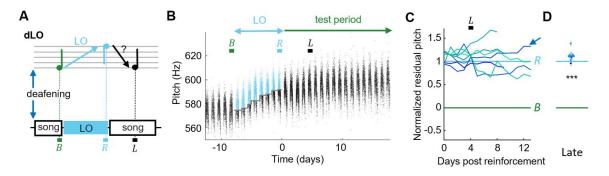
Figure 3. Deaf birds do not recover pitch target after light-induced mismatch. A) *dLO birds were first deafened and then pitch-reinforced using a brief light-off (LO) stimulus. Analysis windows (letters) as in Figure 1.* **B)** *Syllable pitches (dots, blue=LO-reinforced syllables) of example dLO bird that shifted pitch up by $d' = 3.5$ within a week, but showed no signs of pitch recovery during the test period.* **C)** *dLO birds (N=8) do not recover baseline pitch without auditory feedback.* **D)** *Violin plots of same data restricted to the late analysis window (***p<0.001, two-tailed t-test of $NRP = 0$).*
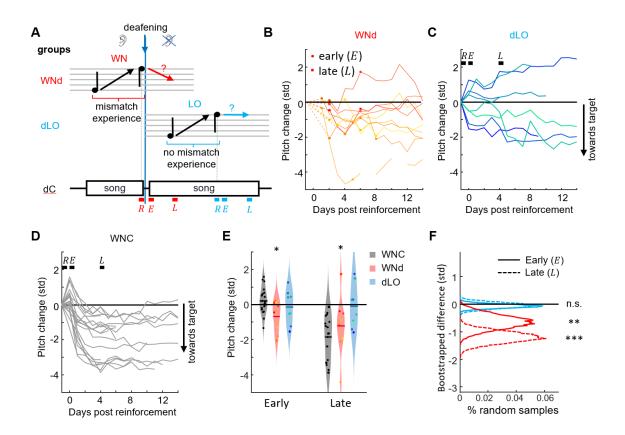

Figure 4. Target mismatch experience is necessary for revertive pitch changes. A) *WNd birds heard a target mismatch during reinforcement whereas dLO birds did not. dC birds were not pitch reinforced, their analysis windows matched those of manipulated birds in terms of time-since-deafening. Analysis windows as in Fig. 1.* **B, C, D)** *Pitch change between R and E windows*

22

*in std for WNd (red, B), dLO (blue, C) and WNC (grey, D) birds. **E)** WNd (red) perform both early and late pitch changes in the direction of the baseline target (by about one standard deviation, * p<0.05, one-tailed t-test), similar to WNC (gray) and unlike dLO (blue) birds without mismatch experience. **C)** Bootstrapped pitch differences between reinforced WNd (blue) and dLO (red) and 10'000 times randomly matched dC birds, shown for early (solid line) and late (dashed line) analysis windows. The stars indicate the bootstrapped probability of a zero average pitch difference between reinforced and dC birds (n.s. not significant, ** p<0.01, *** p<0.001).*
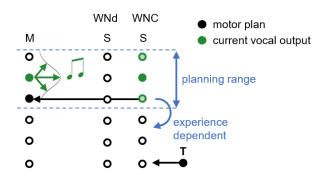

Figure 5. Schematic illustrating the goal-directed planning of vocal changes. *The extent of recent song experience (green arrows, black density) limits (blue dashed lines) the song changes (filled black dot in M) that WNd birds can overtly plan without sensory feedback. Sensory experience (green circles) in WNC birds is a prerequisite (curved blue arrow) for consolidation of motor plans and for reaching a motor target (T) beyond the planning range.*