

Discrete Circuits Support Generalized versus Context-Specific Vocal Learning in the Songbird

Highlights

- Birdsong exhibits context-dependent vocal learning similar to human speech
- Syllable modifications can be specific to or generalize across distinct sequences
- Specificity reflects context-specific biasing from cortical-basal ganglia circuitry
- Generalization reflects changes to a core syllable representation in motor circuitry

Authors

Lucas Y. Tian, Michael S. Brainard

Correspondence

lucas.tian@ucsf.edu

In Brief

Tian and Brainard investigate context-dependent vocal learning in birdsong. They find that learned syllable modifications that differ across sequential contexts reflect sequence-specific biasing from cortical-basal ganglia circuitry, while modifications that generalize reflect changes to a core syllable representation in motor circuitry.

Discrete Circuits Support Generalized versus Context-Specific Vocal Learning in the Songbird

Lucas Y. Tian^{1,2,3,5,*} and Michael S. Brainard^{1,2,3,4}

¹Departments of Physiology and Psychiatry

²Center for Integrative Neuroscience

³Kavli Institute for Fundamental Neuroscience

⁴Howard Hughes Medical Institute

University of California, San Francisco, San Francisco, CA 94158, USA

⁵Lead Contact

*Correspondence: lucas.tian@ucsf.edu

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SUMMARY

Motor skills depend on the reuse of individual gestures in multiple sequential contexts (e.g., a single phoneme in different words). Yet optimal performance requires that a given gesture be modified appropriately depending on the sequence in which it occurs. To investigate the neural architecture underlying such context-dependent modifications, we studied Bengalese finch song, which, like speech, consists of variable sequences of “syllables.” We found that when birds are instructed to modify a syllable in one sequential context, learning generalizes across contexts; however, if unique instruction is provided in different contexts, learning is specific for each context. Using localized inactivation of a cortical-basal ganglia circuit specialized for song, we show that this balance between generalization and specificity reflects a hierarchical organization of neural substrates. Primary motor circuitry encodes a core syllable representation that contributes to generalization, while top-down input from cortical-basal ganglia circuitry biases this representation to enable context-specific learning.

INTRODUCTION

The efficient learning and execution of motor skills, such as speech and musicianship, depends on the ability to flexibly reorder a discrete set of distinct motor gestures (e.g., phonemes in speech or finger movements in piano playing) into a larger set of appropriate sequences (Diedrichsen and Kornysheva, 2015). Reuse of a given gesture in multiple sequential contexts supports efficient learning because it permits a generally applicable adaptive modification to a given gesture—for instance, during initial learning of a skill or in response to weakening of muscles—to be expressed not only in the sequence in which it was learned, but also in other sequences that incorporate the gesture. However, optimal performance of motor sequences

depends not only on the ability to generalize gesture modifications across sequential contexts but also on the ability to modify a given gesture differentially for the distinct contexts in which it is performed. This is prominent in speech, in which the execution of a given phoneme can be systematically varied depending on the word in which it is embedded. Such natural context-dependent modification of gestures (“co-articulation”) is thought to enable the smooth and rapid performance of speech (Bouchard and Chang, 2014) and skills as diverse as piano playing (Engel et al., 1997), sign language (Jerde et al., 2003), and reaching and grasping (Ansuini et al., 2008; Shah et al., 2013; Sosnik et al., 2004).

The idea that a flexible balance of generalization and specificity underlies the reuse of individual motor gestures is strongly supported by human motor adaptation studies. For instance, if consistent external perturbation of speech or reaching movements is imposed in only one sequential context, subjects exhibit corrective adaptations of the movement that tend to generalize to other contexts (Houde and Jordan, 1998; Howard and Franklin, 2015; Rochet-Capellan et al., 2012). However, such generalization is typically only partial, indicating some natural capacity to limit adaptation specifically to the trained context. Moreover, if different directions of perturbation are imposed in distinct sequential contexts, subjects can learn multiple sequence-specific modifications to a given gesture, allowing it to be executed appropriately in each context (Howard et al., 2012; Rochet-Capellan and Ostry, 2011; Wainwright et al., 2005). Collectively, these behavioral observations raise the question of what neural architecture might support the efficient reuse of individual gestures across contexts, while also enabling the modulation of a given gesture to optimize its performance depending on context.

Here we investigate the neural mechanisms underlying the balance between generalization and specificity of learning in adult Bengalese finch song. Bengalese finch song, like human speech, consists of learned sequences formed by reordering a discrete set of vocal gestures, termed “syllables,” so that a given syllable can be expressed in different sequential contexts (Figure 1A; Doupe and Kuhl, 1999). Moreover, experimentally induced sensory errors during the production of a syllable in one sequential context drive adaptation that exhibits partial generalization to the production of the same syllable in other

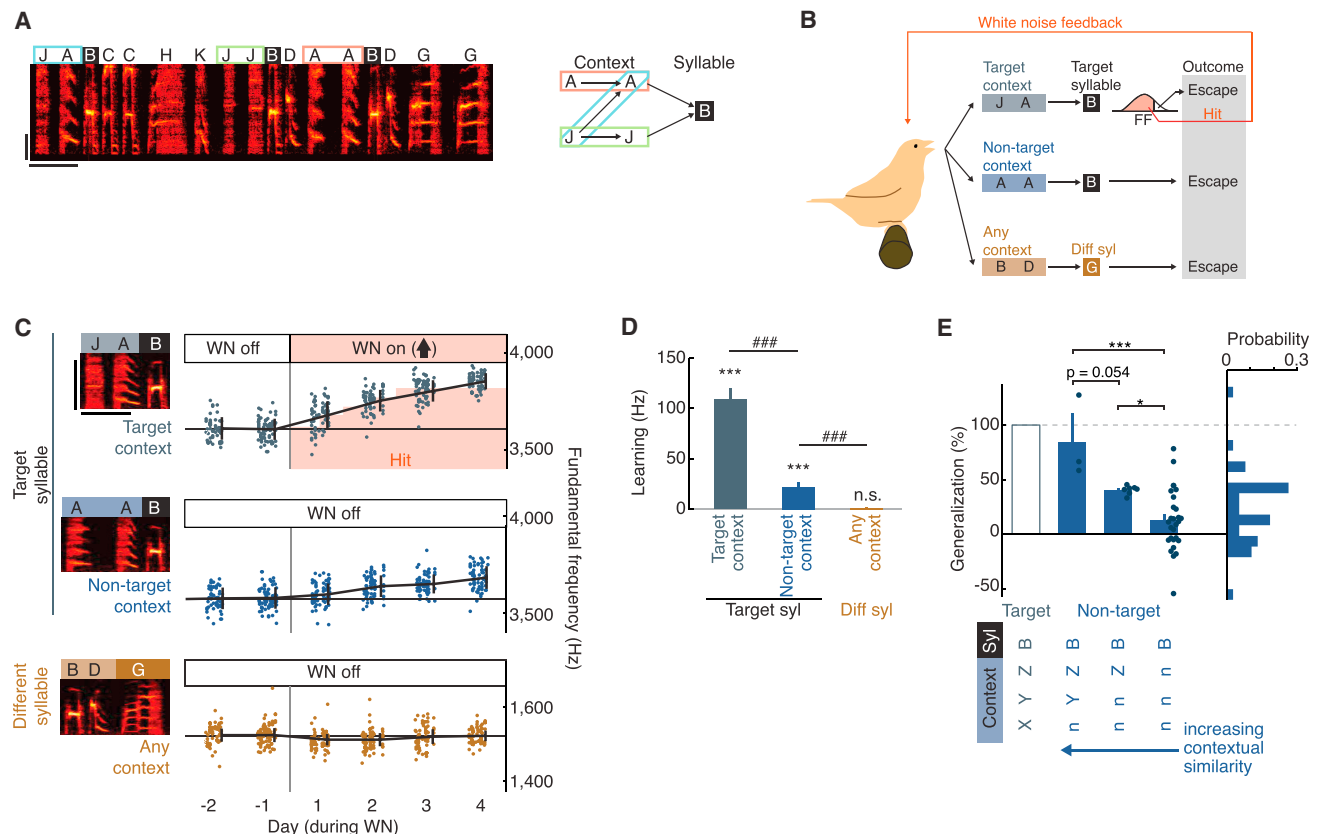


Figure 1. Learning Driven in a Single-Target Context Partially Generalizes to Non-Target Contexts

(A) Spectrogram of an example song with syllables labeled and transition diagram representing three contexts for the syllable *B*. Scale bars, 250 ms (horizontal) and 2 kHz (vertical).

(B) Schematic of training in a single context. White noise (WN) feedback (“hit”) was provided to renditions of the target syllable (*B*) in the target context (*JAB*, gray) when fundamental frequency (FF) of *B* was below a threshold (red fill in histogram). Feedback was not provided (“escape”) when *B* was sung in non-target contexts (blue), or when a different syllable (e.g., *G*) was sung in any context (brown).

(C) Learning over 2 days of baseline (“WN off”) and 4 days of training (“WN on”) for the target context; arrow represents the direction of FF shift that escapes WN feedback for the experiment depicted in (B). Each data point represents a single rendition of the target syllable in the target context (*JAB*, gray), the target syllable in a non-target context (*AAB*, blue), or a different syllable (*BDG*, brown). Renditions within the red shading were below the FF threshold and thus were “hit” with white noise (WN). Mean \pm SD FF for each day is overlaid.

(D) Summary across experiments of learning for target syllables in the target context ($n = 36$), the target syllable in non-target contexts ($n = 48$), and different syllables in any context ($n = 235$). Bars represent mean \pm SEM learning ($n = 36$ experiments, in 12 birds, targeting a syllable in a single context), defined as mean FF on days 3 and 4 of training minus mean FF on the last 2 baseline days. *** $p < 0.0005$, n.s., $p > 0.05$, signed-rank test; *** $p < 0.0005$, rank-sum test.

(E) Left: Generalization as a function of similarity between target and non-target contexts. Contextual similarity was defined by the number of syllables immediately preceding the target syllable that were shared between the target context (*XYZB*) and non-target contexts. Variation in contextual similarity from low (*nnnB*, no syllables shared, $n = 28$), to medium (*nnZB*, 1 syllable shared, $n = 7$), to high (*nYZB*, 2 syllables shared, $n = 3$) accounted for significant variation in the magnitude of generalization (simple linear regression, $p < 5 \times 10^{-5}$, $r^2 = 0.40$, slope = -0.33). Bars represent mean \pm SEM. * $p < 0.05$, *** $p < 0.0005$, corrected for multiple comparisons using the Tukey-Kramer method on results from ANOVA. Right: Histogram of generalization for all cases of the target syllable in non-target contexts (mean, $23.2\% \pm 5.4\%$).

See also Figures S1 and S2.

contexts (Hoffmann and Sober, 2014). In our study, we first show that, as for human speech, Bengalese finches can learn to modify individual syllables differentially depending on context. We then use inactivation of the anterior forebrain pathway (AFP), a cortical-basal ganglia circuit dedicated to song, to reveal a hierarchical organization of neural substrates in which the AFP enables such context-specific learning by biasing a more context-independent syllable representation in downstream motor circuitry. Moreover, when birds are instructed to modify syllables in a general manner across contexts, learning

gradually becomes encoded in primary motor circuitry, but when instruction is context specific, learning remains dependent on biasing signals from the AFP.

RESULTS

Learning Driven in a Single-Target Context Partially Generalizes to Non-Target Contexts

We first evaluated whether birds that have been trained to modify the fundamental frequency (FF), or pitch, of a given syllable in

one context would spontaneously apply the learned changes to the same syllable in other contexts. We used a negative reinforcement paradigm that requires birds to gradually shift the FF of a “target” syllable in order to escape white noise (WN) delivered whenever the FF of a rendition of that syllable exceeds a set threshold (Andalman and Fee, 2009; Charlesworth et al., 2011, 2012; Tumer and Brainard, 2007; Warren et al., 2011). This instructive WN reinforcement was provided to birds only when the target syllable was sung in a single sequential context (Figure 1B, “target context”); reinforcement was withheld when the target syllable was sung in any other sequence (“non-target contexts”) and for all other types of syllables (“different syllables”; see STAR Methods).

Context-dependent reinforcement delivered in a single-target context drove changes in the FF of the target syllable that generalized to non-target contexts (Figure 1C, example experiment; Figure 1D, summary, signed-rank test of FF change in target context, $p < 5 \times 10^{-7}$; signed-rank test of FF change in non-target context, $p < 0.0005$). However, the change in FF in non-target contexts averaged only 23% of the change in the corresponding target contexts, indicating that there is some natural tendency for context specificity in learning (Figures 1C and 1D, $n = 36$ experiments, rank-sum test of FF change in target versus non-target contexts, $p < 5 \times 10^{-9}$; Figure 1E, right, histogram of percent generalization). In contrast to the partial generalization observed for the target syllable, we did not detect any learning for syllables that were categorically different from the target syllable (Figures 1C and 1D, signed-rank test, $p = 0.34$; Figure S1A, Kolmogorov-Smirnov test comparing distributions of learning versus expected drift of FF, $p = 0.39$). Hence, consistent with previous observations in both human and songbird studies (Hoffmann and Sober, 2014; Houde and Jordan, 1998; Rochet-Capellan et al., 2012), we found that learning driven in a single context partially generalizes to other contexts.

We next investigated factors that could account for differences in the magnitude of generalization across experiments (Figure 1E). For each target syllable, we examined a variety of measures of similarity between the target and non-target contexts that have previously been studied for their potential explanatory value with respect to magnitude of generalization (Caudrelier et al., 2016; Hoffmann and Sober, 2014; Howard and Franklin, 2015; Rochet-Capellan et al., 2012; Shadmehr and Mussa-Ivaldi, 1994). We found that the magnitude of generalization for a given non-target context could be explained, to a large extent, by the similarity between the identity of the syllables in the sequences that made up the target and non-target contexts (“contextual similarity,” Figure 1E). Greater contextual similarity corresponded with greater generalization, with only 13% generalization in cases with low contextual similarity but 40% and 84% generalization for cases with intermediate and high levels of contextual similarity (Figure 1E, simple linear regression: $p < 5 \times 10^{-5}$, $r^2 = 0.40$). Further regression analyses confirmed that contextual similarity had strong explanatory power, while other measures we examined provided no significant additional power in accounting for variation in the magnitude of generalization across experiments (Figure S2 reports tests of explanatory value for acoustic distance, rendition-by-rendition correlation, and proximity). This finding parallels observations for human

speech and reach adaptation that generalization tends to be greater when gestures are produced in sequential contexts that are more similar to the context in which learning is driven (Caudrelier et al., 2016; Howard and Franklin, 2015).

Independent Context-Specific Learning for the Same Syllable in Two Contexts

To determine whether partial generalization to non-target contexts reflects an inherently limited ability to express separate learning in different contexts, we asked whether we could override the natural pattern of generalization by instructing opposing modifications of a syllable in two contexts. For each learning trajectory, we first drove learning in only one target context (“single-context phase”), which, as described above, resulted in partial generalization of learning to other contexts (Figure 2A, example experiment; Figure 2B, summary). We then initiated reinforcement in a second context, with the FF contingency opposite of that in the first context while maintaining the contingency in the first context (Figures 2A and 2B, “dual-context phase”). During the dual-context phase, FF in the second context changed in the direction opposing initial learning by an average of 109.8 ± 19.1 Hz (Figure 2C, $n = 13$ experiments, signed-rank test, $p < 0.0005$). By the end of the dual-context phase, FF in the second context had shifted downward past its original baseline (Figure 2B, signed-rank test, $p < 0.05$), and this shift was even more pronounced in the subset of experiments for which training in the dual-context phase was extended past 5 days (Figure S3A). In contrast, learning that had occurred in the first context was maintained with no significant change (Figure 2C, $n = 13$, signed-rank test, $p = 0.31$; we also did not detect any significant changes to FF of different-type syllables, Figure S3B). Correspondingly, the separation between FF of the target syllable in the two contexts increased from 114.4 ± 18.8 Hz at the end of the single-context phase to 211.0 ± 30.0 Hz at the end of the dual-context phase ($n = 13$, signed-rank test, $p < 0.0005$). These results demonstrate that Bengalese finches have a capacity for independent context-specific modifications of a given syllable, mirroring findings for human speech and reach adaptation (Howard et al., 2012; Rochet-Capellan and Ostry, 2011).

A Cortical-Basal Ganglia Circuit, the Anterior Forebrain Pathway, Adaptively Biases Motor Output in a Context-Specific Manner

We next investigated the neural mechanisms underlying generalization and specificity in context-dependent learning. To do so, we took advantage of previous work that has elucidated circuitry for production and plasticity of song. The song motor pathway (Figure 3A) is required for the moment-by-moment production of learned song (Leonardo and Fee, 2005; Nottebohm et al., 1976; Simpson and Vicario, 1990; Vu et al., 1994). In contrast, the AFP (Figure 3A), a basal ganglia-thalamo-cortical circuit specialized for song, is not required for the normal production of adult song but is required both for developmental song learning and modifications to adult song (Andalman and Fee, 2009; Bottjer et al., 1984; Brainard and Doupe, 2000; Warren et al., 2011). Using a similar WN reinforcement paradigm, previous work (Andalman and Fee, 2009; Warren et al., 2011) has shown that during initial stages of learning, inactivation of the

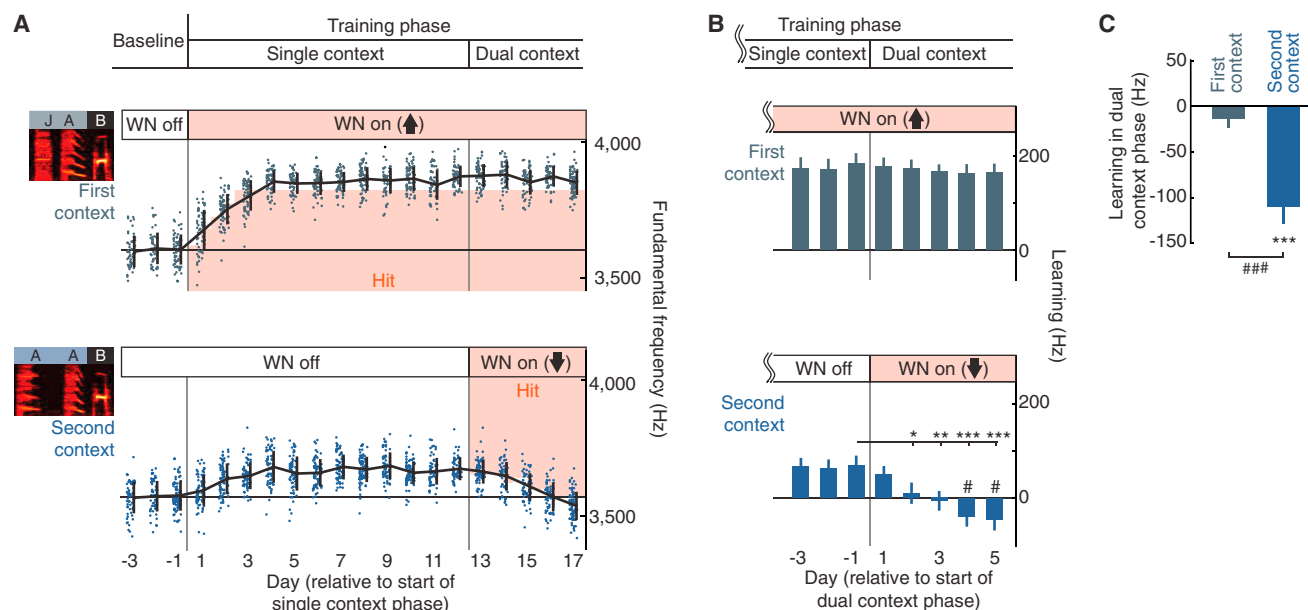


Figure 2. Independent Learning for the Same Syllable in Two Contexts

(A) Example experiment. In the single-context phase, the FF of *B* was driven up in the first context, JAB. In the dual-context phase, the FF of the same syllable, *B*, was driven down in the second context, AAB, while the reinforcement contingency in the first context was maintained. Dots indicate the FF of single renditions, with overlaid thick lines representing mean ± SD.

(B) Across-experiment mean ± SEM learning in the first (top) and second (bottom) contexts. Experiments were aligned to the transition from the single-context phase to the dual-context phase ($n = 13$ experiments, each including a single- and dual-context phase, 9 birds; * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, signed-rank test versus the last single-context day; # $p < 0.05$, signed-rank test versus 0 Hz).

(C) Mean ± SEM learning during the dual-context phase for the first and second contexts. Learning was measured as the change in FF on days 4 and 5 of the dual context phase, relative to FF on the last 2 days of the single-context phase (*** $p < 0.0005$, signed-rank test; ### $p < 0.0005$, rank-sum test).

See also Figure S3.

AFP causes a reversion of FF toward baseline values (Figure 3B, “Early”), but that over a period of maintained learning, the effects of inactivating the AFP gradually diminish (Figure 3B, “Late”). These findings support a model in which WN-driven changes to the FF of targeted syllables are initially directed by biasing signals from the AFP acting upon the downstream motor pathway (Figure 3B, “AFP biasing,” thick green arrow from AFP to RA) but that this learning is gradually transferred to the motor pathway in a process of “systems consolidation” (Figure 3B, “Consolidated to MP,” filled green circle in RA). If these same mechanisms contribute to all adaptive modifications of song, we would expect in our experiments that the early expression of learning in both the target and non-target contexts would rely on biasing signals from the AFP.

To assess the extent to which AFP bias contributes to the expression of learning in target and non-target contexts, we used the previously established approach of AFP inactivation. We first drove learning in a single-target context and then transiently blocked AFP output by infusing the GABA_A receptor agonist muscimol into LMAN. As previously observed for learning in a single context (Andalman and Fee, 2009; Warren et al., 2011), we found that blocking AFP output caused a strong and consistent reduction in the magnitude of learning expressed in the target context (Figure 4A, top, example experiment; Figure 4B, top, summary, $n = 13$ experiments, 48% reversion from a mean of 138.8 ± 11.3 Hz during PBS to

72.2 ± 8.1 Hz following LMAN inactivation, signed-rank test, $p < 0.0005$). This reversion in the expression of learning indicates that the AFP was providing a bias in the target context of ~ 67 Hz in the adaptive direction (i.e., the direction that escapes WN). In striking contrast, although there was significant generalization of learning to non-target contexts, the expression of that generalized learning did not depend on the AFP (Figure 4A, bottom, example experiment; Figure 4B, bottom, summary, $n = 13$ experiments, 14% shift from 42.7 ± 10.3 Hz during PBS to 36.7 ± 12.2 Hz following LMAN inactivation, signed-rank test, $p = 0.50$). A direct comparison of reversion in target and non-target contexts in the same experiments confirmed that AFP bias was highly specific to the target context (Figure 4D, signed-rank test, $p < 0.0005$). Moreover, this specificity did not simply reflect less learning in non-target contexts, as the differential effect of LMAN inactivation on expression of learning in target versus non-target contexts persisted both in analysis of experiments in which there was a large amount of generalization (as in the example experiment of Figure 4A and summary data in Figure S4C) and in analysis of the ratio of effects of inactivation on expression of learning in target and non-target contexts (Figure S4D). LMAN inactivation also did not have a significant effect on FF for different-type syllables (mean learning expressed during PBS infusion, 5.5 ± 2.9 Hz, and during muscimol infusion, 3.88 ± 2.6 Hz, signed-rank test, $p = 0.77$). Thus, the AFP contributes to the

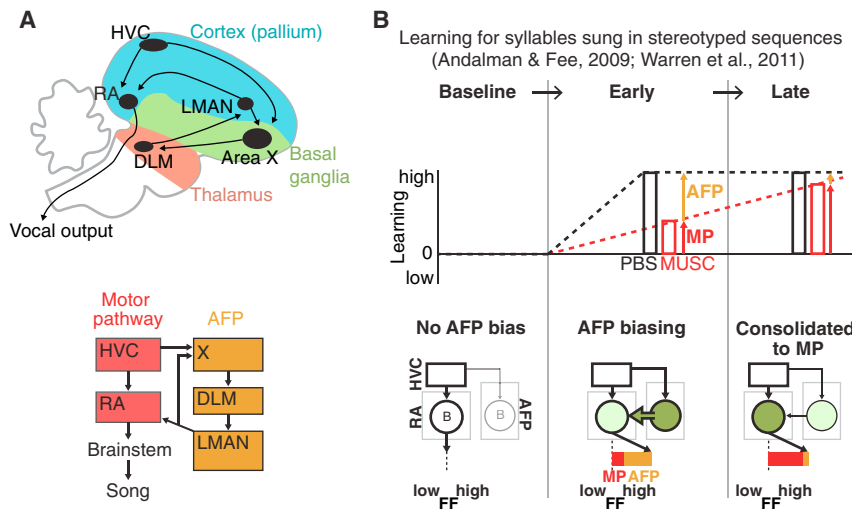


Figure 3. Neural Circuits that Contribute to Song Production and Learning

(A) Top: Song system nuclei schematized according to anatomical organization. Blue, green, and red subdivisions refer to “cortical” (pallial), basal ganglia, and thalamic subdivisions, respectively. Bottom: the motor pathway (red) consists of the cortical nuclei HVC (used as a proper name) and RA (robust nucleus of the arcopallium). The anterior forebrain pathway (AFP, tan) consists of the striatopallidal nucleus Area X (used as a proper name), the thalamic nucleus DLM (medial dorsolateral nucleus of the thalamus), and the frontal cortical nucleus LMAN (lateral magnocellular nucleus of the anterior nidopallium).

(B) Schematic based on previous work of the contributions of the AFP and motor pathway (MP) to the expression of WN-driven learning for a syllable sung in stereotyped sequences (i.e., only ever sung in one context) (Andalman and Fee, 2009; Warren et al., 2011). FF is driven from baseline and then maintained at a fixed value, while LMAN is

periodically inactivated by muscimol infusion. The amount of total learning (black lines and bars, “PBS”) that persists during LMAN inactivation (“MUSC,” red lines and bars) is construed as the motor pathway (MP) contribution to the expression of learning (red arrow), while the difference between total learning and the MP contribution is construed as the AFP contribution to the expression of learning (gold arrow). During “baseline,” LMAN inactivation has no consistent effect on FF, indicating that well-learned song structure is largely encoded in the downstream motor pathway. During “early” learning, LMAN inactivation results in a reversion of learning back toward baseline, indicating that the expression of recent learning depends on biasing signals from the AFP acting on the downstream motor pathway (“AFP biasing,” thick green arrow from AFP to RA). During a “late” period of maintained learning, LMAN inactivation no longer causes a reversion of learning, indicating that learning has been transferred to the motor pathway (“Consolidated to MP,” filled green circle in RA).

expression of learning by providing a motor bias that is highly specific for the target versus non-target context.

These results raise the question of how generalization of learning to non-target contexts arises. We hypothesized that while the AFP provides biasing signals that are context specific, the motor pathway contains a more overlapping representation of the target syllable that is shared across contexts. According to this model (Figure 4C), generalization arises because AFP biasing signals that are specific to the target context drive a gradual modification of the overlapping motor pathway representation through the process of consolidation. Our results thus suggest a hierarchical organization in which the AFP provides context-specific biasing signals that modulate and gradually modify a more context-independent core syllable representation in downstream motor circuitry.

Conflicting AFP Bias Interferes with Consolidation for Context-Specific Learning

Our model makes a prediction about the nature of adaptive modifications that are transferred, or consolidated, to the motor pathway during learning. In particular reduced consolidation should be present for context-specific learning because conflicting context-specific biasing signals would exert interfering influences on the overlapping syllable representation in the motor pathway. In contrast to our model, if the motor pathway contains separate non-overlapping representations of a given syllable in each context, consolidation of learning should proceed equally for context-independent and context-specific learning. To test our model predictions, we carried out experiments in which birds were instructed to either shift FF in the same direction in all contexts (“congruent training”) or shift FF in opposite directions in different contexts (“incongruent training”). We supposed that

for congruent training, the AFP would generate similarly directed biasing signals in each of the two contexts during the early phase of learning (Figure 5Ai, “Early”) that would act coherently to drive a strong transfer of context-independent changes to the downstream motor pathway (Figure 5Ai, “Late”). In contrast, for incongruent training, we supposed that the AFP would generate oppositely directed biasing signals in the two contexts (Figure 5Aii, “Early”) that would antagonize each other in converging onto a shared downstream motor pathway representation of the syllable, thereby interfering with transfer of learning (Figure 5Aii, “Late”).

We first assessed whether congruent versus incongruent training would indeed generate coherent versus antagonistic AFP biasing signals. We measured AFP bias during the first 4 days of maintained learning (Figures 5A and 5B, “Early”), when the expression of learning in a single context has been shown to depend substantially on AFP bias (Warren et al., 2011). Targeted LMAN inactivation during this early period revealed that during congruent training, AFP bias was in the same direction in each context, while during incongruent training, AFP bias was in opposite directions in different contexts (Figure 5C). These results further demonstrate that the presence and direction of AFP bias accurately reflects the presence and direction of context-specific instruction, even in an extreme case in which learning is oppositely directed in distinct contexts. They additionally establish an experimental framework for determining whether conflicting AFP bias during incongruent training interferes with the transfer of learning to the motor pathway.

We assessed consolidation for both congruent and incongruent training during the late period of maintained learning (days 5 and 6), when the expression of learning in a single context has been shown to become largely independent of the

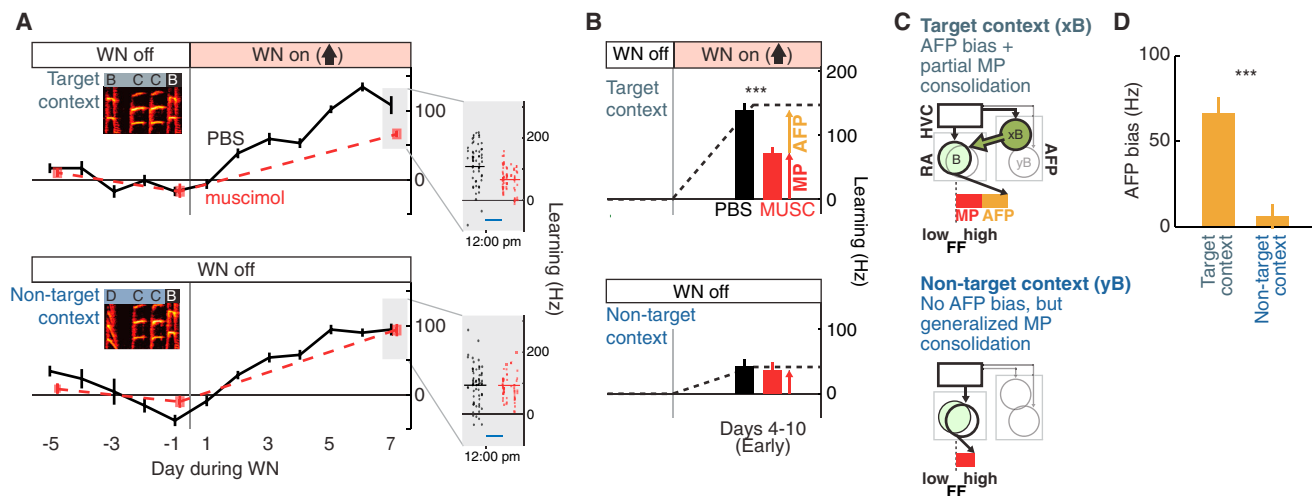


Figure 4. The AFP Adaptively Biases Motor Output in a Context-Specific Manner

(A) Example experiment in which FF of *B* was driven up in the target context (BCCB, top, gray), while reinforcement was withheld in the non-target context (DCCB, bottom, blue). Black lines represent daily mean \pm SEM FF during infusion of vehicle (PBS) into LMAN. Red squares represent daily mean \pm SEM FF during infusion of muscimol. Inset: FF of individual renditions for a single inactivation day; lines represent mean FF, and blue scale bar represents 2 hr.

(B) Muscimol infusion caused significant reversion of learning in the target context (top, *** $p < 0.0005$, signed-rank test), but not in non-target contexts (bottom, $p = 0.50$, signed-rank test) on days 4–10 of training ($n = 13$ experiments in 7 birds). Experimental data (bars representing mean \pm SEM) are overlaid on a schematic of the learning trajectory (dashed lines).

(C) A model for how the AFP and motor pathway contribute to learning in target and non-target contexts. In this model, the motor pathway has a core representation of the target syllable that is largely overlapping between contexts (schematized by overlapping circles in RA), while the AFP has context-specific representations of the appropriate modifications of the syllable for each context (schematized by non-overlapping circles in the AFP). In the target context (“xB,” top), the AFP provides a strong biasing signal to the target syllable *B* (thick green arrow from AFP to RA), and over time this bias begins to drive a consolidation of changes in the motor pathway representation of the target syllable (light green circle in RA reflecting partial consolidation of changes to the MP representation of the target syllable). In the non-target context (“yB,” bottom), there is no AFP bias. However, because the motor pathway representation of the target syllable substantially overlaps between contexts, the gradual modification of the MP representation in the target context contributes to the generalization of learning in the non-target context. As a result, the expression of learning in the target context depends on contributions from both the MP and AFP (red and gold bar, top), but learning that generalizes to the non-target context depends only on contributions from the MP (red bar, bottom).

(D) Mean \pm SEM contribution of the AFP to expression of learning (AFP bias; $n = 13$ experiments, identical to B). AFP bias in the target context ($p < 0.0005$), but not in the non-target context ($p = 0.50$), was significantly different from 0 (signed-rank test). *** $p < 0.0005$, signed-rank test comparing target and non-target contexts. See also Figure S4.

AFP (Warren et al., 2011). For congruent training, consolidation was not significantly different from 100% (Figure 5D, $84.9\% \pm 5.9\%$, mean \pm SEM, signed-rank test, $p = 0.12$) and was indistinguishable from consolidation previously reported for syllables that are sung only in a single context (Figure 5D, $84.9\% \pm 7.1\%$ in Warren et al., 2011). In contrast, for incongruent training, consolidation was both significantly less than 100% and significantly reduced as compared to congruent training (Figure 5D, $44.1\% \pm 12.0\%$, $p < 0.05$, signed-rank test versus 100%; $p < 0.005$, rank-sum test versus congruent). These data indicate that under conditions in which generalization is appropriate, learning rapidly becomes transferred to the motor pathway. In contrast, under conditions in which context-specific modifications to a gesture are required, transfer to the motor pathway is impaired, and ongoing biasing signals from the AFP are required for the expression of learning.

DISCUSSION

The reuse of individual gestures in multiple motor sequences allows efficient generalization of adaptive modifications across

contexts (Diedrichsen and Kornysheva, 2015). At the same time, optimal performance requires that a given gesture be differentially modified depending on the specific context in which it is produced. Using Bengalese finch song as a model system, we demonstrate that the balance between generalization and specificity in the deployment of motor gestures arises from a hierarchical organization within the nervous system; pharmacological inactivation of the AFP revealed that biasing signals from the AFP that are highly specific and appropriate for each context modulate a more context-independent representation of syllable structure in the downstream primary motor pathway (Figures 4C and 5A). When similar modifications to a syllable were instructed across contexts, generalized learning was gradually transferred to the motor pathway, but when distinct modifications were instructed across contexts, this transfer of learning was impaired, and the context-specific expression of learning remained highly dependent on the AFP (Figure 5D). These findings indicate that the primary motor pathway encodes a relatively context-independent or core representation of a given syllable, while frontal cortical-basal ganglia circuitry provides top-down biasing signals that enable

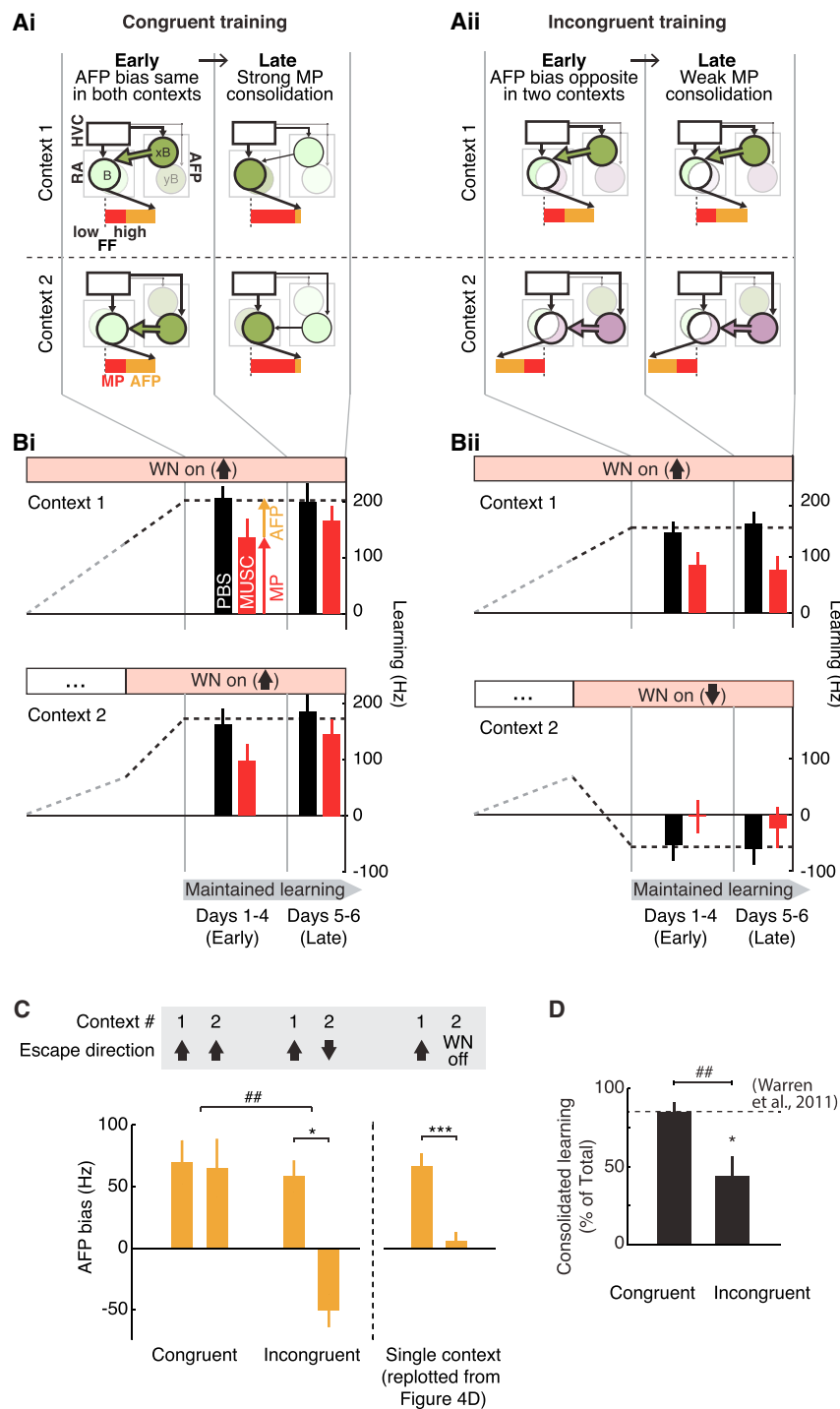


Figure 5. Conflicting AFP Bias Interferes with Consolidation for Context-Specific Learning

(A) Model predictions for congruent and incongruent training. For congruent training (Ai), we predicted that during early learning there would be similarly directed AFP bias in both context 1 (xB) and context 2 (yB) for the target syllable B ("Early," thick green arrows in contexts 1 and 2). These biasing signals would act synergistically to drive strong consolidation in the overlapping downstream motor pathway representation of the syllable ("Late," dark green circles in RA) so that expression of learning would become independent of the AFP. For incongruent training (Aii), we predicted that during early learning there would be oppositely directed AFP bias across contexts ("Early," thick green arrow in context 1 biasing FF upward and thick purple arrow in context 2 biasing FF downward). These biasing signals would drive opposing modifications to the overlapping motor pathway representation and impair consolidation ("Late," light circles in RA) so that expression of learning in both contexts would remain dependent on context-specific AFP biasing signals.

(B) Summary data for congruent and incongruent training ($n = 5$ congruent experiments and 7 incongruent experiments in 6 birds). Bar plots showing mean \pm SEM effects of LMAN inactivation at early and late time points of maintained learning are overlaid on lines schematizing trajectories of learning for congruent (Bi) and incongruent (Bii) experiments (see STAR Methods). Early and late periods are defined relative to a maintained learning period (see STAR Methods).

(C) AFP bias in the early period (days 1–4) of maintained learning was highly context specific and appropriate for each type of training (sample sizes as in B). Bars represent mean (\pm SEM) AFP bias, measured as the amount by which learning reverted toward baseline while LMAN was inactivated. * $p < 0.05$, signed-rank test; ** $p < 0.005$, rank-sum test. AFP bias measured in a separate set of experiments driving learning in only a single-target context is reproduced from Figure 4C and plotted here for comparison ($n = 13$ experiments in 7 birds, *** $p < 0.0005$, signed-rank test).

(D) Consolidation in the late period (days 5 and 6) of maintained learning was strong for congruent training but reduced for incongruent training. Bars represent the mean (\pm SEM) percentage of learning that was still expressed when AFP output was blocked (and thus was dependent on the motor pathway rather than the AFP). The dashed line represents the magnitude of consolidation from a previous study driving learning for syllables that were only sung in stereotyped sequences (Warren et al., 2011). Data are shown for learning in the first context, because the magnitude and trajectory

of learning in the first context was matched between training types (see STAR Methods); however, the significance of these results was unaffected when we used both contexts (Figure S5A). * $p < 0.05$, signed-rank test versus 100%; ** $p < 0.005$, rank-sum test. See also Figure S5.

appropriate context-specific modulation and updating of this core representation.

Our finding that the AFP injects a context-specific biasing signal into the motor pathway indicates a role for the AFP in inter-

grating contextual signals (reflecting the current syllable and sequence) with instructive signals (reflecting the appropriate FF for each context) to enable context-dependent vocal learning (Figures 4C and 5A). Signals encoding sequential context may

be conveyed from neurons in the cortical nucleus HVC that send an efference copy of premotor commands to the basal ganglia nucleus Area X (HVC_X neurons) (Fee and Goldberg, 2011; Fujimoto et al., 2011; Mooney, 2014); the firing patterns of these neurons reflect not only the identity of the syllable currently being produced but also that of preceding syllables (Fujimoto et al., 2011). Signals encoding rendition-by-rendition variation in the FF of targeted syllables are potentially generated within Area X (Woolley et al., 2014) or relayed to Area X by inputs from the motor pathway (Charlesworth et al., 2012) or LMAN (Fee and Goldberg, 2011; Kao et al., 2005). Signals encoding outcomes—such as whether or not a given rendition escapes WN—plausibly derive from rich neuromodulatory inputs to the AFP, including from midbrain dopaminergic neurons (Gadagkar et al., 2016; Hoffmann et al., 2016). The association between contextual signals (HVC_X activity) and appropriate motor-biasing AFP activity could then be mediated by plasticity at cortical-striatal (HVC_X-X) synapses (Fee and Goldberg, 2011), as has been implicated for decision-making tasks in mammals (Xiong et al., 2015).

Our finding that generalization of learning persists following pharmacological inactivation of the AFP (Figure 4) indicates that this generalization largely depends on the modification of a core syllable representation in the downstream motor pathway. The presence of such a core representation is consistent with recordings in the motor pathway nucleus RA showing that similar populations of neurons are active during the production of a given syllable regardless of the sequence in which it is sung (Leonardo and Fee, 2005; Wohlgemuth et al., 2010). We hypothesize that this motor pathway representation is gradually modified in response to biasing signals from the AFP in a process of systems consolidation (Andalman and Fee, 2009; Fee and Goldberg, 2011; Warren et al., 2011). To the extent that the overlapping neural elements (such as synapses from HVC afferents onto RA neurons) are active during the production of a syllable in multiple contexts, modification of those shared elements, driven by AFP bias in one context, would naturally contribute to circuit changes that generalize to the production of the syllable in other contexts.

Consistent with this model, we found that the degree of transfer of learning to the motor pathway depends on the extent to which biasing signals from the AFP are coherent across contexts (Figure 5). Our results indicate that when it is optimal to generalize modifications across contexts—for example, during initial learning or in response to weakening of musculature or other perturbations that affect control of a syllable regardless of context—consistent biasing signals from the AFP will promote an updating of the core MP representation. In contrast, when context specificity is appropriate—for example, to modify central commands in a manner that accounts for context-dependent dynamics of the musculoskeletal system (Bouchard and Chang, 2014; Ostry et al., 1996; Schmidt and Wild, 2014; Wohlgemuth et al., 2010)—conflicting biasing signals will interfere with consolidation, and learning will continue to rely on moment-by-moment modulation by the AFP. Such a dependence of consolidation on the consistency of AFP bias therefore may be a natural way for the nervous system to transfer modifications that are generally appropriate to primary motor circuitry while reserving

frontal “executive” circuitry for dynamically adjusting performance in response to context-specific requirements (Duan et al., 2015; Hilaro et al., 2012; Kim and Hikosaka, 2013; Miller and Cohen, 2001; Narayanan and Laubach, 2006).

More broadly, a similar balance between generalization and specificity of learning in human motor skill adaptation (Houde and Jordan, 1998; Howard and Franklin, 2015; Howard et al., 2012; Rochet-Capellan and Ostry, 2011; Rochet-Capellan et al., 2012) may also reflect separate contributions of primary motor representations and flexible top-down bias from frontal cortical-basal ganglia circuits. Indeed, neural signals indicating sequential context are present in mammalian cortical-basal ganglia circuitry (Dudman and Krakauer, 2016; Mello et al., 2015; Mushiaki and Strick, 1995; Tanji and Shima, 1994; Turner and Desmurget, 2010), and the contributions of basal ganglia circuitry to motor production may include a role in flexible fine-timescale modulation of movement kinematics (Dudman and Krakauer, 2016; Rueda-Orozco and Robbe, 2015; Turner and Desmurget, 2010). Hence, the critical contributions of frontal cortical-basal ganglia circuits to sequence-dependent vocal learning in the songbird may reflect a general role of these circuits in integrating contextual cues to enable adaptive context-dependent learning and execution of motor skills.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at <https://doi.org/10.1016/j.neuron.2017.10.019>.

AUTHOR CONTRIBUTIONS

Conceptualization, L.Y.T. and M.S.B.; Methodology, L.Y.T. and M.S.B.; Software, L.Y.T.; Formal Analysis, L.Y.T.; Investigation, L.Y.T.; Resources,

M.S.B.; Writing – Original Draft, L.Y.T.; Writing – Review & Editing, L.Y.T. and M.S.B.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Calcitonin Gene Related Peptide	Sigma	RRID: AB_259091
Chemicals, Peptides, and Recombinant Proteins		
Muscimol	Tocris	Cat#0289
Experimental Models: Organisms/Strains		
Bengalese finch (<i>Lonchura striata domestica</i>)	This lab	N/A
Software and Algorithms		
MATLAB	Mathworks	https://www.mathworks.com/
Custom-written MATLAB code for data analysis	This paper	Request from Lead Contact
Labview	National Instruments	http://www.ni.com/en-us/shop/labview.html
Custom-written Labview software for song acquisition and WN-driven training	Tumer and Brainard, 2007; This paper	Request from Lead Contact
Other		
Guide cannula (CMA 7)	Harvard Apparatus	Cat#8010684
Microdialysis probe (CMA 7, 1mm)	Harvard Apparatus	Cat#P000082

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Lucas Tian (lucas.tian@ucsf.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal models

We used 12 adult (range: 141 to 671 days old at start of experiment) male Bengalese finches (*Lonchura striata domestica*) that were bred in our colony and housed with their parents until at least 60 days of age. During experiments, birds were housed individually in sound-attenuating chambers (Acoustic Systems) on a 14h/10h light/dark cycle with food and water provided *ad libitum*. All experiments were performed on undirected song (i.e., with no female present). All procedures were in accordance with protocols approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

METHOD DETAILS

Song recording and computerized training paradigm

We used a custom-written Labview program (National Instruments) to record song and deliver white noise feedback during training (Charlesworth et al., 2011, 2012; Tumer and Brainard, 2007; Warren et al., 2011). Briefly, song was recorded with an omnidirectional lavalier microphone (Countryman), bandpass filtered between 75 Hz and 10 kHz, and digitized at 32 kHz. To detect a specific segment of a specific syllable for targeted reinforcement, the spectrum of each successive 8ms segment of ongoing song was tested for a match to a preconstructed spectral template (based on the Euclidian distance between those spectra). Upon a match, the fundamental frequency (FF) of that segment was compared to a preset FF threshold. To drive upward shifts in FF, feedback was delivered with <1 ms latency if FF was below threshold; to drive downward shifts, feedback was delivered only if FF was above threshold. Feedback was a 40–60 ms burst of white noise (WN) at 90–95 dB(A). To provide context-dependent reinforcement, we modified the training paradigm so that delivery of WN was contingent not only on the FF of the target syllable but also on the identity of the syllables preceding the target syllable (the “sequential context,” as described further below).

Determining sequential context for each rendition of a given syllable

Syllables were classified manually by visual inspection of spectrograms. Similar to a previous study in Bengalese finches (Wohlgemuth et al., 2010), for a given bird we detected cases in which the same syllable type was sung across different sequential contexts using a method based on the Acoustic Distance (a measure of difference in acoustic structure) between syllables in a pair (see “Multiple regression analysis of generalization” for calculation of Acoustic Distance). The distribution of Acoustic Distances across syllable pairs was bimodal. The Acoustic Distance at which the distribution had a local minimum between these two modes was used as a classification threshold; hence, any syllable pairs with Acoustic Distance within the first mode of the distribution were classified as same-type syllables. The result of this method is similar to that from subjective hand labeling (Wohlgemuth et al., 2010). We then defined “motifs” as stereotyped sequences of syllables that were reused across song bouts and were preceded, and sometimes followed, by introductory notes or song termination. The sequential context for each rendition of a given syllable was then defined by the directly preceding syllables in that rendition’s motif (including introductory notes preceding the motif). For example, if a bird had a repertoire consisting of two motifs, *AABHCD* and *AHCGDC*, then across all song bouts, that bird could sing *C* in three potential contexts (i.e., following either *BH*, *AH*, or *GD*). In cases of “repeated” syllables (i.e., a syllable repeated successively > 3 times in the same motif, such as *B* in *ACBBBB*; $n = 6$), we only included the first rendition of the repeat to avoid over-representing the syllable.

Single context training

Birds were trained to shift the FF of a syllable in one sequential context, and no reinforcement was provided in any other context. We performed a total of 36 single context experiments in 12 birds. In 29/36 cases we targeted a unique syllable/context combination (in 11 cases we targeted a syllable that had previously been targeted, but in a different context [Figure S1B]; in six cases we targeted a previously targeted syllable/context combination, but drove learning in the opposite direction). In 22 experiments we drove FF up; in the other 14 experiments we drove FF down. For presentation of results, the direction of “learning” is defined as the direction that escaped WN.

At the onset of training, the FF threshold for reinforcement was set at the 70th percentile of FF determined from the last baseline day, so that ~70% of renditions were “hits” and ~30% were “escapes.” WN training began when lights turned on in the morning of the first training day. Learning was quantified as the mean FF (see “FF calculation” below) across renditions on days 3 and 4 of training minus the mean FF across renditions on the last two baseline days. Because successful learning results in a reduced hit rate, the FF threshold was adjusted 1–2 times a day over 2–4 days to maintain a hit rate of ~70% (in 6 cases until day 2; in 9 cases until day 3, in 21 cases until day 4). In a small number of experiments, the bird’s singing rate dropped dramatically for 2–4 days when training was initiated ($n = 5$ experiments, < 5 catch bouts/day); in these cases the first day with substantial singing was treated as the first day of training.

To estimate the amount of change in FF that could occur due to “drift” under control conditions we collected at least six days of continuous baseline singing data in 24 experiments directly preceding the start of WN. For these experiments, we measured the amount of change in FF that occurred in the absence of WN over the same duration used in the analysis of learning; this “baseline drift” was computed as the difference between mean FF on days 5 and 6 and the mean FF on days 1–2 of baseline recordings.

Generalization was defined for non-target syllables/contexts as the change in FF calculated as a percent of the change in FF for the target syllable in the target context in the same experiment. For analyses of generalization, we only used experiments with significant learning in the target context, because generalization is not well defined in the absence of learning in the target context. The criterion for significant learning was that the shift in FF exceeded the 97.5th percentile of baseline drift pooled across syllables and birds ($n = 30/36$ experiments met that criterion). For all other analyses, we included all 36 experiments.

To determine whether “off target” delivery of WN could have influenced measured values of generalization, we measured the frequency with which WN was delivered to targeted syllables in non-target contexts within the set of songs that were used to quantify learning over days 1–4 of WN training. In 36 out of 48 cases the frequency of off-target hits was 0% (Figure S1Cii, blue histogram). In the remaining 12 non-target context cases the median frequency of mistargeting was 1.2%, with a range of 0.2% to 2.7%. Similarly, for different-type syllables, for 227/235 cases the frequency of off-target hits was 0% (Figure S1Cii, brown histogram). In the remaining 18 cases for different-type syllables, the median hit frequency was 1.0% with a range of 0.2% to 5.6%. Moreover, regression analyses confirmed that the rare off-target hits do not explain the patterns of generalization that we report in our manuscript (Figures S1Ciii and S1Civ).

To test what features of syllables, when sung in different contexts, best predict the magnitude generalization across contexts, we fit a multiple linear regression model to examine the extent to which a linear combination of three variables—contextual similarity, acoustic distance, and FF correlation—predicted the response variable (generalization). For details, see “Multiple regression analysis of generalization” below.

Dual context training

In a subset ($n = 13$ experiments, 9 birds) of the single context experiments described above, we extended the duration of single context training (mean \pm SD = 12.4 ± 6.4 total days, with 4.1 ± 2.1 days of incremental adjustment of FF threshold at the start of training). This “single context phase” was immediately followed by a “dual context phase” during which a contingency was introduced to shift FF of the target syllable in a second context in the direction opposite that in the first context. Over ~3–5 days of

the dual context phase we incrementally adjusted the FF threshold in the second context 1–2 times a day to maintain a ~70% hit rate. Throughout that period we did not change the FF threshold in the first context, except in cases where FF in the first context shifted toward baseline to a point where >70% of renditions were being hit. In that case, in order to maintain an instructive reinforcing signal in the first context, we adjusted the FF threshold to maintain the hit rate at ~70%.

LMAN inactivation

We used microdialysis to infuse the GABA_A receptor agonist muscimol (Tocris, Catalog #: 0289) into LMAN to transiently silence neural activity during learning (Lindfors et al., 1989; Warren et al., 2011). Bilateral guide cannulas (CMA 7, Harvard Apparatus) were first stereotactically implanted over LMAN. During implantation, the bird was positioned so that the ventral surface of the upper beak was 40° below horizontal. Cannulas were centered at 5.45–5.65 mm rostral and 1.5 mm lateral to the caudal point of the intersection of the midsagittal and transverse sinuses (i.e., “Y₀”), and lowered to a depth such that the tip of the probe that would subsequently be inserted into the cannula would be 2.4 mm deep relative to the surface of the brain. Our goal was to position the tip of the probe at the center of LMAN in the rostral-lateral plane and ~200 μm below the dorsal surface of LMAN. After birds recovered from surgery and were singing (~2 days), we inserted microdialysis probes (CMA 7, 1 mm membrane length, diameter 0.24 mm, 6 kDa cutoff) into the cannulas. The output of one probe was used as the input to the other probe. Probes were connected to pumps via flexible tubing and PBS was continuously infused, except during LMAN inactivation when muscimol was infused (see below). Solutes diffuse through the membrane while maintaining zero net volume transfer. In some cases, the tubing was interfaced with a dual channel liquid commutator (Instech Labs 2-Channel Microdialysis Swivel). In all cases birds could comfortably move and sing during infusion. The pump was outside the sound-attenuating chamber, allowing us to switch solutions without disturbing the bird. Flow rate was maintained at 0.3–0.5 μl/min and increased to 0.8–1.0 μl/min during muscimol infusion. The concentration of muscimol (dissolved in PBS) ranged from 100 μM to 700 μM across experiments, and was calibrated before each experiment to elicit a reduction in FF variability (a marker of successful LMAN inactivation) before training began (see below and Figures S4A and S4B).

LMAN inactivation was performed in a similar time window on each inactivation day for a given experiment (~12:30 pm to ~4:00 pm). We analyzed songs starting after a lag from the switch to muscimol, which accounts for flow of drug through tubing and diffusion within tissue. The duration of that lag was separately determined for each experiment based on the amount of time it took from the start of infusion to observe an ~30% reduction in FF coefficient of variation (CV, standard deviation divided by the mean) - that effect is a consistent indicator of lesion (Hampton et al., 2009) or inactivation (Warren et al., 2011) of LMAN. The duration, based solely on baseline days, from the start of infusion until FF CV was reduced to a stable value was used as the lag duration for the entire experiment (Figure S4A; mean lag, 94.2 min; SD, 31.2 min). Muscimol infusion successfully reduced FF CV across syllable types and contexts, both during baseline and training (Figure S4B). In the 12/14 experiments in which we restricted analyses to catch bouts (see “FF calculation”), starting from the time when muscimol data were collected (i.e., the end of the lag period), we transiently increased the catch rate (on average increased to 0.8 from 0.15) to allow us to collect a sample of catch song bouts of comparable size to the sample collected pre-inactivation. This was necessary because the duration of singing during inactivation was lower than before inactivation. The catch rate was decreased back to its normal value at the end of muscimol infusion. FF during PBS was quantified in a time window starting at ~8:30 am (lights were turned on at 7:00 am) and ending at the PBS-to-muscimol switch time.

FF shifts during PBS and muscimol infusion were normalized relative to their respective baselines (number of days directly preceding start of training, range: 3–7 days; muscimol inactivation baseline data were collected in a subset of those days, range: 2–4 days). PBS shift was defined as FF during PBS infusion minus baseline FF during PBS infusion, while muscimol shift was defined as FF during muscimol infusion minus baseline FF during muscimol infusion. The differences between baseline FF during muscimol and PBS infusion were small and not in a consistent direction; therefore all of our results held if we instead normalized muscimol FF to baseline FF during PBS infusion.

LMAN inactivation during single context training

Based on a previous LMAN inactivation study (Warren et al., 2011), we defined a maintained learning period as a period of at least five days during which (1) the FF threshold for WN was no longer being adjusted and (2) each day’s mean FF was within a window defined by the mean FF across all days ± 0.75 times the mean of within-day standard deviations of FF. On average, the maintained learning period for single context experiments started on day 5.2 (SD = 2.8) relative to start of training. The period in which LMAN inactivation data were obtained started on day 4 of training, when a large change in FF in the target context had been reached, and ended on day 10 of training or day 4 of the maintained shift period, whichever was earlier. We defined this as an “early” period in the learning trajectory, during which the AFP has been shown to contribute significantly to the expression of learning (Warren et al., 2011). Our main results held when we used other windows (first and last day modified by ± 1–2 days). LMAN inactivation days were usually separated by at least one day and data from multiple inactivation days were averaged (separately for baseline and learning days). For comparison of effects of LMAN inactivation for target versus non-target contexts, effects for multiple non-target contexts were averaged to get a single mean value for each experiment.

LMAN inactivation during Congruent and Incongruent training

We measured the contribution of the AFP to expression of learning for Congruent and Incongruent training experiments, in both early and late periods in the learning trajectory. For 12 experiments, learning was driven and maintained in context 1, following which

learning was then driven in the second context in either Congruent (i.e., same direction as in context 1, $n = 5$) or Incongruent (i.e., opposite direction from context 1, $n = 7$) directions, while maintaining the reinforcement in the first context ($n = 6$ birds, with 5 birds that contributed Congruent experiments also contributing 6 Incongruent experiments). Effects of LMAN inactivation were assessed relative to the onset of a period of maintained learning, defined as the intersection of maintained learning periods separately determined for each context (as described above for single context experiments). AFP bias and consolidation, inferred from effects of LMAN inactivation on learning, were grouped and averaged over an early period (days 1–4) and late period (days 5 and 6) of maintained learning. For 3 out of 12 experiments, feedback in context 2 was provided in the opposite direction prior to the onset of Congruent ($n = 2$) or Incongruent ($n = 1$) training. The exclusion of those 3 experiments did not alter the significance of the effects of LMAN inactivation (Figure S5B).

Localization of probes

We performed post-mortem histology on sectioned (40 μm thick, coronal) tissue to confirm placement of probes within or directly adjacent to LMAN. Tissue damage, revealed by Nissl or DAPI stain, indicated the location of the probe. LMAN was visualized by immunostaining for calcitonin gene related peptide (Sigma, RRID: AB_259091, 1:5000 to 1:10000) (Bottjer et al., 1997).

QUANTIFICATION AND STATISTICAL ANALYSIS

Overview

Unless noted otherwise, to compare two samples we used the nonparametric two-sided Wilcoxon rank-sum test and for paired samples we used the nonparametric two-sided Wilcoxon signed-rank test. Within-group variances were similar for groups being compared. All regression analyses were performed using the ordinary least-squares method. Tests were deemed statistically significant if $p < 0.05$. Statistical details for all experiments are included in their corresponding figure legends. For experiments corresponding to Figures 1–4, no randomization was required in allocating animals to experimental groups because each animal contributed to both experimental groups (dimension 1: target versus non-target context; dimension 2: PBS versus muscimol infusion). For the experiment in Figure 5, randomization in allocation to experimental types (Congruent versus Incongruent) was not required because almost all (5/6) animals contributed data to both experimental types. Syllable labeling was performed blind to magnitude of learning and LMAN inactivation effects. For LMAN inactivation experiments, experimenters were not blinded to whether data were from PBS or muscimol infusion periods, as muscimol infusion causes changes to pitch CV that are conspicuous even during visual inspection of spectrograms. No datasets were excluded unless appropriate as described elsewhere [i.e., in calculation of percent generalization (see “Single context training” above) or in control analyses which were restricted, by design, to a subset of experiments (Figures S3A, S4C, and S5B)]. Sample sizes were not predetermined but were comparable to previous related studies (Andalman and Fee, 2009; Charlesworth et al., 2011, 2012; Tumer and Brainard, 2007; Warren et al., 2011). All analyses were performed using custom-written MATLAB (Mathworks) software.

FF calculation

All analyses were performed on FF values that were calculated offline. In 33/36 experiments we analyzed only “catch” bouts, which were a randomly interleaved 7%–16% of song bouts in which reinforcement was withheld. In the other three experiments, we analyzed both catch bouts and a subset of bouts in which reinforcement occurred normally (“training bouts”). In experiments in which we analyzed training bouts, we excluded from analysis the two syllables directly following the target syllable, to avoid potential acute effects of WN on the FF of those syllables (Sakata and Brainard, 2006). For each rendition, we calculated a spectrogram using a Gaussian-windowed ($\sigma = 1$ ms) short-time Fourier transform (window size = 1,024 samples; overlap = 1,020 samples; sampling rate = 32 kHz). Within each time bin, FF was defined as the frequency corresponding to peak power of the first harmonic, estimated using parabolic interpolation. FF for the rendition was then calculated as the mean FF across time bins for a fixed window defined relative to syllable onset (mean window size = 14.4 ms). All syllables consisting of largely broadband noise (e.g., introductory note J in Figure 1A) were excluded from learning analyses.

Multiple regression analysis of generalization

We fit a multiple linear regression model to examine the extent to which a linear combination of three variables (contextual similarity, acoustic distance, and FF correlation) predicted the response variable (generalization) in experiments driving learning in only a single context (see “Single context training” above).

Contextual similarity was coded as a discrete variable with values 0, 1, or 2 corresponding to the number of syllables, directly preceding the target syllable, that were shared in the target and non-target contexts (see main text and Figure 1E for details).

Acoustic distance was measured between the target syllable when sung in the target and non-target contexts as the mean Euclidian distance in an 8-dimensional feature vector space. The acoustic features used were FF, duration, spectral entropy, temporal entropy, spectro-temporal entropy, amplitude slope, frequency slope and time to half-peak amplitude. All features were calculated as in (Wohlgemuth et al., 2010), with slight differences for FF (described in “FF calculation”) and frequency slope (as in Sakata and Brainard, 2006). For each syllable in each context, we calculated a mean feature vector across renditions from baseline recordings. The feature vectors for each syllable were normalized (via z-score relative to a global reference distribution of feature

vectors from 110 randomly sampled baseline renditions from each syllable in each context), and acoustic distance between any two syllables was calculated as the distance between the mean z-scored feature vectors for those syllables.

FF correlation was measured as the Pearson's correlation of FF for a syllable in two different contexts across song bouts. If a syllable in a specific context was sung more than once in a given song bout, we first took the average across those renditions to obtain one value of FF for each context for that song bout. Therefore, each pairwise correlation was calculated between two vectors, one for each context in the pair, each with length equal to the number of song bouts in the dataset.

The parameters in the model were fit using the ordinary least-squares method. The continuous predictor variables (acoustic distance and FF correlation) were first scaled such that a unit change in the scaled variable corresponded to a change of 1.59 times the sample standard deviation of that variable. This was performed to facilitate comparison with the regression coefficient for contextual similarity, since a unit change in contextual similarity corresponded to a change of 1.59 times its sample standard deviation.

DATA AND SOFTWARE AVAILABILITY

Data and custom-written software are available upon request.

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Supplemental Information

**Discrete Circuits Support Generalized versus
Context-Specific Vocal Learning in the Songbird**

Lucas Y. Tian and Michael S. Brainard

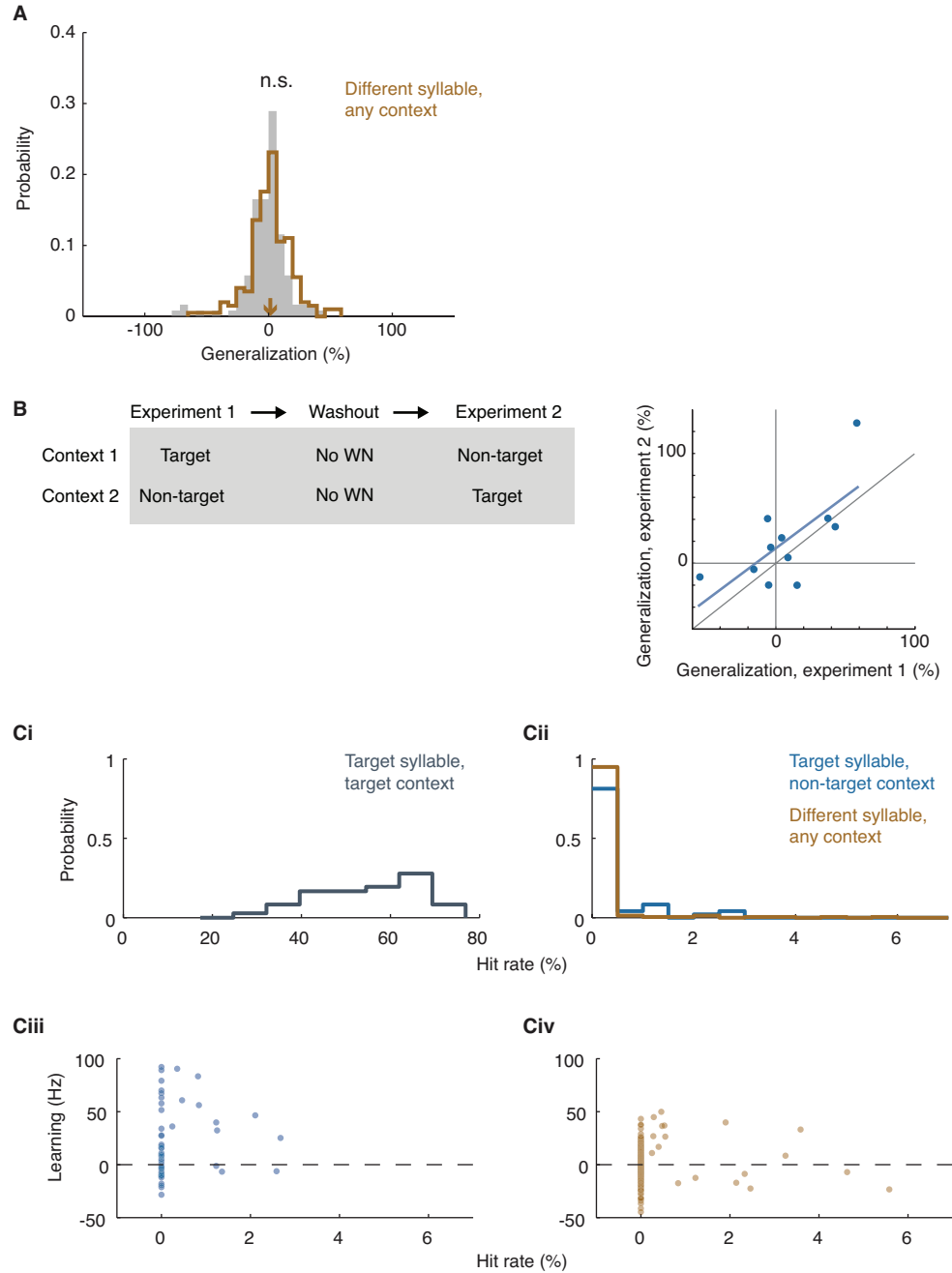


Figure S1. Learning driven in a single target context partially generalizes to non-target contexts. Related to Figure 1.

(A) Histogram of generalization of learning for different-type syllables ($n = 199$), overlaid on the distribution of baseline drift (gray, estimated by measuring day-to-day fluctuations of mean FF in baseline data, see STAR Methods). Arrow represents mean generalization, which was not significantly different from 0 by signed-rank test ($p < 0.34$). The lack of generalization for different-type syllables is consistent with a previous study using WN-driven learning in Bengalese finches (Tumer and Brainard, 2007). However, another study, using a different training paradigm in which learning was driven by sensory errors imposed by earphones, found that different-type syllables could show negative generalization (Hoffmann and Sober, 2014). The reported negative generalization was small in magnitude and inconsistent across days following onset of training. Hence, this difference from our study could reflect either a difference in generalization of learning driven by sensory errors and binary reinforcement

(Hoffmann and Sober, 2014; Izawa and Shadmehr, 2011), or other more subtle methodological differences that could have contributed to variation in the detection of small effects.

(B) Patterns of generalization, for the target syllable in non-target contexts, across multiple experiments within birds indicate that generalization is symmetric between contexts. Left: learning was driven in a single target context; in the first experiment Context 1 was the target context and Context 2 was the non-target context, and vice versa for experiment 2. Experiments 1 and 2 were separated by a “washout” period in which WN feedback was turned off, allowing FF to return to baseline. Right: the slope of generalization in experiment 2 vs. generalization in experiment 1 was not different from unity (simple linear regression: $p = 0.017$, $r^2 = 0.48$, slope = 0.95 (CI: 0.21 to 1.68)).

(Ci-iv) WN feedback was successfully localized to the target syllable in the target context.

(Ci) Histogram of hit rate for the target syllable in the target context; each datapoint is one experiment. Hit rate was calculated as the fraction of renditions that elicited WN feedback, averaged over the four days of training (see STAR Methods). The hit rates were set to ~70% at the beginning of each experiment and are generally lower than 70% in this plot due to learning.

(Cii) Histogram of hit rates across non-target syllables/contexts shows that off-target WN hits were rare. For the target syllable in a non-target context (blue histogram), in 36 out of 48 cases the frequency of off-target hits was 0%. In the remaining 12 cases the median frequency of mis-targeting was 1.2%, with a range of 0.2% to 2.7%. Similarly, for different-type syllables (brown histogram), for 227/235 cases the frequency of off-target hits was 0%. In the remaining 18 cases, the median hit frequency was 1.0%, with a range of 0.2% to 5.6%.

(Ciii, Civ) Generalization of learning to the target syllable in non-target contexts (Ciii) or to different-type syllables (Civ) could not be explained by the presence of off-target hits; there was no detected relationship between the amount of learning and rate of off-target hits (simple linear regression, $p > 0.5$ for same-type; $p > 0.91$ for different-type). Moreover, there was significant generalization to the target syllable in nontarget contexts ($p < 0.05$, signed-rank test) even if we exclude data points with nonzero hit rate.

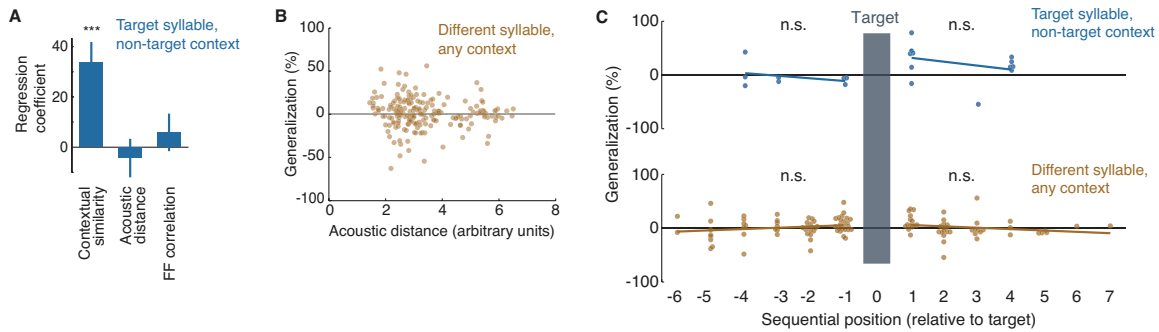


Figure S2. Examination of factors that explain patterns of generalization. Related to Figure 1.

(A) We fit a multiple regression model, with generalization to the target syllable in non-target contexts as the response variable and three predictor variables (STAR Methods): *contextual similarity* (see main text and Figure 1E), *acoustic distance* (a measure of the difference in mean acoustic structure between the target syllable when sung in two contexts) and *FF correlation* (bout-by-bout correlation between FF of target syllable renditions in the target vs. non-target contexts). The overall fit was significant ($p < 5 \times 10^{-6}$, $R^2 = 0.42$). Contextual similarity had a strong effect on generalization (***, $p < 0.0005$), while neither acoustic distance ($p = 0.58$) nor FF correlation ($p = 0.44$) had significant effects. Bars represent estimated effects with standard errors. Each regression coefficient is the expected change in the response variable (generalization, in %) for a change in the predictor variable of 1.59 times its sample standard deviation, with the other two predictor variables held constant. This results from scaling of the raw data for the continuous predictor variables (acoustic distance and FF correlation). Scaling was performed in order to facilitate comparison of the regression coefficients for acoustic distance and FF correlation with that for the third (discrete) predictor variable, contextual similarity, since a unit change in contextual similarity corresponds to a change of 1.59 times its sample standard deviation.

(B) Consistent with the lack of generalization on average for different-type syllables (Figure S1A) there was no correlation between generalization and acoustic distance to the target syllable (simple linear regression: $p = 0.25$) for different type syllables. This result parallels Hoffmann and Sober, 2014.

(C) We found no linear relationship between generalization and either the sequential position of the target syllable in a non-target context (top) or the sequential position of different-type syllables (bottom). We only included contexts that had a consistent position relative to the target syllable in the target context (i.e., in “stereotyped” sequences relative to the target). None of the linear regression analyses, performed separately for cases grouped by syllable type (top: same-type; bottom: different-type) and whether they preceded (left) or followed (right) the target, resulted in a significant effect of position on generalization (four p-values range between 0.11 and 0.40). This result is consistent with (Hoffmann and Sober, 2014), who did not find a significant relationship between sequential position and generalization when they used a similar analysis to ours, in which the mean effect for each syllable at each position from the target is considered one observation (their “ M_{syls} ”). However, Hoffman and Sober reported that there was a trend towards a relationship between generalization and sequential position using “ M_{syls} ” that became significant if they construed each iteration of each syllable as an independent observation (their “ $M_{\text{iterations}}$ ”). We did not apply this analysis because each rendition of a given syllable at a given point in our experiments was not an independent observation and therefore treating the multiple renditions of a given syllable as independent observations would have artificially inflated significance.

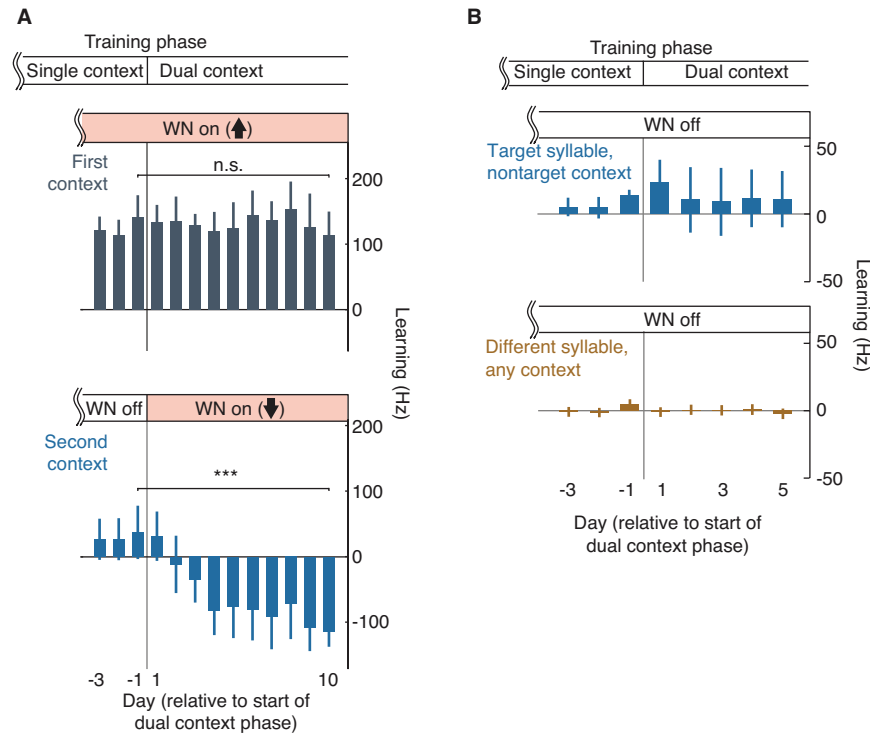


Figure S3. Independent learning for the same syllable in two contexts. Related to Figure 2.

(A) Summary of dual context training for experiments in which training was extended to 10 days ($n = 4$), plotted the same way as for the entire set of experiments in Figure 2B, which shows training up to 5 days (***, $p < 0.0005$, Student's t -test vs. day -1). FF in the second context was significantly below baseline FF on each of the last 2 days, $p < 0.05$, Student's t -test.

(B) We did not detect any change in FF, during the dual context phase, for renditions of the target syllable in a third context that does not receive WN (i.e., not in the first or second context, top, $n = 5$), or for different-type syllables (bottom, $n = 89$). For both datasets, FF does not significantly deviate from FF on the last day of the single context phase during any of the dual context days. However, we do not construe this result as conclusive for the target syllable in a third context (top) due to low sample size.

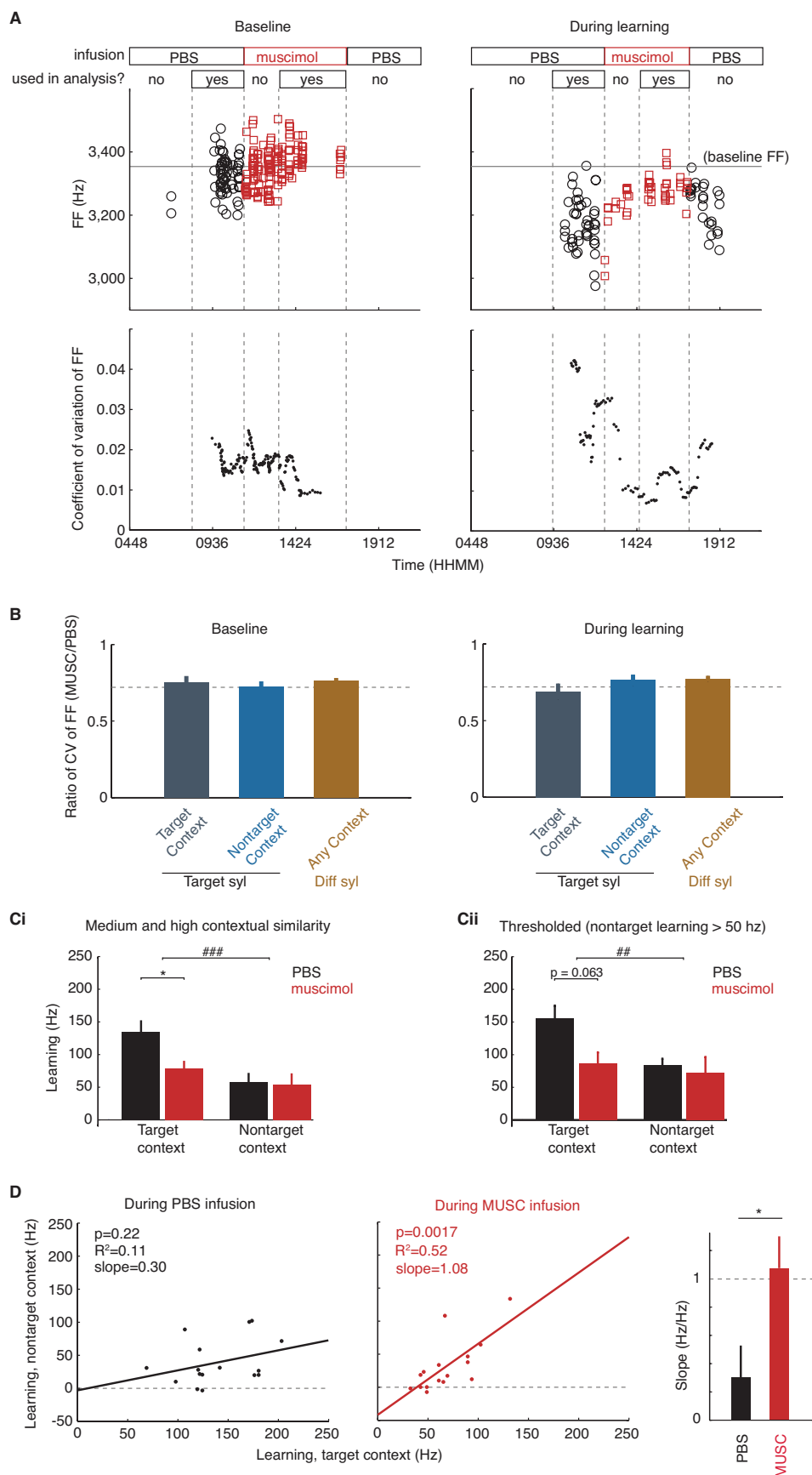


Figure S4. The AFP adaptively biases motor output in a context-specific manner. Related to Figure 4.

(A) Example of a single day of inactivation during baseline (left) and learning (right) in the same experiment (for the target syllable in the target context). The top two plots show FF for individual renditions and bottom two plots show the running average of the coefficient of variation for FF (CV, standard deviation/mean, using windows of 15 renditions and plotted at the mean time of those renditions). On the baseline day, infusion was switched from PBS to muscimol at 11:25. FF during muscimol was defined as the mean FF of songs starting after a lag of 120 minutes from start of muscimol infusion, when the CV for FF was fully reduced (a marker of successful LMAN inactivation, see STAR Methods), and ending when infusion was switched back to PBS. FF during PBS was defined using a window starting three hours before the switch and ending at the time of the switch. For all inactivation days during baseline and learning the temporal window for PBS and muscimol data were defined in the same manner. In this experiment the bird was trained to shift FF lower. The plot on the right, representing a day during learning, depicts expression of learning before inactivation, transient reversion of learning during inactivation, and recovery of learning once muscimol was washed out.

(B) LMAN inactivation caused a consistent reduction in the CV of FF. Mean \pm SEM across all syllable types of the ratio of the CV during muscimol infusion to the CV during PBS infusion, separately plotted for baseline (left) and learning (right) days. In all cases the ratio was significantly lower than one (signed-rank test: $p < 0.05$), and was similar to the ratio in previous LMAN inactivation experiments in Bengalese finches (dashed line, from Warren et al., 2011).

(Ci, ii) LMAN inactivation had a differential effect on expression of learning in target and non-target contexts, even when restricting analysis to experiments with large magnitude of generalized learning in the non-target context. This restriction was performed two different ways.

(Ci) First, we took advantage of our finding that generalization is greatest in cases of higher contextual similarity (see Main Text and Figure 1E). We therefore considered pairs of target and non-target contexts that shared at least one syllable directly preceding the target syllable ($n = 8$ experiments). There was significant reversion for target contexts (*, $p < 0.05$, signed-rank test) and no significant reversion for non-target contexts ($p > 0.5$), and reversion was significantly greater for target contexts (###, $p < 0.0005$, signed-rank test). Mean reversion for the target context was 57.0 ± 9.4 Hz out of 135.4 ± 17.1 Hz total learning (42% reversion) and for the non-target context was 4.3 ± 10.3 Hz out of 58.0 ± 14.0 Hz total learning (7% reversion).

(Cii) Second, we restricted our analysis to pairs of target and non-target contexts for which learning in the non-target context was greater than 50 Hz ($n = 5$); this value was chosen because previous experiments indicate that we would expect to detect reversion for learning of this magnitude or greater [published data from a study in zebra finches (Andalman and Fee, 2009)]. This analysis also resulted in significantly greater reversion for target versus non-target contexts (##, $p = 0.008$, signed-rank test), with no significant reversion in the non-target context ($p = 0.48$). Mean reversion for the target context was 68.7 ± 15.1 Hz out of 155.3 ± 19.9 Hz total learning (44% reversion) and for the non-target context was 11.8 ± 17.1 Hz out of 84.4 ± 9.5 Hz total learning (14% reversion).

(D) LMAN inactivation increases the ratio of learning expressed in the non-target context relative to the target context, consistent with the effect of LMAN inactivation, and therefore AFP bias, being highly specific to the target context. The ratio of learning expressed in the non-target context, relative to the target context, is estimated as the slope from a simple linear regression analysis comparing non-target context learning vs. target context learning across experiments. This slope is calculated separately for learning during PBS (black, left) or muscimol (middle, red) infusion in the same experiment. The increase in this slope during muscimol infusion, relative to PBS infusion (right, bar plots, *, $p < 0.05$, analysis of covariance) indicates that LMAN inactivation leads to a greater proportional reduction of learning in the target context than in the non-target context.

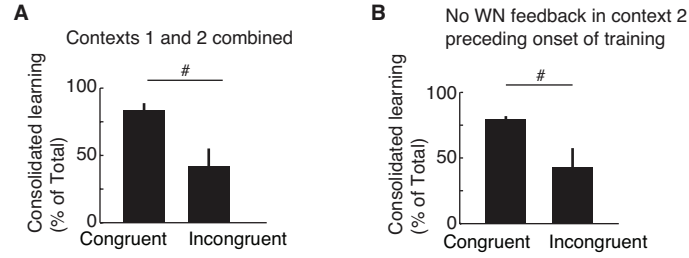


Figure S5. Conflicting AFP bias interferes with consolidation for context-specific learning. Related to Figure 5.

(A) Consolidation in the late period (days 5-6) of maintained learning was reduced in Incongruent ($n = 7$) experiments relative to Congruent experiments ($n = 5$) when measurements were combined between context 1 and 2 (#, $p < 0.05$, rank-sum test). This panel is analogous to Figure 5D, in which consolidation in only the first context is presented.

(B) Consolidation in the late period of maintained learning was reduced in Incongruent ($n = 6$) experiments relative to Congruent experiments ($n = 3$) even when only including experiments for which training in the second context was preceded by a period with no WN feedback. #, $p < 0.05$, rank-sum test.