Neural mechanisms of motor skill flexibility in songbirds

by
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Dedicated to Jeffrey Knowles

He only needs to block his face with the nearest plastic visor he can get his hands on and the blindness, if he is lucky, becomes reality. So grab a hand and drive through this land that envelopes scientists and artists and prisoners alike, be them a million years or million angstroms apart.

Neural mechanisms of motor skill flexibility in songbirds

by Lucas Tian

Abstract

Even well-learned motor skills must be performed differently depending on context (e.g., the skills involved in riding a mountain bike and road bike will generally differ). At the same time, learning must take advantage of the similarities between similar skills performed in different contexts by transferring, or generalizing, learning gained in one context to performance of similar skills in other contexts (e.g., it would be useful if learning first how to ride a road bike makes it easier to then learn how to ride a mountain bike). The flexibility of motor skills, therefore, depends on the ability for the nervous system to adaptively balance the generalization and specificity of learned modifications.

This adaptive balance is perhaps best illustrated in sequential motor skills, such as speech or dance (indeed "sequential" applies to a wide range of skills). These 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 - this "coarticulation" is thought to enable the smooth and rapid production of skills. A diversity of experimental studies on humans have revealed that learned modification to a given gesture tends to generalize when the same gesture is used in other contexts; however, there is an additional capacity to learn highly context-specific modifications to individual gestures if such learning is the optimal way to respond in a given sensory environment. How this adaptive

balance between generalization and specificity is implemented in neural mechanisms is largely unclear.

In this dissertation I report on experiments describing the neural mechanisms enabling generalization and specificity of vocal learning in birdsong. Bengalese finch song consists of variable sequences of discrete vocalizations called "syllables." I first showed that at the behavioral level, Bengalese finches balance generalization and specificity of learned modifications to syllables in a manner that looks remarkably similar to the balance previously demonstrated for humans in similar motor adaptation experiments. In particular, 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 highly-specific for each context, to an extent unexpected given the original propensity to generalize.

I then used localized inactivation of a cortical-basal ganglia circuit specialized for song to find that this balance between generalization and specificity reflects a hierarchical organization of neural substrates. Primary motor circuitry [the "motor pathway" (MP)] encodes a core syllable representation that contributes to generalization, while context-specific input from cortical-basal ganglia circuitry [the "anterior forebrain pathway" (AFP)] biases this representation to enable context-specific learning.

Finally, I performed neural recording experiments with the goal of further understanding *how,* in terms of changes to neural activity, this context-specific pitch bias is implemented. By analyzing the correlation between spiking activity in LMAN (the output of the AFP) and RA (the primary motor circuitry within the MP crucial for encoding syllable acoustic structure) during singing and learning, we found evidence suggesting the presence

of a premotor signal conveyed from LMAN to RA, generated during learning, which acts to bias pitch through biasing of motor activity in RA. This biasing signal may be the outcome of the integration of signals encoding context, performance, and feedback in the AFP.

Taken together, these results (1) establish Bengalese finch song as a model system to study the flexibility of motor skill learning, (2) localize two key behavioral components of flexibility - generalization and specificity - to two different circuits, and (3) provide empirical support for the neural mechanisms by which these two circuits interact to adaptively balance generalization and specificity of learning.

Beyond birdsong, these findings may suggest broader principles regarding the neural mechanisms of flexibility in the learning and execution of motor skills, the contributions of cortical-basal ganglia circuitry to such flexibility, and the neural mechanisms involved in the control and adaptation of sequenced motor skills.

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Chapter 1: Introduction

I will first provide a broad overview of the problems in the neuroscience of motor skills that are driving the work in this dissertation. I then introduce birdsong and motivate its use as a model to study these problems. Because Chapters 2 and 3 each have their own introduction and discussion sections, in this chapter I have tried, for points that will be made in Chapters 2 or 3, to limit the amount of detail included here. In Chapter 4 I try to tie together all the findings and relate them to some more general research problems.

1.1. Motor skill flexibility

An experimental approach to motor skill flexibility.

Motor skills allow us to interact with the world in a goal-directed manner. The ability to precisely coordinate muscle activity to generate movement enables communication, tool use, locomotion, sport, dance, and contributes to virtually all animals' behaviors. Motor skills depend on the ability for the brain to generate patterned activity, appropriate for a given context, goal, action, that is conveyed through the rest of the nervous system to drive appropriately patterned activity of muscles.

A remarkable feature of motor skill is its flexibility (Adolph and Eppler, 2002; Bernstein, 1996; Clearfield and Thelen, 2001; MacKay, 1982; Wolpert and Kawato, 1998; Wolpert et al., 2001). The movements underlying a given skill are not generally produced in an identical fashion from use to use; instead the movements are adaptively modified in a manner appropriate for all the different contexts and situations in which it may be expressed. Somehow the nervous system must "know" how to modify the way a tennis

racket is swung depending on context unique to each instance (e.g., body posture, location of opponent, spin on the ball, etc.). On the other hand, some movements *should[1]* be performed in a similar way across different contexts because these contexts do not differ in their demands on the movement (e.g., as a tennis player tires gradually over the course a match, she should generally compensate in a similar way across all movements in order to counteract this fatigue); as a result it would be optimal for any learned modifications to those movements to transfer, or generalize to all contexts in which this movement is performed. In general, the ability to transfer learned modifications allows for the performance of new skills with as little relearning as possible, and also the ability to build on prior learning to acquire increasingly complex skills.

In this dissertation, the term "flexibility" will be used to denote the ability to adaptively balance these seemingly competing aspects of motor skills: generalization and specificity. Flexibility has been studied both in naturalistic behaviors (Adolph and Eppler, 2002; Bernstein, 1996; Clearfield and Thelen, 2001; MacKay, 1982; Wolpert et al., 2001) as well as in motor adaption studies in which the experimenter has precise control over the types of movements being performed and, crucially, the type of sensory feedback subjects receive during movements (details below). Experimentally-induced systematic perturbation of sensory feedback acts as the training signal, which drives a gradual

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[1] From the standpoint of what might be "optimal," which, admittedly, the nervous system does not *a priori* have to care about.

[2] Two main types of perturbations are used: one is a predictable force field that perturbs

modification of behavior in a manner that counteract that perturbation[2]. This form of motor learning is called "motor adaptation."

Flexibility in motor adaptation is assessed by testing generalization and specificity of learning across contexts. Subjects tend to exhibit partial generalization of learning across contexts if training signals are provided when a motor gesture is performed in only one context. For example, training in one sequential context, defined by the sequence of gestures in which the gesture "targeted" for learning is embedded[3], tends to generalize to when the targeted gesture is produced in other sequential contexts (Houde and Jordan, 1998; Howard and Franklin, 2015; Rochet-Capellan et al., 2012)]. However, subjects reveal a capacity for learning modifications that are highly specific to context if different types of incompatible training signals[4] are provided in multiple contexts (Gandolfo et al., 1996; Howard et al., 2012; Nozaki et al., 2006; Pearson et al., 2010; Rochet-Capellan and Ostry, 2011; Wainscott et al., 2004). People therefore demonstrate an adaptive balance between generalization and specificity; this balance depends on the extent to which feedback signals differ across contexts[5]. The overarching question in this dissertation is: what mechanisms in the brain enable this balance of generalization and specificity?

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[2] Two main types of perturbations are used: one is a predictable force field that perturbs movements and the other is disruption of sensory feedback during the movement.

[3] E.g., the word in which a phoneme is embedded, or the sequence of arm movements directly preceding and following a discrete reaching movement.

[4] Incompatible in the sense that the modifications they are driving are opposite to each other.

[5] A more extensive discussion of the study of flexibility in motor adaptation is in Chapter 4.

Motor skills depend on the reuse of a smaller set of motor "primitives" in a larger set of contexts.

Posing this question about generalization and specificity implicitly assumes, to some extent, that motor skills are organized in a manner that involves the reuse of a set of motor gestures or primitives in a large set of contexts. What evidence is there that skills are controlled in this hierarchical manner?[6] Indeed, it is difficult to directly observe hierarchical structure from inspection of behavior partly because well-learned skills are often produced in a highly smooth manner that belies the involvement of discrete motor gestures. However, there are various lines of evidence consistent with motor skills depending on the recombination of motor gestures: (1) There is the common-sense argument that, given the extremely large set of slightly different skilled sequences that one could perform, it would be impossible to learn to produce each through practice - there must be some reuse of a smaller set of primitives [e.g., (Bernstein, 1996; Jordan, 1986)]. (2) There is evidence from stimulation studies and analyses of muscle electromyography that movements may be constructed by the superposition and sequencing of primitives defined by muscle co-activation patterns, or "synergies" (d'Avella et al., 2003, 2015; Berger et al., 2013; Dominici and Lacquaniti, 2011). (3) Neural recordings and imaging studies of brain circuits have found evidence for hierarchical representations of motor sequences

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[6] "Hierarchical" in the sense that representations of motor gestures make up the lower level, and sequencing/selection/action/planning processes make up the higher level. See Chapter 4 for a discussion of different types of hierarchies in motor skills.

(Averbeck et al., 2002; Diedrichsen and Kornysheva, 2015; Tanji and Shima, 1994)[7]. (4) It is possible for some brain manipulations to specifically impair sequencing, but leave intact execution of individual actions [e.g., (Aldridge and Berridge, 1998; Cromwell and Berridge, 1996; Markowitz et al., 2018; Shallice and Burgess, 1991)] and for other manipulations to specifically impair execution while minimally affecting sequencing (Long et al., 2016). (5) Analysis of how learned adaptations to an action, trained in one context (e.g. body posture, action sequence) automatically generalizes to production of the same action in other contexts indicates that similar neural substrates are involved even in different contexts [see (Poggio and Bizzi, 2004; Shadmehr and Mussa-Ivaldi, 1994) and discussion in this Chapter and Chapter 4). (6) Detailed analysis of speech behavior, especially of errors in motor skill production, provide evidence for hierarchical mechanisms [(Hickok, 2012; Sternberg and Wright, 1978); for an alternative view on speech errors see (Mowrey and MacKay, 1990)]. While a strict hierarchical model is unlikely to entirely account for motor skills, not the least because feedback from lower to higher levels is crucial, the evidence seems to be strong enough such that one can frame the problem of motor skill flexibility as one of understanding the balance between generalization and specificity of modifications to motor gestures.

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[7] Although note that there is also neural recording evidence that even regions that one might position "lower" in the hierarchy often exhibits activity that encodes "higher" features (Ben-Shaul et al., 2004; Lu and Ashe, 2005; Ostry et al., 1996). This argues that even in a hierarchical model, there is an important place for feedback and non-feedforward processing.

Neural mechanisms of motor skill flexibility

There is extensive research in human (and to a lesser extent non-human primate) motor adaptation studies on patterns of how adaptation to a given gesture (e.g., a reaching movement performed to one target) generalizes to similar gestures (e.g., a reaching movement to another nearby target, or the same reaching movement but in a different sequential context). Any natural tendency to partially generalize is thought to reflect the similarity of the underlying neural substrates, or somewhat synonymously, the coordinate system in which the action is controlled) (Mattar and Ostry, 2007; Poggio and Bizzi, 2004; Shadmehr and Mussa-Ivaldi, 1994).

A crucial finding is that the motor system is not hard-wired to generalize. In fact it has a remarkable ability to learn specific adaptations associated with different contextual cues, if those cues are predictive of different types of feedback or constraints on movement. From an ethological standpoint the efficacy of a contextual cue in supporting context-dependent adaptation seems to relate to whether it would normally be associated with different movement requirements; for example, arbitrary visual cues are not effective (Gandolfo et al., 1996; Howard et al., 2013; Osu et al., 2004), while some of the most effective cues are differences in posture (Gandolfo et al., 1996) and sequential context (Howard et al., 2012; Rochet-Capellan and Ostry, 2011)[8]. This might reflect that in the real world execution often should differ based on body state or posture or action sequence[9].

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[8] In Chapter 4 is a more extensive discussion of context-dependent adaptation. See (Ayala, 2015) for a recent survey.

[9] Interestingly, people seem much better at using visual cues if those cues are somehow realistically incorporated into something that looks like a virtual tool (Cothros et al., 2009;

How does the brain learn context-dependent adaptations[10]? Partly because these studies focus on human subjects, there is not much direct understanding of neural mechanism. In brief, there is an expectation that the brain contains internal models that it can switch between depending on contexts, thus enabling context-dependent behavior. These ideas, and their relation to the experimental findings in this dissertation, will be discussed in Chapter 4.

1.2. The songbird as a model for motor skill learning and production

The songbird has for decades been a prominent model system for studying the neural mechanisms of motor skill learning and production. There are a few features that position birdsong as a useful model system.

First, birdsong is learned through a process that is similar that that underlying the learning of complex motor skills in other species, and has particularly striking parallels to the learning of speech (Doupe and Kuhl, 1999). Learning involves a development process

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Howard et al., 2013). A similar "selection" for sensory cues by ethological relevance occurs in other behaviors (Garcia and Koelling, 1966).

[10] Note that the above discussion focused entirely on laboratory studies of motor adaptation, which focus on the modification of movement execution, in experimental paradigms that control for modification to other features of motor skills (e.g., sequencing). However, the neuroscience of motor learning and control spans a wide range of organisms and tasks; some tasks, such as the learning of a new skill, may involve a wide range of interacting learning processes often studied in separate subfields (e.g., action selection, sequence learning, motor adaptation). Chapter 4 includes a discussion of how these multiple aspects of motor learning may involve similar underlying processes in the basal ganglia (building on the role of basal ganglia in birdsong learning).

that occurs in multiple, overlapping stages (Doupe and Kuhl, 1999; Immelmann, 1969; Konishi, 1965; Marler; Price, 1979; Thorpe, 1958). In the "sensory" stage, juvenile birds listen to and memorize the songs of adults - this auditory memory forms a "template"; the goal of song learning is to mimic this template. Next, during the "sensorimotor stage", through extensive practice, birds transition from singing an unstructured "subsong" (akin to "babbling" in speech), to a crystallized adult song. Adult song can be described as consisting of relatively stereotyped sequences of discrete vocalizations called "syllables" (Figure 2.1). This sensorimotor practice requires both motor production and hearing, and is thought to depend on a trial-and-error process by which sensory-motor circuits in the brain evaluate sensory feedback in order to appropriately modify subsequent motor output.

Second, song is relatively unique among complex motor skills in its amenability to detailed quantitative monitoring and analysis. The "goal" of the motor system, for birdsong, is to produce sounds of certain acoustic quality in its frequency content, loudness, and temporal modulation all of which can be recorded in its entirety and quantified precisely. This amenability to quantitative monitoring allows for the detailed study of the behavioral learning trajectory, and the analysis of behavioral variation and how it correlates with neural signals and is affected by various nervous system manipulations. Moreover, the ability to synthetically mimic song by playing sounds through a speaker allows for the experimental manipulation of auditory tutoring and feedback.

Third, songbirds are tractable for experimental manipulations in the brain due to their small size and the anatomy of the brain regions for song learning and control. These regions form a circuit consisting of discrete interconnected nuclei (see Figure 2.3 and

corresponding text for details). The fact that these nuclei seem to only be involved in song and no other behaviors, and are anatomically separated from each other, allows for experimental manipulations of relatively high specificity.

The role of the Anterior Forebrain Pathway (AFP) in song plasticity

Lesions in the anterior forebrain pathway (AFP) dramatically impair song development in juveniles (Bottjer et al., 1984; Scharff and Nottebohm, 1991; Sohrabji et al., 1990)). Early on, it was shown that part of the contribution of the AFP to song plasticity is likely in providing trophic support of the developmental changes in the motor pathway (MP) that are crucial for song learning (e.g., release of growth factors) (Akutagawa and Konishi, 1994, 1998; Johnson and Bottjer, 1994; Johnson et al., 1997; Kittelberger and Mooney, 1999, 2005). In addition, it was hypothesized that the AFP may also play an active role in conveying signals reflecting the outcome of an error computation process (either in the AFP or upstream). The AFP would therefore contribute a biasing signal that pushes behavior closer towards mimicking the memorized tutor song template [e.g., (Bottjer et al., 1984; Doupe and Konishi, 1991; Scharff and Nottebohm, 1991)].

The strongest evidence that the AFP not only provides trophic support, but also biases behavior as part of an active error-driven process, came arguably from recent studies on the role of the AFP in adult song plasticity. This line of research was ignited by findings that adult song, previously thought to be immutable, would, in fact, demonstrate significant plasticity in response to altered sensory feedback (Leonardo and Konishi, 1999; Nordeen and Nordeen, 1992; Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997). This is thought to reflect a natural ongoing process of sensory-feedback dependent motor

calibration present throughout life (Sober and Brainard, 2009). Furthermore, it was determined that this adult plasticity depends on the AFP, mirroring juvenile plasticity. This was inferred from studies in which lesions to the AFP blocked adult plasticity that is normally induced by deafening (Brainard and Doupe, 2000), denervation of the vocal musculature (Williams and Mehta, 1999), or developmental manipulations that lead to juvenile-like learning in adults (Morrison and Nottebohm, 1993).

A training paradigm to study learned pitch modifications in adult birds.

The finding of adult song plasticity allowed for the development of controlled training paradigms analogous to the controlled motor adaptation paradigms in humans (as described above). Adult song is stable enough to implement closed-loop feedback systems that are able to disrupt sensory feedback in a controlled manner; moreover this stability is crucial in allowing experimenters to measure subtle changes to behavior that reflect motor adaptation driven by this disrupted feedback.   The first publication to use such a paradigm trained adult birds to modify the acoustic structure of song, over the course of hours, by providing aversive auditory feedback in a manner contingent on the pitch (or fundamental frequency) of a chosen "Target" syllable (Tumer and Brainard, 2007). Birds will gradually (over the course of hours) modify the pitch of their song to reduce the probability of eliciting this disruptive feedback (see Figure 2.1). This paradigm offers remarkable control over learning at the level of (1) what song element is being modified (i.e., which syllable, or subsyllabic timepoint, is being targeted), (2) which direction pitch should be modified (e.g., if high pitch renditions are targeted with feedback, then learning proceeds downwards), and (3) the magnitude of learning can be controlled - greater

learning is elicited if during the experiment the pitch threshold is gradually shifted to "follow" the bird's pitch as it is gradually changing, so that the bird has a constant drive to increasingly shift pitch.

The combination of this training paradigm with methods for disrupting neural activity, over a large number of studies, have led to remarkable insight into contributions of the AFP and MP to adult pitch modifications. Some important points are summarized below.

Silencing LMAN, the output nucleus of the AFP, blocks initial learning and the expression of recent learning.

If LMAN is silenced pharmacologically, WN-drive pitch modifications cannot occur (Charlesworth et al., 2012). If learning is first initiated, and then once pitch has changed appreciably before LMAN is silenced, learning that was obtained recently is eradicated - i.e., learning reverts towards its baseline (pre-training) value (Andalman and Fee, 2009; Warren et al., 2011). These findings indicate that the AFP contributes to learning in adult birds by provide a bias to motor output, such that pitch is shifted in a direction that increases the likelihood of escaping aversive feedback[11]. The neural mechanisms by which this bias occurs is largely unknown and is a question motivating Chapter 3.

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[11] Learning seems to be better explained as "learning to do more of what led to escapes" as opposed to "learning to do less of what led to WN feedback" (Charlesworth et al., 2011).

Reversion is incomplete, suggesting a two-stage mechanism that maps onto the AFP and the motor pathway (MP).

Inactivation of LMAN during learning does not cause complete reversion of learning. Moreover, dependency of learning on LMAN activity decreases over days[12] (Andalman and Fee, 2009; Warren et al., 2011). These results indicate that learning gradually becomes dependent on modifications downstream of the AFP. There are a few reasons to believe that these modifications occur in the MP. First, there is evidence in brain slices for a plasticity mechanism in which the relative strength of LMAN's and HVC's inputs to a given RA projection neuron depends on the relative temporal patterning of those inputs (Mehaffey and Doupe, 2015). This dependence on LMAN's inputs may enable LMAN to bias plasticity at HVC-RA synapses, thus enabling the "transfer" of learning-related plasticity from the AFP to the MP. Second, variation in RA activity is likely able to influence variation in behavior (Sober et al., 2008), increasing the plausibility that plasticity in RA could encode pitch modifications. Finally, developmental plasticity also undergoes a process by which song production gradually shifts from being AFP- to MP-dependent [e.g., see (Aronov et al., 2008; Kittelberger and Mooney, 1999)]. Indirect evidence that adult pitch modifications reflect a serial transfer of changes from the AFP to the MP, as opposed to a process where modifications occur in parallel but at different rates, is that learning does not occur at all unless LMAN is active (in both juveniles and adults).

A model recapitulating these points on the role of the AFP and the MP in adult pitch modifications is presented in Figure 2.3 and the accompanying text. All of these ideas will be discussed in greater detail in Chapter 4.

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[12] Again, see Figure 2.3 and accompanying text.

1.3. Pitch learning in adult Bengalese finches as a model for generalization and specificity of motor skill learning

A particular feature of Bengalese finch song positions this behavior as a potentially powerful model to study neural mechanisms underlying the patterns of generalization and specificity of motor skill learning. In particular, Bengalese finch song exhibits sequence variability, such that a given syllable can be naturally sung in multiple sequential contexts (Okanoya, 2004; Warren et al., 2012; Woolley and Rubel, 1997). The pitch training paradigm, which normally occurs by providing altered feedback to drive learning for one syllable, can be modified to direct context-dependent learning by targeting the pitch-contingent reinforcement to a given syllable only when it is sung in one preselected "Target" context. In turn, one can imagine varying the pitch-contingency of feedback across contexts in any arbitrary way. One can provide a strongly context-dependent task by providing aversive feedback driving pitch up in one context and down in another. Alternatively, one can provide feedback that is general by providing the same direction feedback across contexts. I take advantage of this ability to experimentally "tune" the complexity[13] of feedback in the behavioral experiments in Chapter 1 to assess the extent to which birds exhibit generalization and, in addition, the ability to modify patterns of generalization depending on the pattern of feedback across contexts.

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[13] Here and in other sections, I sometimes use "complex" to mean tasks in which training feedback differs across contexts (i.e., more specificity of learning is required in a complex task).

I will briefly note that the use of this experimental strategy on a sequenced motor skill mirrors prior motor adaptation studies in humans in reaching and speech sequences (see Chapter 2); thus behavioral results with birds may potentially be productively compared to those for related human tasks.

Finally, the known contributions of the AFP and MP to pitch modifications allow me to build on that prior work to ask how the song system enables generalization and specificity of pitch modifications, as in Chapters 2 and 3.

1.4. Summary of this dissertation

Chapter 2 presents two sets of experiments. The first set characterizes the patterns of generalization of learning across sequential contexts as a function of the extent to which the information encoded in feedback signals differ across contexts. By revealing both a "default" tendency to exhibit generalization and an additional capacity for highly specific learning, these experiments set up Bengalese finch song as a potentially powerful model to study the neural mechanisms underlying the adaptive control of generalization.

The second set of experiments in Chapter 2 use transient, localized, pharmacological silencing of LMAN during learning to reveal that separate circuits contribute to generalization and specificity; in particular, modifications to circuits downstream of LMAN, presumably in the motor pathway, lead to generalization of learning, while the AFP generates a context-specific motor pitch bias that promotes the expression of context-specific learning.

Experiments in Chapter 3 seek to characterize the neural mechanisms of this context-specific motor bias from the AFP. By examining the correlations between spiking

neural activity in LMAN and RA during singing and learning, and how those correlations relate to behavior, I provide evidence that the pitch bias generated by the AFP (defined with respect to behavior) reflects fast synaptic transmission of signals from LMAN that convey an instructive premotor influence biasing RA premotor activity.

Chapter 4 integrates the results from the prior chapters into a discussion regarding birdsong and motor skills in general.

Chapter 2: Discrete circuits support generalized vs. context-specific vocal learning

This chapter was previously published as:

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The content of this chapter is identical to the publication except in three ways: figure numbers have been modified to indicate they are in Chapter 2, some footnotes have been added, and references have been moved into the general bibliography at the end of the dissertation.

2.1. Abstract

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, a skill that, 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 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.

2.2. 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 ("coarticulation") 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, then 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; Wainscott et al., 2004)[14]. Collectively, these behavioral observations raise the question of what neural architectures 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 2.1A; (Doupe and Kuhl, 1999)]. Moreover, experimentally

[14] A related paradigm looks at the patterns of generalization when modifications are learned to either discrete movements or rhythmic movements formed by repeating the discrete movement - the movement can be construed as being performed in two sequential contexts (discrete vs. in a repeat). Motor adaptation studies indicate that learning shows specificity for this type of sequential context (Howard et al., 2011; Ikegami et al., 2010).

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 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 used 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.

2.3. Results

Learning driven in a single target context partially generalizes to non-target contexts

We first evaluated whether birds 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 2.1B, "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 2.1C, example experiment; Figure 2.1D, 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 was some natural tendency for context specificity in learning (Figure 2.1C, D, n = 36 experiments, rank-sum test of FF change in target vs. non-target context: $p < 5 \times 10^{-9}$; Figure 2.1E 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 (Figure 2.1C, D, signed-rank test: p = 0.34, Figure S2.1A, Kolmogorov-Smirnov test comparing distributions of learning vs. 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 2.1E). 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 2.1E). 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 2.1E, 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 S2.2 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 2.2A, example experiment; Figure 2.2B, summary). We then initiated reinforcement in a second context,

with the FF contingency opposite to that in the first context, while maintaining the contingency in the first context (Figure 2.2A, B, "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 2.2C, 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 2.2B, 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 five days (Figure S2.3A). In contrast, learning that had occurred in the first context was maintained with no significant change (Figure 2.2C, n = 13, signed-rank test: p = 0.31; we also did not detect any significant changes to FF of different type syllables, Figure S2.3B). 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 (p < 0.0005, n = 13, signed-rank test). 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 2.3A) 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 anterior forebrain pathway (AFP, Figure 2.3A), 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 AFP causes a reversion of FF towards baseline values (Figure 2.3B, "Early"), but that over a period of maintained learning, the effects of inactivating the AFP gradually diminish (Figure 2.3B, "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 2.3B, "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 2.3B, "Consolidated to MP", filled green circle in RA). If these same mechanisms contribute to all adaptive modifications of song, then 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 2.4A top, example experiment; Figure 2.4B 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 2.4A bottom, example experiment; Figure 2.4B bottom, summary, n = 13 experiments, 14% shift from 42.7 ± 10.3 Hz during PBS versus 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 2.4D, 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 vs. 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 2.4A and summary data in Figure S2.4C) and in analysis of the ratio of effects of inactivation on expression of learning in target and non-target contexts (Figure S2.4D). 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 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 2.4C), generalization arises because AFP biasing signals 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 consolidate, to the motor pathway during learning; in particular, for context-specific learning there should be reduced consolidation, 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, then 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 2.5Ai, "Early") that would act coherently to drive a strong transfer of context-independent changes to the downstream motor pathway (Figure 2.5Ai, "Late"). In contrast, for Incongruent training, the AFP would generate oppositely directed biasing signals in the two contexts (Figure 2.5Aii, "Early"), that would antagonize each other in converging onto a shared downstream motor pathway representation of the syllable, and thereby interfere with transfer of learning (Figure 2.5Aii, "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 four days of maintained learning ("Early" in Figures 2.5A, B), when previous work has shown that the expression of learning depends 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 2.5C). 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 5-6), when previous work indicates that the expression of learning in a single context becomes largely independent of the AFP (Warren et al., 2011). For Congruent training, consolidation was not significantly different from 100% (Figure 2.5D, 84.9 ± 5.9%, mean ± SEM, signed-rank test: p = 0.12), and was

indistinguishable from consolidation previously reported for syllables that are sung in only a single context [Figure 2.5D, 84.9 ± 7.1% in (Warren et al., 2011)]. In contrast, for Incongruent training, consolidation was both significantly less than 100% and significantly reduced relative to that for Congruent training (Figure 2.5D, 44.1 ± 12.0%, p < 0.05, signed-rank test vs. 100%; p < 0.005, rank-sum test vs. Congruent). These data indicate that under conditions in which generalization is appropriate, learning rapidly becomes transferred to the motor pathway. In contrast, under conditions when context-specific modifications to a gesture are required, transfer to the motor pathway is impaired and there is an ongoing requirement of biasing signals from the AFP for the expression of learning.

2.4: 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 anterior forebrain pathway (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 2.4C, 2.5A). 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 2.5D). 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 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 integrating contextual signals (reflecting the current syllable and sequence) with instructive signals (reflecting the appropriate FF for each context) to enable context-dependent vocal learning (Figure 2.4C, 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 – 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 2.4) 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 2.5). 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 coherence of AFP bias may therefore 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; Hilario 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; Mushiake 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 time-scale 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.

2.5. Acknowledgements

2.6. Methods

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 upwards shifts in FF, feedback was delivered with <1 ms latency if FF was below threshold; to drive downwards 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. This 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 S2.1B); in six cases we targeted a previously targeted syllable/context

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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 1-2x 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 5-6 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 97.5[th] 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 S2.1Cii, blue histogram). In the remaining 12 non-target context cases the median frequency of mis-targeting 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 S2.1Cii, 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 (Figure S2.1Ciii, iv).

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 ± 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 followed immediately 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 1-2x 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 towards 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 (Lindefors et al., 1989; Warren et al., 2011). Bilateral guide cannulas (CMA 7, Harvard Apparatus) were first stereotaxically 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 midsaggital and transverse sinuses (i.e., "Y_0"), 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 S2.4A, B).

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 S2.4A; 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 S2.4B). 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 i) the FF threshold for WN was no longer being adjusted and ii) 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 (S.D. = 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 or 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 vs. 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 5-6) 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 S2.5B).

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 2.1-2.4, no randomization was required in allocating animals to experimental groups because each animal contributed to both experimental groups (dimension 1: target vs. nontarget context; dimension 2: PBS vs. muscimol infusion). For the experiment in Figure 2.5, randomization in allocation to experimental types (Congruent vs. 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 S2.3A, S2.4C, S2.5B)]. 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 (σ = 1 ms) short-time Fourier transform (window size = 1024 samples; overlap = 1020 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 2.1A) 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 2.1E 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.
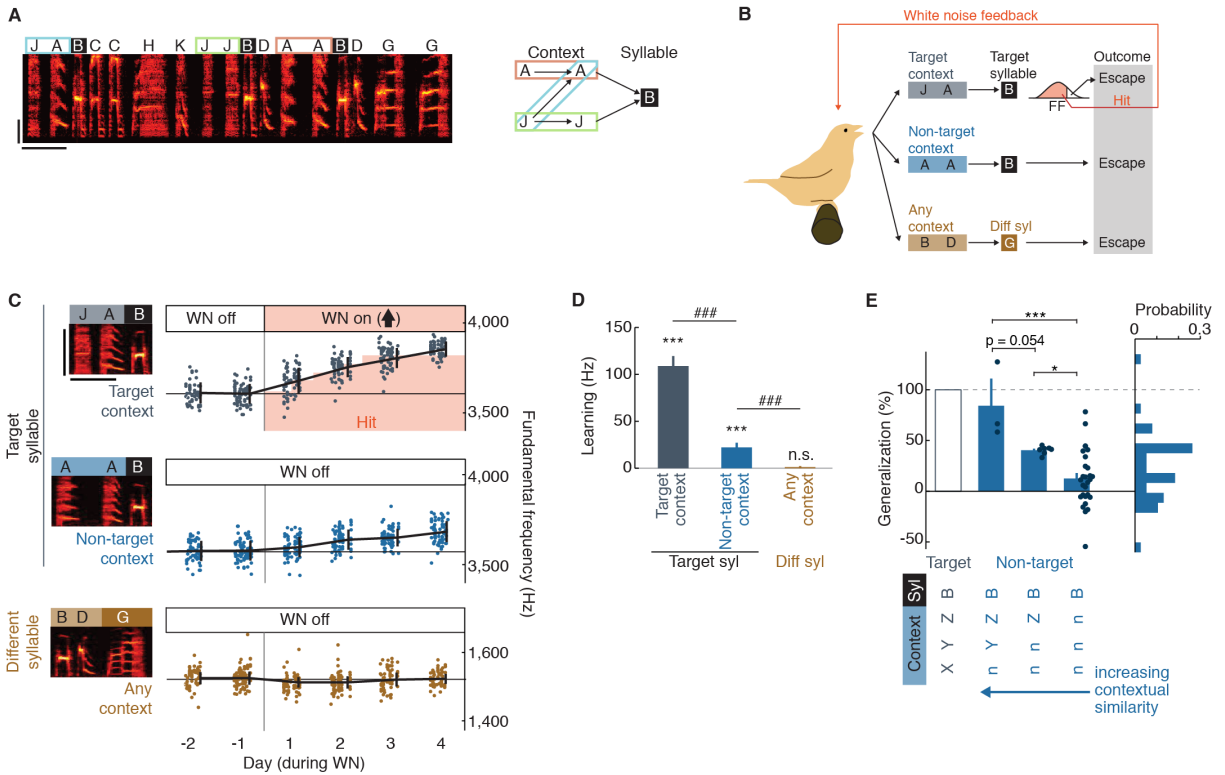
2.7. Figures

Figure 2.1. 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 feedback ("hit") was provided to renditions of the target syllable **B** in the target context JA**B** (grey) 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 two days of baseline ("WN off") and four days of training ["WN on" for the target context; arrow direction represents the direction of FF shift that escapes WN feedback] for the experiment depicted in (B). Each datapoint represents a single rendition of the target syllable in the target context (JA**B**, grey), the target syllable in a non-target context (AA**B**, blue), or a different syllable (BD**G**, brown). Renditions within the red shading were below the FF threshold and were thus "hit" with white noise (WN). Mean ± 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 ± SEM learning (n=36 experiments, in 12 birds, targeting a syllable in a single context), defined as mean FF on days three and four of

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training minus mean FF on the last two 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 ("XYZ**B**") and non-target contexts. Variation in contextual similarity from low ("nnn**B**", no syllables shared, n=28), to medium ("nnZ**B**", 1 syllable shared, n=7), to high ("nYZ**B**", 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 ± SEM. *, ***, $p < 0.05$, 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 ± 5.4%).

See also Figures S2.1 and S2.2.
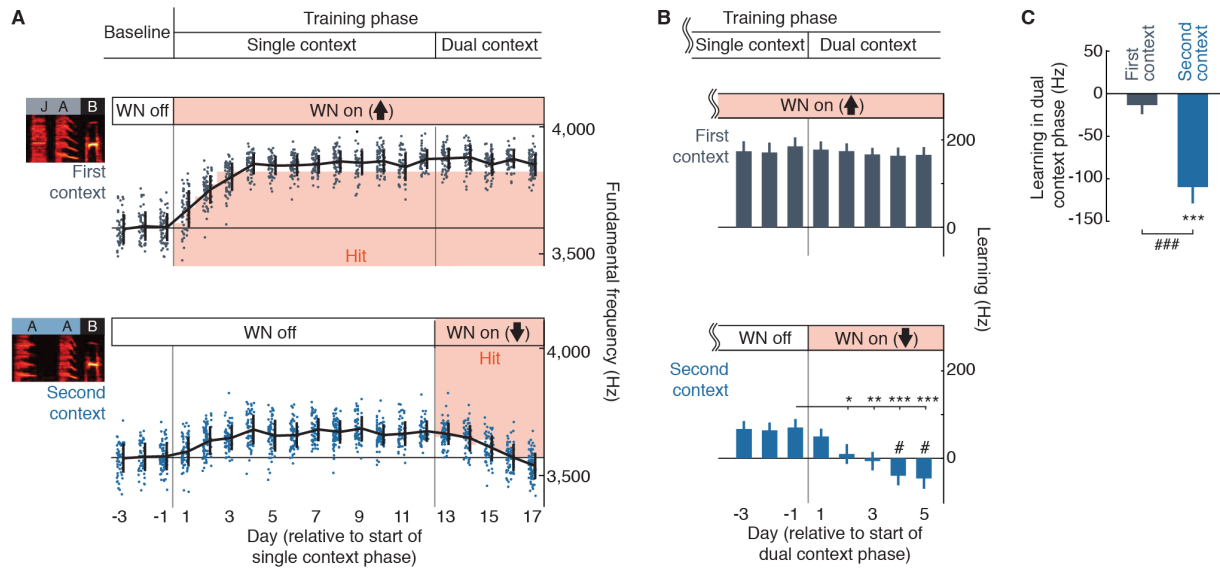
Figure 2.2. Independent learning for the same syllable in two contexts.

(A) Example experiment. In the single context phase, the FF of **B** was driven up in the first context, JA**B**. In the dual context phase, the FF of the same syllable, **B**, was driven down in the second context, AA**B**, 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, 0.005, 0.0005, signed-rank test vs. the last single context day; #, p < 0.05 signed-rank test vs. 0 Hz).

(C) Learning during the dual context phase for the first and second contexts. Learning was measured as the change in FF, on days 4-5 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 S2.3

Figure 2.3. 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 pr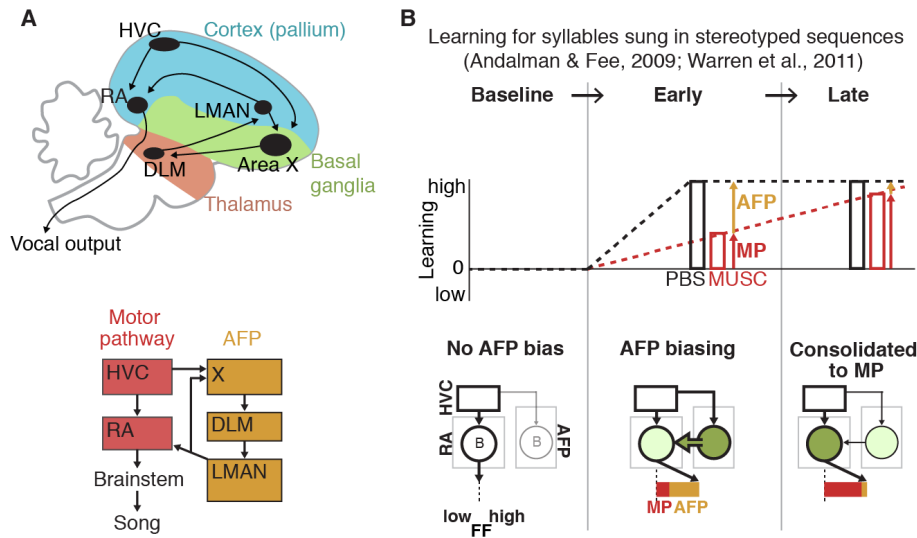oper name), the thalamic nucleus DLM (medial dorsolateral nucleus of thalamus), and the frontal cortical nucleus LMAN (lateral magnocellular nucleus of the anterior nidopallium).
(B) Schematic based on previous work of the contributions of the AFP and motor pathway (MP) to the expression of WN driven 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 (red lines and bars, "MUSC") 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 towards 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).
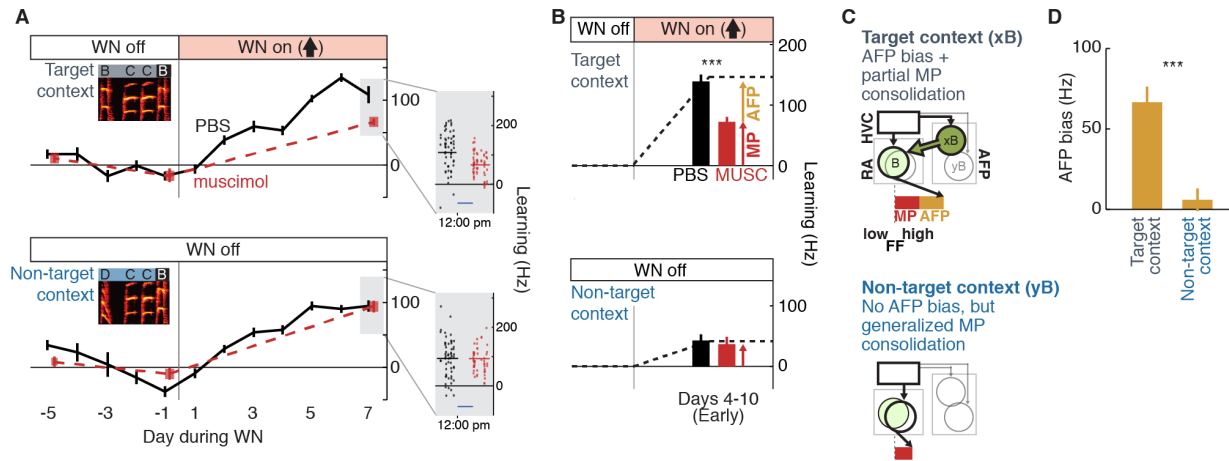
48

Figure 2.4. 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 (BCC**B**, top, grey), while reinforcement was withheld in the non-target context (DCC**B**, bottom, blue). Black lines represent daily mean ± SEM FF during infusion of vehicle (PBS) into LMAN. Red squares represent daily mean ± 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 hrs.

(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) 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 (top, "x**B**") 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 (bottom, "y**B**"), there is no AFP bias. However, because the motor pathway representation of the target syllable overlaps substantially 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 ± 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 S2.4.

Ai Congruent training

Early → Late
AFP bias same in both contexts | Strong MP consolidation

Context 1
RA HVC
xB AFP
B yB

low high
FF

Context 2

MP AFP

Aii Incongruent training

Early → Late
AFP bias opposite in two contexts | Weak MP consolidation

Context 1

Context 2

Bi

WN on (↑)

Context 1

PBS MUSC MP AFP

Bii

WN on (↑)

Context 1

... WN on (↑)

Context 2

... WN on (↓)

Context 2

Maintained learning

Days 1-4 (Early)   Days 5-6 (Late)

Learning (Hz)

Maintained learning

Days 1-4 (Early)   Days 5-6 (Late)

C

| Context # | 1 | 2 | | 1 | 2 | | 1 | 2 WN off |

Escape direction

AFP bias (Hz)

##

*

***

Congruent   Incongruent   Single context (replotted from Figure 4D)

D

Consolidated learning (% of Total)

##

(Warren et al., 2011)
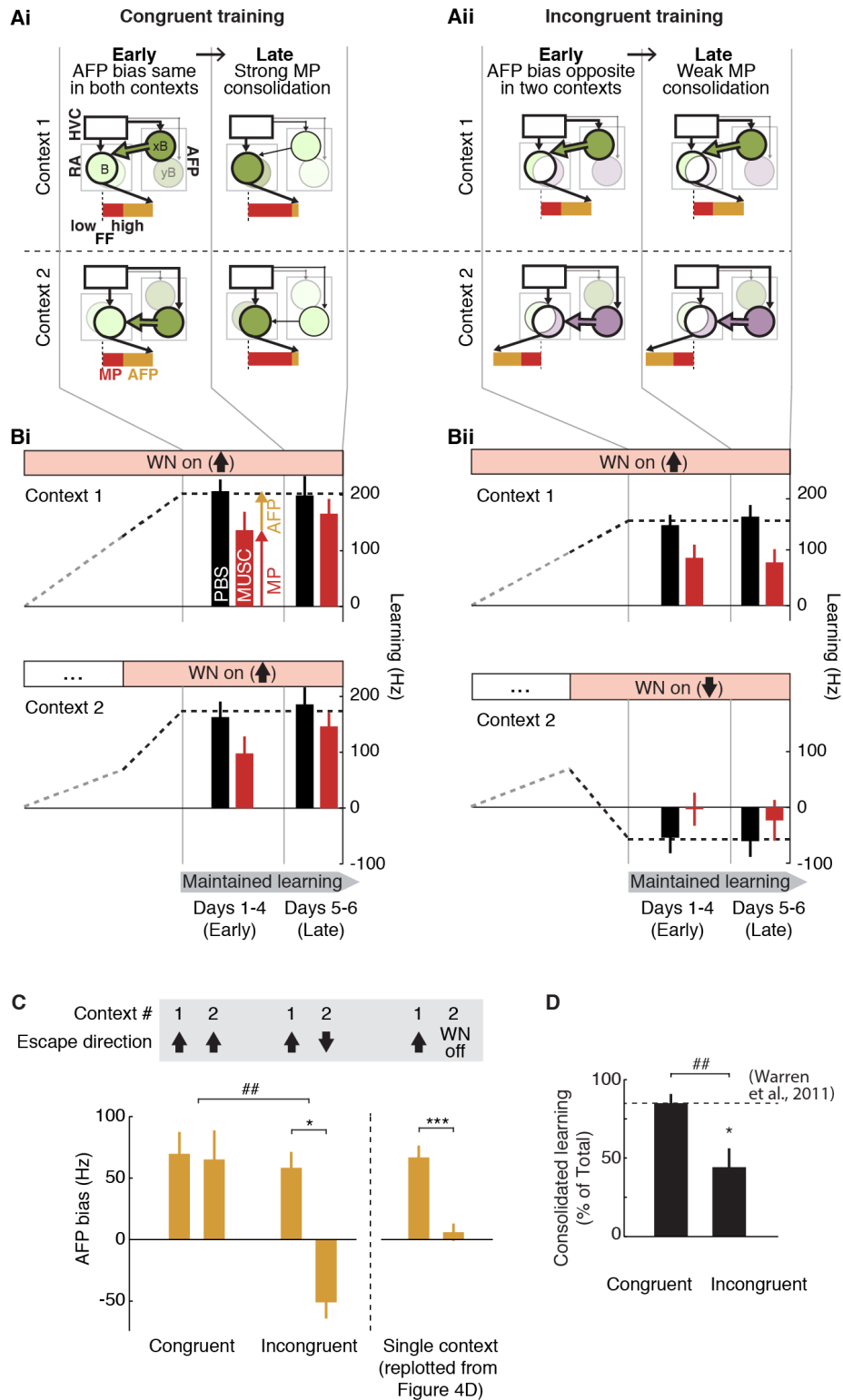
*

Congruent   Incongruent

50

Figure 2.5. Conflicting AFP bias interferes with consolidation for context-specific learning

(Ai, Aii) 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(x**B)** and context 2 (y**B)** for the target syllable **B** ("Early", thick green arrows from both 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 upwards, and thick purple arrow in context 2 biasing FF downwards). 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.

(Bi, Bii) Summary data for Congruent and Incongruent training (n = 5 Congruent experiments and 7 Incongruent experiments in 6 birds). Bar plots showing mean ± 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 Methods). Early and late periods are defined relative to a maintained learning period (see 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 (± SEM) AFP bias, measured as the amount by which learning reverted towards 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 2.4C and plotted here for comparison (n = 13 experiments in 7 birds, ***, p < 0.0005, signed-rank test).

(D) Consolidation in the late period (days 5-6) of maintained learning was strong for Congruent training, but reduced for Incongruent training. Bars represent the mean (± SEM) percentage of learning that was still expressed when AFP output was blocked (and was thus dependent on the motor pathway and not on the AFP). Dashed line represents 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 Methods); however, significance of these results were unaffected if we used both contexts (Figure S2.5A). *, p < 0.05, signed-rank test vs. 100%; ##, p < 0.005, rank-sum test.

See also Figure S2.5.
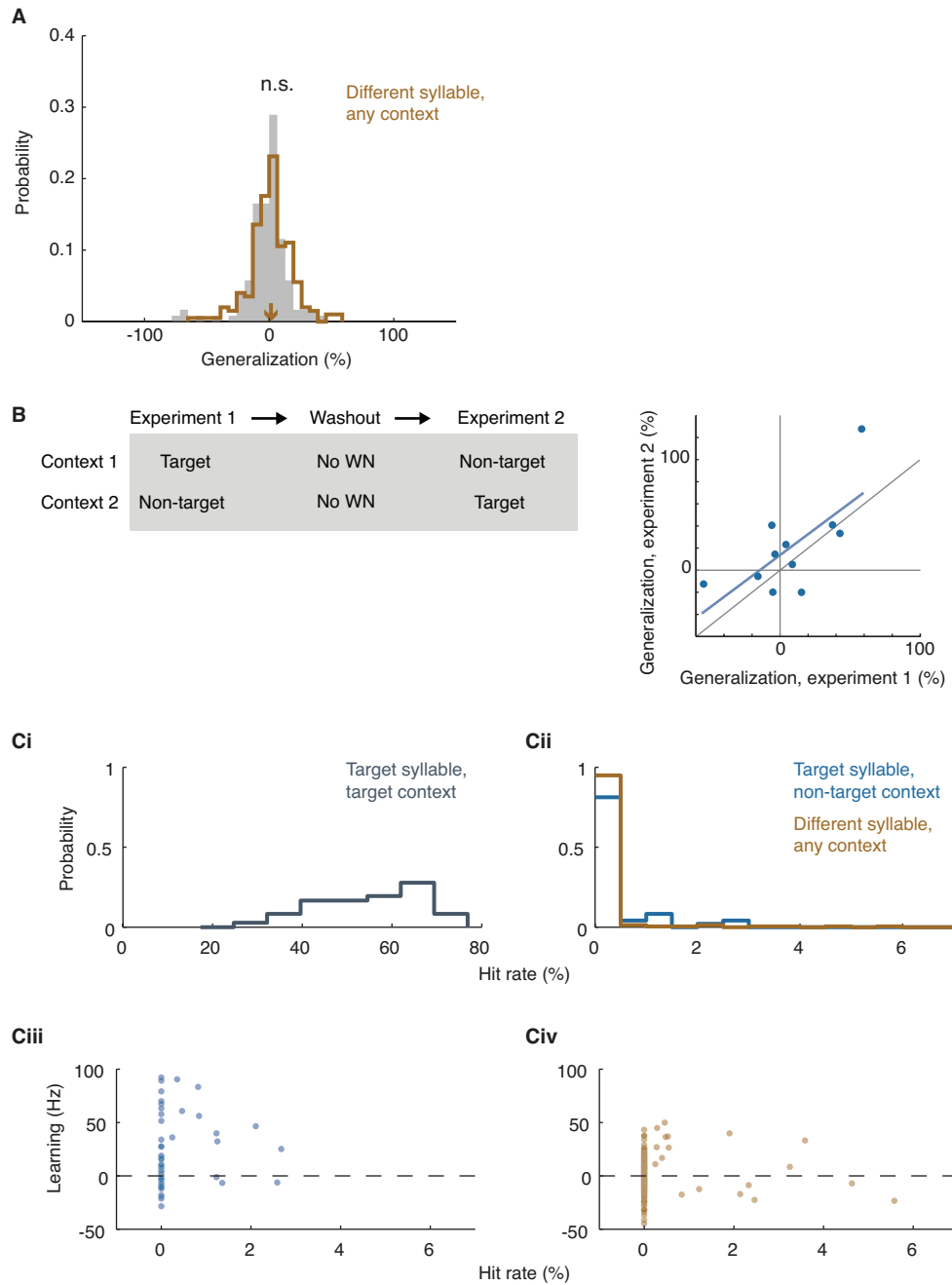
Figure S2.1. Learning driven in a single target context partially generalizes to non-target contexts. Related to Figure 2.1.

(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.
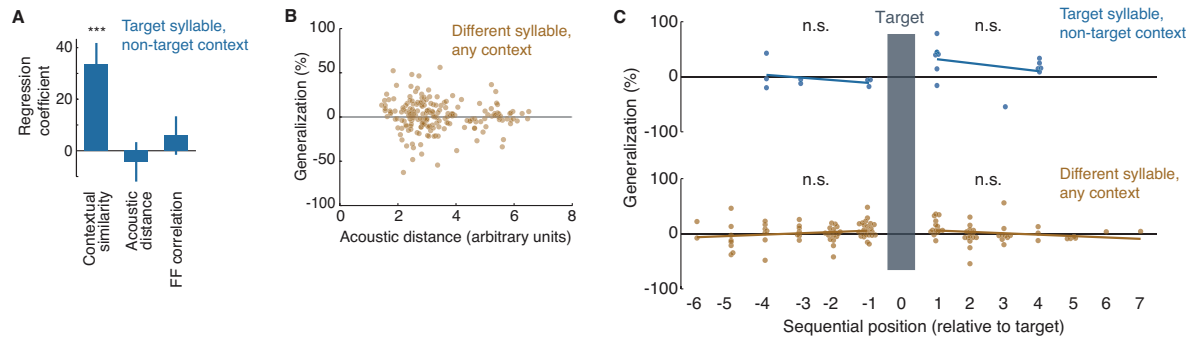
Figure S2.2. Examination of factors that explain patterns of generalization. Related to Figure 2.1.

(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 2.1E), *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 S2.1A) 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 "$M_{iterations}$"). 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.
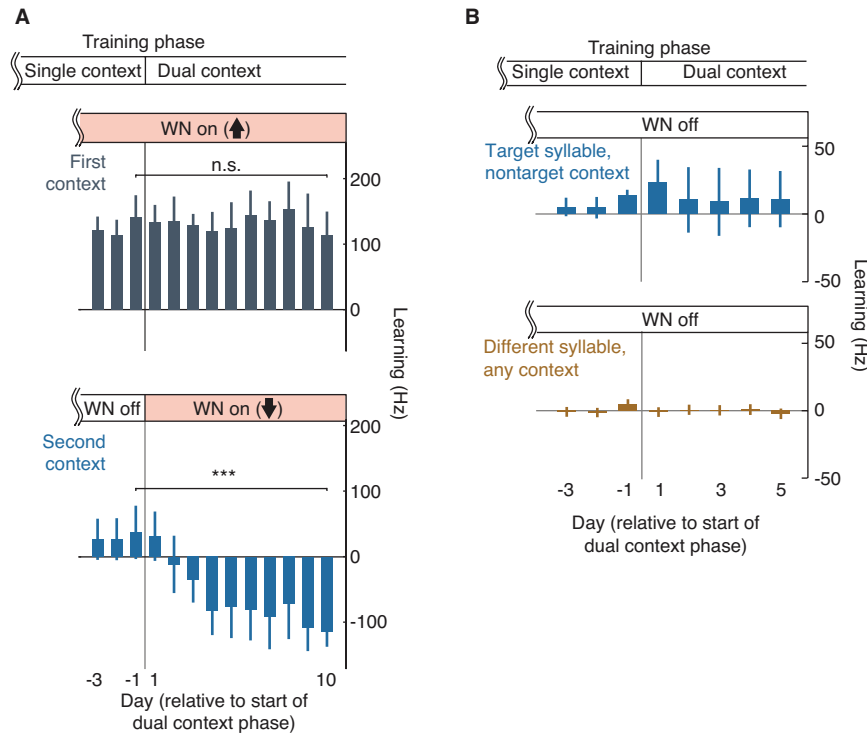
Figure S2.3. Independent learning for the same syllable in two contexts. Related to Figure 2.2.

(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 2.2B, 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.

A

Baseline | During learning

infusion: PBS | muscimol | PBS   PBS | muscimol | PBS

used in analysis? no | yes | no | yes | no   no | yes | no | yes | no

FF (Hz): 3,400 / 3,200 / 3,000   (baseline FF)

Coefficient of variation of FF: 0.04 / 0.03 / 0.02 / 0.01 / 0

Time (HHMM): 0448 0936 1424 1912

B

Baseline | During learning

Ratio of CV of FF (MUSC/PBS): 1 / 0.5 / 0

Target Context | Nontarget Context | Any Context

Target syl | Diff syl

Ci Medium and high contextual similarity

Learning (Hz): 250 / 200 / 150 / 100 / 50 / 0

| *

PBS
muscimol

Target context | Nontarget context

Cii Thresholded (nontarget learning > 50 hz)

Learning (Hz): 250 / 200 / 150 / 100 / 50 / 0

| p = 0.063

PBS
muscimol

Target context | Nontarget context

D

During PBS infusion

p=0.22
R²=0.11
slope=0.30

Learning, nontarget context (Hz): 250 / 200 / 150 / 100 / 50 / 0 / -50

During MUSC infusion

p=0.0017
R²=0.52
slope=1.08

Learning, target context (Hz): 0 50 100 150 200 250

*
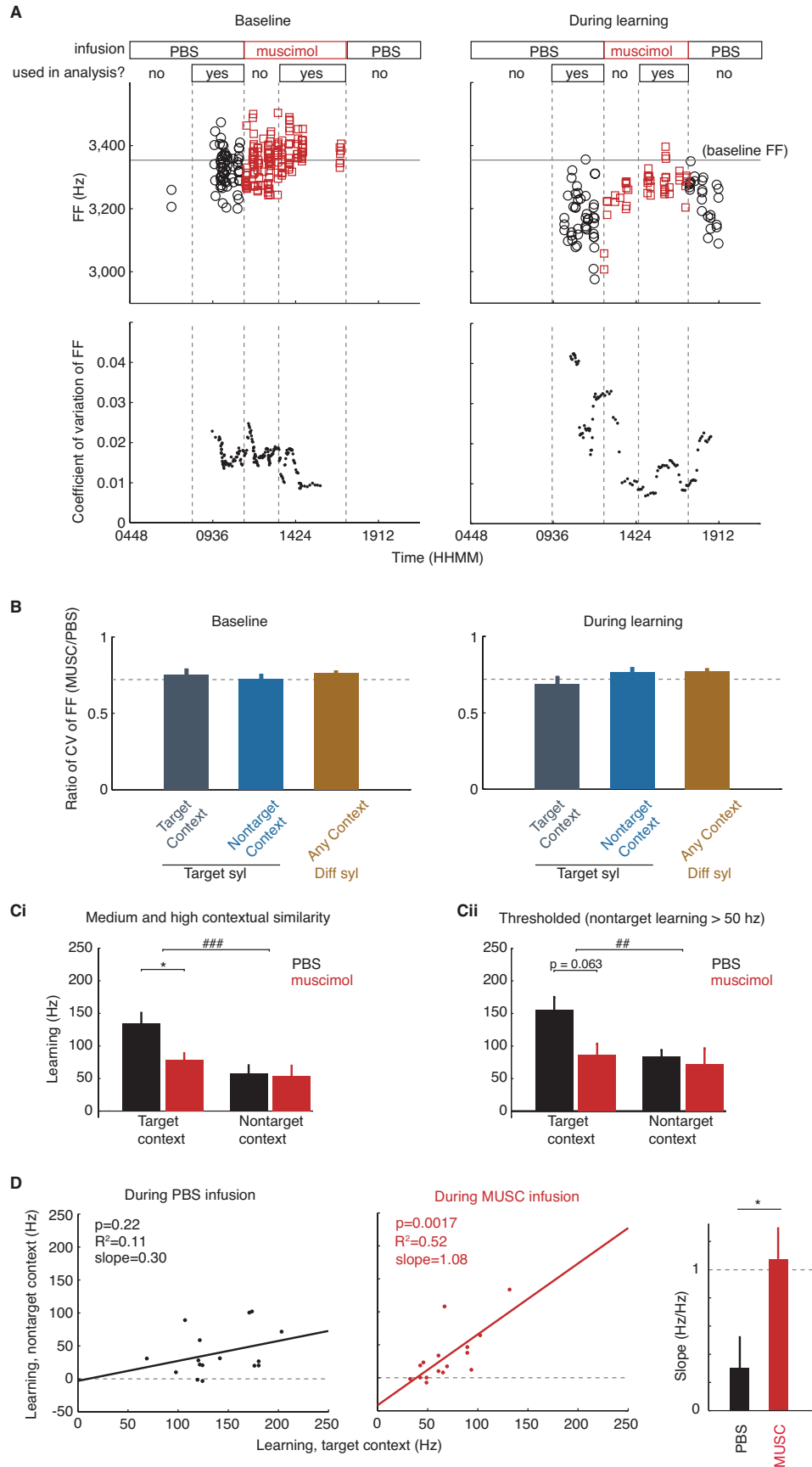
Slope (Hz/Hz): 1 / 0

PBS | MUSC

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Figure S2.4. The AFP adaptively biases motor output in a context-specific manner. Related to Figure 2.4.

(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 ± 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 2.1E). 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.

A Contexts 1 and 2 combined

B No WN feedback in context 2 preceding onset of training
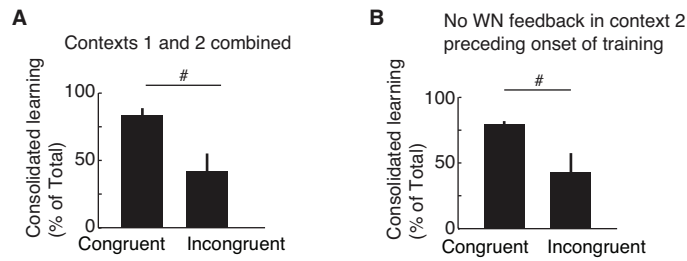
Figure S2.5. Conflicting AFP bias interferes with consolidation for context-specific learning. Related to Figure 2.5.

(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 2.5D, 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.

Chapter 3: A neural signature of a context-specific premotor pitch bias

The work in this chapter was done in collaboration with W. Hamish Mehaffey and Michael S. Brainard. I built the electrode-array microdrives, and performed all experiments and analyses. W.H.M. developed the custom-made headstages used for neural recordings, which, by being miniaturized relative to the commercial version, reduced the physical burden on birds to ensure that birds were comfortable and sang enough to exhibit learning within a single day. All authors contributed to the conceptualization of the ideas in this chapter. M.S.B. funded and supervised all aspects of the project.

3.1. Introduction

In Chapter 2, I described experiments in which we assessed the effects of pharmacological inactivation of LMAN on the expression of context-dependent learning (Figure 3.1A, B). We found that transient LMAN inactivation leads to reversion of learning in a manner highly specific to the Target context (in Chapter 2 see Figures 2.4, 2.5; also see summary in this chapter in Figures 3.1A-D). This finding indicated that the AFP contributes a learned bias to pitch that is expressed only when the Target syllable is sung in the Target context; in other words, AFP pitch bias promotes greater specificity of learning (Figure 3.1C, D). The goal of those previous experiments was to elucidate neural mechanisms underlying generalization and specificity of learning at the level of *where*, in terms of anatomy, and *what*, in terms of contributions of those anatomical regions to behavior. The focus of this chapter is on *how* this bias is implemented in neural activity.

One model for how this bias is implemented is the "premotor instruction" model (Andalman and Fee, 2009; Bottjer et al., 1984; Brainard and Doupe, 2000; Fee and Goldberg, 2011; Mooney, 2018; Scharff and Nottebohm, 1991; Warren et al., 2011; Williams and Mehta, 1999). In this model, premotor neural activity in LMAN (i.e., activity that occurs directly before and during movement and causally influences movement) modulates RA activity, and therefore biases pitch, through fast synaptic transmission. These instructive signals may arise as a result of plastic changes in the AFP. In other words, on a rendition-to-rendition basis, pitch bias would be encoded in the activity conveyed from LMAN to RA. There are a few indirect lines of evidence in support of this model: (1) LMAN's pitch bias depends on fast synaptic transmission from LMAN to RA; this is shown by experiments in which antagonizing NMDA receptors in RA, which accounts for almost all of the synaptic input from LMAN to RA (Mooney and Konishi, 1991), causes reversion of learning (Warren et al., 2011) (2) Rendition-by-rendition variation in LMAN activity seems sufficient to drive variation in adult pitch. Evidence for this comes from findings that (i) transient electrical stimulation of LMAN is sufficient to systematically perturb pitch (Kao et al., 2005), (ii) natural social-context-dependent fluctuation in the variability of pitch can be accounted for by an underlying social-context-dependent fluctuation in the variability of LMAN activity (Hessler and Doupe, 1999; Kao et al., 2005, 2008) and (iii) LMAN projects directly to RA, where neural activity is known to correlate with pitch (Sober et al., 2008).

While this "instructive" model offers a parsimonious explanation of existing results, a plausible alternative class of models argues for a "permissive" role for the AFP in the expression of learning. In these models, no learning-related change in the information conveyed from LMAN to RA is necessary for LMAN inactivation to still cause reversion of

recent learning. Broadly, there are two types of permissive models. First, LMAN may release growth factors in RA that are required for plasticity; indeed, there is evidence LMAN releases trophic factors crucial for normal development of RA (Akutagawa and Konishi, 1994, 1998; Johnson and Bottjer, 1994; Johnson et al., 1997; Kittelberger and Mooney, 1999, 2005). However, the short latency between LMAN inactivation and reversion (on the order of minutes), and the fact that blocking LMAN-to-RA synaptic transmission causes reversion, both argue against this sort of permissive role for LMAN in explaining pitch bias. A second type of model allows for fast synaptic transmission from LMAN to RA to play a crucial role in expression of learning, but does not suppose that the activity conveyed from LMAN directly encodes pitch bias. This situation could occur, for instance, if learning depends entirely on plasticity in the motor pathway, and that plasticity is only expressed in the context of active LMAN inputs (e.g., activity at newly formed synapses in RA may depend on a baseline level of NMDA receptor-mediated transmission from LMAN). A general framework for this type of permissive role is that LMAN's inputs establish a certain configuration in RA defined by the activity and baseline electrophysiological properties (e.g., excitability) of neurons in the population; silencing LMAN's inputs may then reconfigure activity enough to impair the expression of recent plasticity in RA (Harris-Warrick and Marder, 1991). This impairment may affect only recent learning if, for instance, the relevant synapses are strengthened over time in a process of "synaptic consolidation" such that expression of learning becomes less susceptible to perturbation of LMAN's inputs (Dudai, 2004; Fusi et al., 2005). We currently lack recordings of LMAN and RA activity during learning, which may provide evidence in support of either an instructive or permissive role for LMAN in learning (Figure 3.1E).

Here we recorded neural activity in LMAN and RA concurrently throughout a day of learning. We reasoned that if LMAN conveys a premotor instructive signal to RA, then this signal may be reflected in the moment-to-moment correlation between LMAN and RA activities and in how that correlation relates to pitch changes during learning. By analyzing the cross-covariance between spiking activity in LMAN and RA, we found that LMAN and RA activity are correlated during singing; analysis of the lag in the cross-covariance suggests that this relationship reflects a moment-to-moment influence of LMAN on RA. The strength of this cross-covariance increased during learning. Consistent with this increase reflecting the emergence of an instructive premotor signal from LMAN, we found that rendition-to-rendition variability in LMAN-RA cross-covariance predicted rendition-to-rendition variability in the expression of learning. Finally, in experiments that drove learning specifically in one "Target" sequential context, the increase in cross-covariance was specific to that Target context. Our results provide evidence for a context-specific premotor instructive signal during learning.

3.2. Results

Neural evidence for a premotor influence of LMAN on RA during singing

We recorded neural activity simultaneously from multiple spatially separated sites in LMAN and RA during singing (range of number of simultaneous sites per session: N = 1-2 in LMAN, N = 1-5 in RA, in 11 recording sessions, in 3 birds). For a given site, all extracted spikes were combined into one multi-unit dataset (Figure 3.2A-C). Comparison of the range of mean firing rates obtained in these multiunit sites with previously reported single unit recordings [LMAN in zebrafinch (Kao et al., 2008); RA in Bengalese finch (Sober et al.,

2008)] suggest that each multiunit site sampled activity from at least ~4-8 single units, depending on the site.

We first asked whether we could detect any evidence of an influence of LMAN on RA by measuring the cross-covariance between activity in LMAN and RA before the onset of training (i.e., the "Baseline" period) (Figure 3.2A). This was motivated by previous studies which have indicated that LMAN activity contributes to moment-to-moment variability in adult song structure even during baseline singing (Kao et al., 2005; Kojima et al., 2018; Olveczky et al., 2005). One function of LMAN activity at baseline is to increase the variability of acoustic structure presumably to facilitate behavioral exploration in the service of trial-and-error learning. The use of cross-covariance was motivated by previous studies, which indicate that this measure reflects the influence of LMAN activity on RA activity (Hahnloser et al., 2006; Kimpo et al., 2003) Cross-covariance indicates the extent to which moment-to-moment variation in LMAN activity predicts, or is predicted by, moment-to-moment variation in RA activity; this is done by computing the similarity between LMAN and RA spike patterns as a function of the lag between the LMAN and RA activity segments that are being compared. This similarity is normalized to the similarity that would arise simply due to the fact that both LMAN and RA have similar average song-locked activity (see Methods; example in Figure 3.2B-D, for example of average song-locked activity, see the top of Figure 3.2C). Cross-covariance is inherently measuring correlations, and so biological interpretation depends on interpreting the combination of sign, magnitude, and time lag of non-zero values combined with prior knowledge of anatomy and physiology (see Discussion). In general, non-zero values in cross-covariance with short time lags, when the leading region is known to directly project to the lagging region, are suggestive of an

influence of the first region on the second.

We measured the cross-covariance between each simultaneously recorded pair of LMAN and RA units, separately for each distinct syllable, using neural activity in a premotor window, during which activity has the potential to causally influence syllable pitch (Figure 3.2C). We found that on average LMAN-RA cross-covariance was significantly positive, consistent with prior demonstration that the inputs from LMAN are excitatory (Mooney and Konishi, 1991) (Figure 3.2E). Although there was some variation in the magnitude and time lag of cross-covariance across syllables and unit pairs (see light grey curves in Figure 3.2E), non-zero values were generally positive, and there was a distinct positive peak in the average cross-covariance occurring at a time lag of ~3 ms with LMAN leading (Figure 3.2E). Although this LMAN-leading peak in the cross-covariance function likely reflects a mixture of different circuit-level mechanisms (see Discussion), given that LMAN neurons sends excitatory projections to RA, a parsimonious explanation is that this LMAN-leading positive peak is an indirect measure of the strength of the influence of the local ensemble in LMAN on the local ensemble in RA (with "local ensemble" including neurons not recorded but whose activity is correlated the neurons being recorded). This result is consistent with LMAN modulating RA activity at baseline to inject variability into song.

LMAN-RA cross-covariance increases during learning

We next tested whether there is a systematic change in LMAN-RA cross-covariance during WN-driven pitch modification, which might reflect a learning-related change in the influence of LMAN on RA. For each experiment, we tracked neural activity for a small number of sites in LMAN and RA over a day of training (Figures 3.3A, B).

We measured LMAN-RA cross-covariance for renditions before the onset of WN ("Baseline", Figure 3.3C) and for the set of renditions at the end of training ("Trained", Figure 3.3C). Comparison of these two sets of cross-covariance functions revealed that cross-covariance increased on average over the course of a day of training (Figures 3.3C). For each pair of units we computed a single value representing its increase in LMAN-RA cross-covariance by taking the average cross-covariance in a short window centered at a lag corresponding to the peak in the average cross-covariance function (shaded area in Figure 3.3C, right). Across all experiments, there was a preponderance of unit pairs that exhibited increases in average cross-covariance over learning (Figure 3.3D)

This change exhibited several features that parallel the behavioral effects of learning. First, the average time course of this increase in LMAN-RA cross-covariance exhibited a gradual increase over the day (Figure 3.3E), matching the average time course of changes to pitch (Figure 3.3F). Second, increase in cross-covariance was specific for the syllable that was being targeted for learning (Figure 3.3G, H).

Evidence that the increase in LMAN-RA cross-covariance reflects premotor instruction

This increase in LMAN-RA cross-covariance during learning may be due to LMAN contributing a premotor instructive signal that, from rendition-to-rendition, biases RA activity to produce pitch that is shifted away from baseline in the direction of learning (i.e., "instructive" model in Introduction). If this were the case, we reasoned that inspection of renditions at the end of training, when learning should be strongly dependent on AFP bias, would reveal a relationship between the natural rendition-to-rendition variation in the strength of the LMAN-RA cross-covariance and pitch. This would occur because variation in

both pitch and the measured LMAN-RA cross-covariance would be driven by the same underlying neural process that generates the instructive signal. For the block of renditions at the end of training, we placed renditions into one of two groups based on the direction in which pitch deviated from the median pitch. We reasoned that any underlying neural process contributing to expression of learning was likely to be, on average, more strongly expressed on renditions deviating in the direction that escapes WN ("Stronger" expression of learning, Figure 3.4A) compared to renditions that deviated in the opposite direction ("Weaker" expression of learning, Figure 3.4A).

Consistent with that prediction, renditions with stronger expression of learning exhibited on average greater learning-related increase in LMAN-RA cross-covariance (example experiment in Figure 3.4B, C; summary in Figure 3.4D). This result is consistent with the possibility that the increase in LMAN-RA cross-covariance represents the magnitude of a premotor instructive signal that directly contributes to the expression of pitch changes.

We sought further evidence in support of this conclusion. We reasoned that if the increase in LMAN-RA cross-covariance reflects an instructive premotor signal, then this increase should exhibit temporal specificity for a time window during which neural activity in LMAN influences pitch (estimated from stimulation of LMAN to be about ~30-50 ms preceding syllable onset (Kao et al., 2005). To test this possibility, we measured the timing of the change in LMAN-RA cross-covariance in multiple smaller time windows spanning the premotor window for the Target syllable. This finer resolution measurement of the increase in LMAN-RA cross-covariance, compared to the single measurement averaged over the entire premotor window used in Figure 3.4D, revealed that the relationship

between the expression of learning and magnitude of LMAN-RA cross-covariance was greatest at a timepoint directly preceding syllable onset (Figure 3.4E). Taken together, these results indicate that enhanced LMAN-RA cross-covariance during learning is a signature of an instructive premotor signal that adaptively biases pitch.

Increase in LMAN-RA cross-covariance is context-specific

We previously reported that for sequential-context-dependent learning the contribution of AFP bias to learning is highly specific to the context targeted with WN feedback. In contrast, the expression of learning that spontaneously generalizes from the "Target" context to other "Non-target" contexts does not depend on an instructive premotor bias from LMAN; instead, generalized learning depends on modifications that have already transferred downstream to be encoded, presumably, in the motor pathway (these findings were reported in Chapter 2; for a brief summary see Introduction for this chapter and Figure 3.1A-D). This finding of differential specificity of the contributions of AFP bias and motor pathway changes to learning leads to another prediction that tests whether changes to LMAN-RA cross-covariance is a neural signature of AFP bias. In particular, the increase in LMAN-RA cross-covariance should also be specific to the Target context.

To test this prediction, for a subset of the experiments we drove learning for the targeted syllable in only one specific Target context. We found that the increase in LMAN-RA cross-covariance was specific to this Target context (Fig. 3.5). On average, LMAN-RA cross-covariance did not change during learning for the Non-target context. This remarkable specificity of the LMAN-RA cross-covariance further supports the conclusion

that the increase in LMAN-RA cross-covariance corresponds to the neural implementation of the context-specific pitch bias previously measured by the effect of LMAN inactivation on behavior.

3.3. Discussion

Previous work transiently inactivating LMAN during learning has shown that LMAN contributes a context-specific pitch bias crucial for the expression of learning. It remained unclear *how* this bias is implemented in changes to neural activity. Here we presented evidence for a sequential-context-specific premotor instructive signal conveyed from LMAN to RA. By supporting a role for the AFP in generating an instructive premotor signal, our findings also provide evidence against an entirely permissive role for LMAN in adult pitch modifications (see Introduction for description of "instructive" and "permissive" models). The strength of this conclusion rests on two key assumptions: first, that the mechanism underlying the cross-covariance of spike trains in LMAN and RA is related to fast synaptic transmission from LMAN to RA; second, at a functional level a rendition-to-rendition relationship between pitch and LMAN-RA cross-covariance can be taken as evidence for the presence of an underlying neural process - an instructive signal from LMAN to RA - driving that relationship. Below we discuss the implications of our findings, and along the way address the extent to which these assumptions are warranted, and what caveats remain.

What mechanisms underlie non-zero LMAN-RA cross-covariance? The direct implication is that variation in activity in LMAN is predictive of variation in activity in RA. This predictive ability may in principle be a result of monosynaptic input from LMAN to RA.

In sampling simultaneously from multiple neurons in LMAN and RA it may be possible that we sampled from some cases of directly connected neurons. However, this is unlikely to account entirely for the effect across all experiments, since not all cases of positive cross-covariance exhibited a sharp LMAN-leading peak (see Figure 3.2E). Such a peak would likely be more prominent if our effects were driven largely by monosynaptic influence. Instead, the shape of the cross-covariance function was somewhat variable across syllables and pairs of sites. It is therefore more likely that the effect depends on a combination of the following mechanisms: (1) monosynaptic connections between neurons being sampled, (2) strength of the influence of the local ensemble in LMAN on the local ensemble in RA (with "local ensemble" including neurons not recorded but whose activity is correlated the neurons being recorded), and (3) polysynaptic connections, through local circuitry in RA, between the recorded LMAN and RA neurons. Importantly, it is plausible that learning-related changes in LMAN's influence on RA could be detected in all of these scenarios. Moreover, the fact that the average cross-covariance function had a short-latency LMAN-leading peak argues against this signal reflecting a polysynaptic input from RA to LMAN through DLM. This peak is also unlikely to reflect common shared input from HVC, since prior work has shown that an LMAN-leading peak similar to the one we described remains unaffected in anesthetized birds after silencing HVC. The LMAN-RA cross-covariance likely indicates, to some extent, a causal influence of LMAN on RA, through some combination of direct (i.e., #1) or indirect mechanisms (i.e., #2 and #3).

What mechanisms may underlie the increase in LMAN-RA cross-covariance during learning? One possibility is that the increase reflects modification (presumably net strengthening) of synapses from LMAN to RA; indeed in slice preparations plasticity at

these synapses can be elicited in a manner that depends systematically on the relative timing of HVC's and LMAN's inputs to a given RA neuron (Mehaffey and Doupe, 2015). A second possibility is that there is no plasticity at synapses from LMAN to RA, but instead there are changes to LMAN activity that results in modifications to the signals transmitted to RA. Indeed, in other systems variation in the millisecond-level correlation of neurons within an ensemble can influence the extent to which activity influences downstream regions (Alonso et al., 1996; Kumar et al., 2010; Zandvakili and Kohn, 2015). Distinguishing between these two possibilities may require more dense recordings of LMAN and RA activity during learning, and possibly the ability to assess changes in LMAN-RA synaptic strength as a function of learning.

We interpreted the rendition-to-rendition relationship between expression of learning and LMAN-RA cross-covariance as evidence that the increase in LMAN-RA cross-covariance reflects the development of an instructive motor signal. Our reasoning (see Results section and schematic in Figure 3.4A) was that if it is indeed true that the magnitude of LMAN-RA cross-covariance reflects the strength or efficacy of an underlying premotor instructive signal, then any natural rendition-to-rendition variability (or "noise") in this signal would drive correlated variability in both the measured LMAN-RA cross-covariance and pitch. Therefore renditions exhibiting greater increases in LMAN-RA cross-covariance would be expected, on average, to be renditions with stronger expression of learned pitch changes. Our results were consistent with this prediction.

What other non-instructive models may account for this finding? In principle, it is possible that LMAN-RA cross-covariance reflects a signal conveyed from LMAN that is not directly influencing pitch on a given rendition, but is instead important for driving

plasticity in RA. In this model, LMAN input on a given rendition acts to "stamp in" the activity driven by RA in that rendition. This would require LMAN to somehow be able to "predict" whether a given rendition is likely to be one that is good (escapes WN) or bad. This predictive ability could be learned by a process that associates input from HVC, encoding pitch, with performance-related feedback so that on future renditions similar HVC input to the AFP would elicit LMAN output that drives plasticity in RA in an appropriate manner.

An alternative, non-instructive model is that the increase in LMAN-RA cross-covariance reflects a common input that drives pitch bias (likely by influencing RA activity) and some change to activity in LMAN, RA, or both that leads to increased LMAN-RA cross-covariance (e.g., through one of the mechanisms discussed above). In that case pitch and LMAN-RA cross-covariance would show a rendition-to-rendition relationship even if LMAN does not provide premotor instruction. Following this line of reasoning, one possibility is that the increase in LMAN-RA cross-covariance may be due to increased excitability of RA neurons, so that a given input from LMAN is more likely to drive spiking in RA. Learning would, for instance, depend on the increase in excitability of a specific ensemble of RA neurons. While this remains in principle possible, it seems unlikely because there was no difference in the mean firing rate of RA neurons for renditions with stronger vs. weaker expression of learning (Figure S3.1). The most parsimonious model seems to be one in which an instructive premotor signal from LMAN exists and is driving shared variation in pitch and LMAN-RA cross-covariance.

How might the processes underlying the increase in LMAN-RA cross-covariance encode a pitch bias? One possibility is based on the idea that different ensembles in LMAN

influence or encode different acoustic parameters by virtue of how they influence ensemble activity in RA. These functional "channels" could consist of distinct populations of neurons, or alternatively overlapping sets of neurons but with different ensemble-level patterns of activity, which controls how activity in each channel affects acoustic structure. Learning would depend on modification of the relative influence of different channels such that the net effect is to appropriately bias behavior. The tuning of these channels could occur through the selective strengthening of synapses from LMAN to RA, or alternatively through modifications in the AFP (as discussed above). Assuming this model is correct, it is possible that each of our multiunit sites sampled activity from multiple channels - an increase in strength of any of these channels would potentially lead to an increase in cross-covariance. While it is possible that there is actually a preference for strengthening over weakening of channels, we cannot rule out other reasons why potential decreases in strength of channels may be poorly measured in LMAN-RA cross-covariance (e.g. due to a floor effect). While our study does not provide direct evidence for how the processes underlying the increase in LMAN-RA cross-covariance encode a pitch bias, it is consistent with a model in which pitch information is encoded in the relative strength with which different patterns of ensemble activity can drive downstream RA activity.

If it is the case that AFP bias reflects modifications in the AFP, then what are those modifications? What learning rules and plasticity mechanisms within the AFP lead to the generation of context-specific premotor instruction? This mechanism must account for the finding that the increase in LMAN-RA correlation showed remarkable specificity for sequential context. At an abstract level, the AFP must be able to precisely monitor the association between the ongoing song sequence (i.e., context), performance (i.e., pitch) and

feedback (i.e., hit or escape rendition), and use that information to "learn" the appropriate modifications. A simple model for how this learning occurs is described in the Discussion for Chapter 2 and also in Chapter 4.

This evidence that the AFP, a cortical-basal ganglia circuit, conveys an instructive premotor signal to downstream primary motor circuits suggests that a general function of cortical-basal ganglia circuits may be to instruct activity in downstream circuits to bias behavior in a context-dependent manner. Other evidence that this is a general function of cortical-basal ganglia circuits is discussed briefly in the Discussion for Chapter 2 and will be expanded on in Chapter 4.

Various forms of learning may depend, initially, on the engagement of one set of brain circuits that act to bias activity in another set of circuits. In many cases, the set of brain circuits that are selectively engaged during initial learning may also be circuits that are crucial for varying behavior depending on context. This would mirror the idea that the AFP contributes to initial learning by conveying context-specific instructive signals to RA. This idea will be further discussed in Chapter 4.

3.4. Methods

Animal subjects

We used 3 adult 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.

Training pitch modifications using WN feedback

WN-driven pitch modification (i.e., "pitch training") was performed in a manner identical to that described in Methods for Chapter 2 in the sections "Song recording and computerized training paradigm", "Determining sequential context for each rendition of a given syllable", Single context training", except for the following differences:

Training was driven both for cases in which a given syllable was only ever sung in one context and also for cases in which a syllable was sung in multiple contexts (in which case WN feedback was provided in only a single "Target" context). All analyses treat these two types of experiments identically, except for the analysis for Figure 3.5, where, because of the hypothesis being tested, we only considered experiments where multiple contexts existed for the targeted syllable. Any analyses comparing the target syllable to different syllables ignored Nontarget contexts for the Target syllable.

All training trajectories were over the course of a single day. WN was turned on after sufficient "baseline" data were collected (generally on the order of 15 to 40 song bouts). In some cases training extended until lights off, while in other experiments training ended earlier because neural signal was lost.

FF calculation

All analyses were performed on FF values that were calculated offline. 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 (σ = 1 ms) short-time Fourier transform (window size = 1024 samples; overlap = 1020 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. All syllables consisting of largely broadband noise (e.g. introductory notes) were excluded from learning analyses.

Electrode array microdrives

Custom-made tungsten electrode (0.5MOhm, Microprobes WE30010.5F) arrays were interfaced with custom-made microdrives [modified from (Vandecasteele et al., 2012)] that allowed manual adjustment of arrays during recording sessions. Electrode arrays for the two microdrives (LMAN and RA) were wired to the same connector

(Omnetics, A79042-001). During recordings sessions this connector interfaced with an Intan headstage (see below).

Microdrive implantation

Implants were performed by first locating the region of interest either using electrophysiological landmarks [RA, tonic activity, recorded using carbon fiber electrodes (Kation Scientific)] or stereotaxical coordinates (LMAN). We implanted in the left hemisphere in all birds. A reference electrode was implanted in either the region centrally located between LMAN and RA or in cerebellum.

Electrophysiological recordings

Voltage signals were amplified, filtered (1Hz to 12000Hz pass band), and multiplexed on a headstage interfaced to a connector implanted on the bird's skull. Signals were stored on a computer for offline analysis. The head stages were custom-made by W.H.M. On each recording session we slowly lowered (<20um/sec) the electrodes from their "resting" position towards either LMAN or RA. Localization within these nuclei was assessed by evaluating tonic activity (RA), song-locked firing rate modulation, and stereotaxic coordinates (i.e., depth). After the recording sessions electrodes were raised to a position with the tip at >300um above the region of interest.

Spike detection

Spikes were detected using Waveclus (Chaure et al., 2018). In brief, Waveclus is a spike clustering algorithm that detects putative spikes by amplitude, transforms those

spikes into a feature space defined by wavelet coefficients, and then clusters spikes using superparamagnetic clustering. The number of clusters was manually set to one, resulting in multiunit datasets.

Normalized cross-covariance

Normalized cross-covariance was performed by first computing cross-covariance as in (Kimpo et al., 2003). Cross-covariance was calculated over multiple sliding 60ms windows preceding the onset of the syllable. The earliest window spanned from 100ms to 40ms preceding syllable onset, and the latest window from 60ms to 0ms preceding syllable onset (sliding by 5ms). Each cross-covariance calculation was performed on binned spike trains (2.5ms bins); cross-covariance functions were then smoothed using a Gaussian kernel (SD = 5ms). The cross-covariance functions over all 60ms windows were averaged to generate one cross-covariance function for a given 100ms premotor window. To normalize cross-covariance so that it is comparable across pairs of units and experiments, we normalized this function, by taking the z-score relative to the distribution of cross-covariances calculated in a control dataset. This control dataset consists of the same data, but shuffled so that, instead of comparing LMAN and RA spike trains from the same rendition, spike trains from different (directly adjacent) renditions are compared. This distribution represents the mean and variance of cross-covariance functions that would be expected if there were no relationships between LMAN and RA activity beyond that arising from both signals being modulated, on average, by singing.

Statistical tests

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. Tests were deemed statistically significant if $p < 0.05$. All analyses were performed using custom-written MATLAB (Mathworks) software.
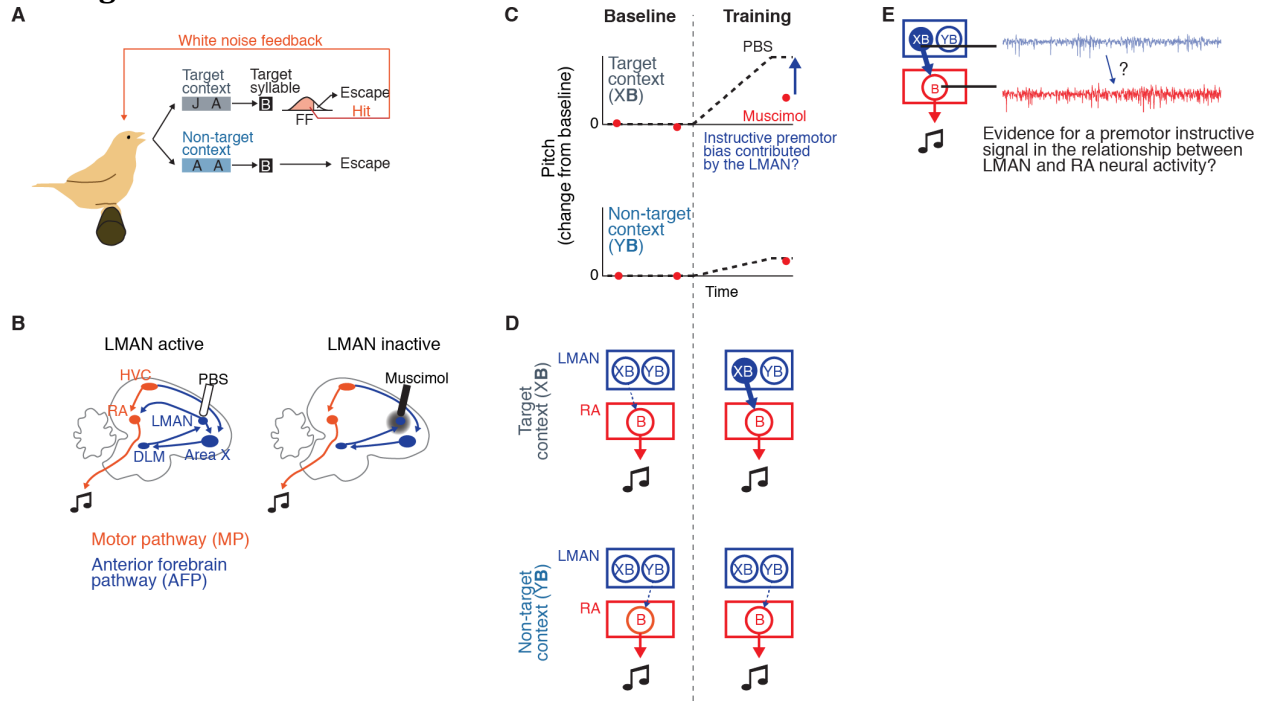
3.5. Figures

Figure 3.1. Summary of prior experiments: the AFP biases pitch in a context-specific manner during learning.

(A - D) Summary of experiments and findings from Chapter 2.

(A) Schematic of an example context-dependent training session. Aversive white noise feedback ("hit") was provided to renditions of the target syllable **B** in the target context JA**B** (grey) 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).

(B) Transient (over a few hours) infusion of muscimol was used to silence LMAN activity at various timepoints during learning. This effectively blocked the output of the anterior forebrain pathway (AFP, blue). At all other times a control solution (phosphate buffered saline, PBS) was infused. Schematics illustrating infusion are overlaid on a schematic of song system nuclei.

(C) Schematic summarizing effect of muscimol infusion on expression of context-dependent learning. Muscimol is infused a few days into training; this causes reversion of learning in the target context but not the nontarget context, consistent with the AFP contributing a pitch bias specifically when the Target syllable is sung in the Target context. See Figure 2.4 for details.

(D) A model for how the AFP contributes to learning in the target context. See Figure 2.4C for a detailed explanation. For the purpose of this chapter, the key point is that during learning the AFP contributes a pitch bias (thick blue arrow) specifically when the Target syllable is sung in the Target context.

(E) Schematic summarizing the question motivating the experiments in this chapter.
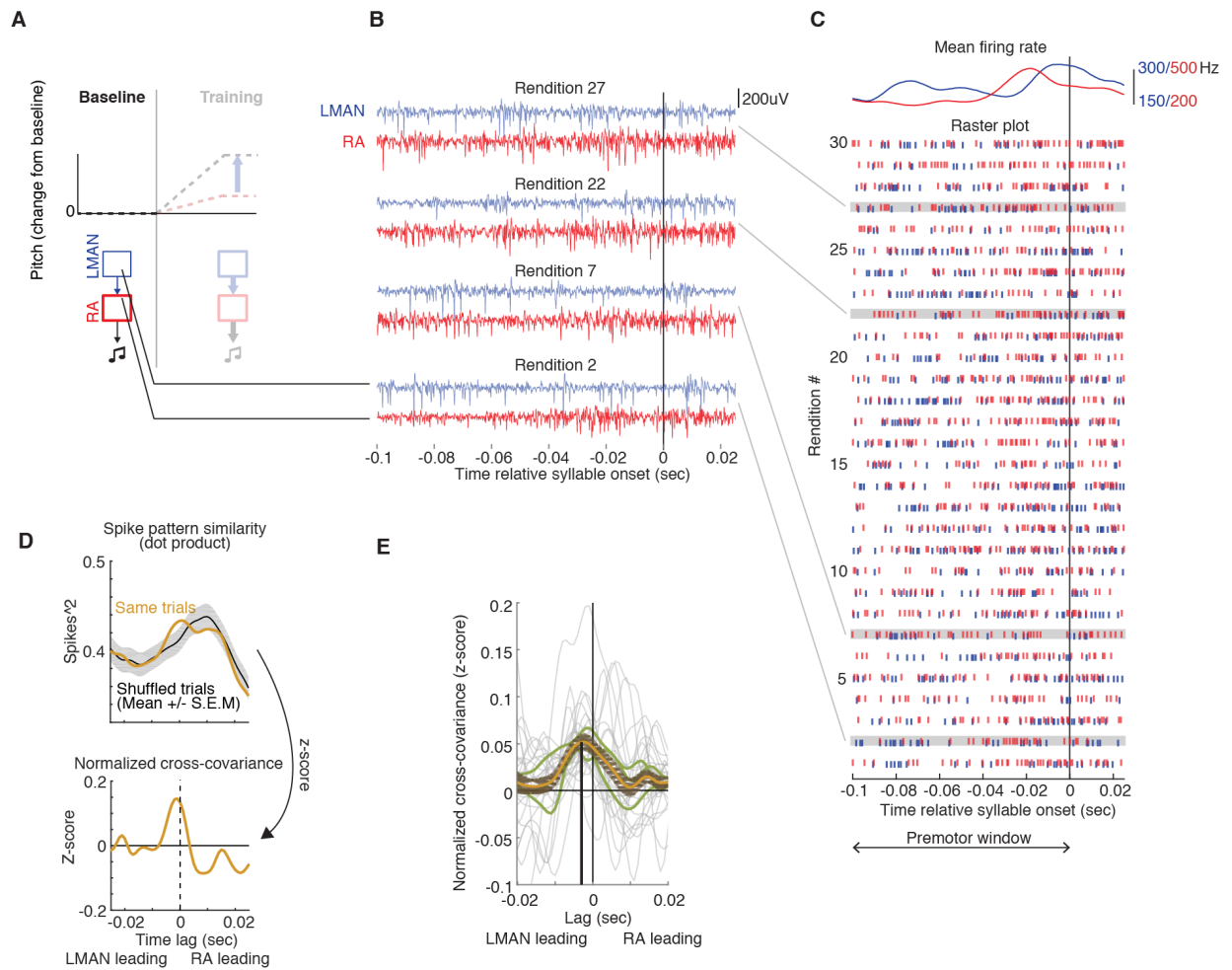
Figure 3.2. Neural evidence for a premotor influence of LMAN on RA during singing.
(A) For this figure, analyses focus on simultaneously recorded multi-unit activity in LMAN and RA during singing at Baseline (preceding onset of training).
(B) Activity in LMAN and RA aligned to the onset of the same syllable across four example renditions.
(C) Raster plot and mean smoothed firing rates (top) across a subset of renditions, ordered chronologically. For all analyses in this chapter, unless otherwise noted, data used to calculate cross-covariance were sampled from the "Premotor window" (-0.1 sec to 0 sec relative to syllable onset)
(D) Calculation of normalized cross-covariance for the example units and syllable in (B) and (C). Top: the sliding dot product was calculated on spike trains for both the actual dataset and a control dataset in which the LMAN and RA activity were shuffled across trials (see Methods). Bottom: the sliding dot product was z-scored relative to the shuffle distribution to compute a normalized cross-covariance function.
(E) Normalized cross-covariance across all syllables and pairs of units. The light grey curves represent individual syllables (N = 50 channel pairs, multiple channel pairs were averaged to obtain one trace per syllable); the green lines represent individual birds (N = 3), and the orange trace represents the mean +/- S.E.M. across all syllables.
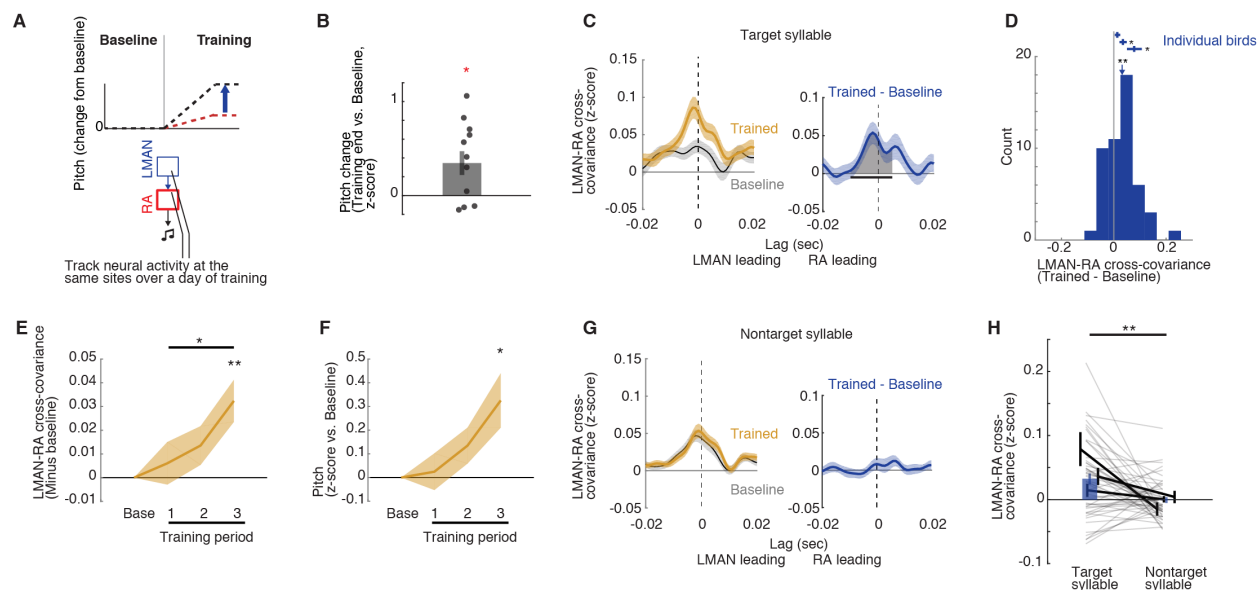
Figure 3.3. LMAN-RA cross-covariance increases during learning

(A) Schematic of experiment.

(B) Summary of pitch change across experiments (N = 11 experimental trajectories over 3 birds).

(C) Left: mean cross-covariance for Baseline and Trained (last quarter of trials during the training session) across experiments (N = 50 unit pairs, 11 experiments, 3 birds). Right: Change in cross-covariance during training. The shaded region indicates the window over which the average cross-covariance was taken in order to extract a single value representing change in cross-covariance for each pair of units. The window is 15 msec long centered at the peak of the cross-covariance at the end of training.

(D) Histogram of learning-related change to cross-covariance. The crosses at top represent mean +/- SEM for each individual bird.

(E) Average time course of change to LMAN-RA cross-covariance. Renditions during training were split into three bins of equal sample size (Training periods 1-3).

(F) Average time course of change to pitch.

(G) Same as (C), but for syllables that were not targeted with WN feedback. Each unit pair contributed one datapoint after averaging over all Nontarget syllables (N = 50 unit pairs, 11 experiments, 3 birds).

(H) Change in cross-covariance for Target vs. Nontarget syllables. Each light grey line represents a single unit pair (multiple Nontarget syllables were averaged into one datapoint). Black lines represent individual birds.
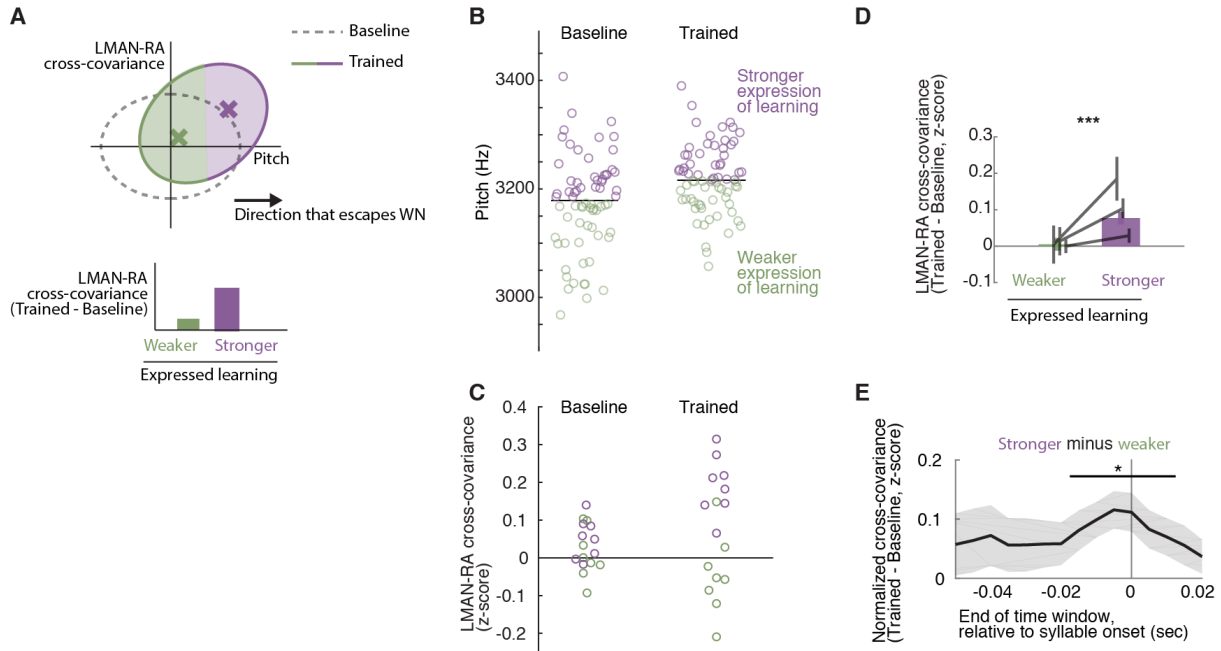
Figure 3.4. Evidence that the increase in LMAN-RA cross-covariance reflects premotor instruction.
(A) Schematic illustrating predicted outcome if LMAN-RA cross-covariance reflects a premotor instructive signal that biases pitch. See main text for details. The important point is that, if the increase in LMAN-RA cross-covariance during learning reflects a signal that causally drives pitch, then natural variability in this signal should correlate to some extent with variability in pitch (top); in particular, the increase in LMAN-RA cross-covariance should be greater for renditions that more strongly express learning (top & bottom).
(B) Example experiment illustrating pitch of individual renditions during Baseline and at the end of training (Trained). Renditions are ordered chronologically. Renditions are grouped into two sets ("stronger" vs. "weaker" expression of learning) based on the deviation of the rendition's pitch from the median pitch (black horizontal line). In this experiment WN feedback targeted lower pitch renditions.
(C) Same example experiment as in (B), showing LMAN-RA cross-covariance for individual pairs of units (circles). Each datapoint represents the mean cross-covariance for a given pair of units in the set of renditions defined by pitch ('Stronger" or "weaker" expression of learning) and phase of training (Baseline vs. Trained). The renditions used are exactly those plotted in Figure 3.4B.
(D) Change in LMAN-RA cross-covariance during training, measured separately for trials expressing "stronger" or "weaker" learning. Baseline subtraction was performed relative to the same group of renditions at Baseline (N = 50 unit pairs, 11 experiments, 3 birds; global mean +/- SEM is taken over unit pairs)
(E) LMAN-RA cross-covariance calculated as in (D), but at finer temporal resolution at multiple time bins spanning the premotor window. Each calculation was perfomed on a 60 msec window; the resulting cross-covariance value was plotted at the latest timepoint in that window. The asterisk and black line indicate timepoints when values were significantly greater than 0.
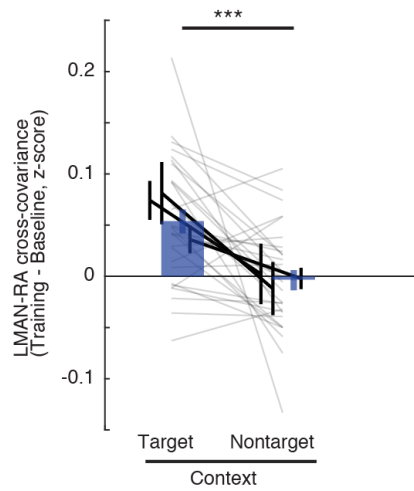
Figure 3.5. Increase in LMAN-RA cross-covariance is context-specific.
Increase in LMAN-RA cross covariance during training for experiments in which feedback was targeted to only a single sequential context (Target), and at least one other sequential context exists (Nontarget) (N = 29 pairs, 7 experiments, 3 birds). If multiple Nontarget contexts existed, then the datapoint represents the mean over those contexts. Plotting conventions identical to those in Figure 3H.
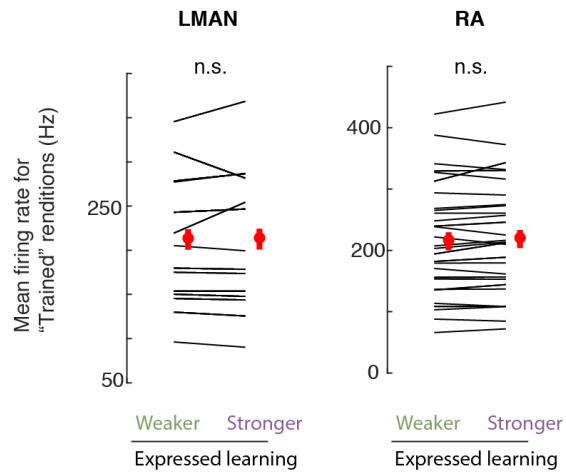
Figure S3.1. No significant difference in the mean firing rate for renditions expressing stronger vs. weaker learning.
Mean firing rate in the premotor window is not significantly different between two groups of renditions split based on strength of expression of learning. See Figure 3.4 for details on how renditions were split into "Stronger" and "Weaker" learning groups, and to contrast the greater increase in LMAN-RA cross-covariance seen there for Stronger learning with the lack of a similar effect on firing rate here.

Chapter 4: General discussion

4.1. A neural mechanism for a particular form of motor skill flexibility in birdsong.

Summary of findings

At the behavioral level, we found that pitch modifications reflect an adaptive balance between generalization and specificity across contexts (Chapter 2, Figures 2.1, 2.2). Generalization occurs when WN feedback is targeted to one context, but birds have an additional capacity for highly context-specific learning when opposite-direction feedback is provided in two contexts. Using transient pharmacological inactivation of LMAN during learning, we found that this balance reflects separate contributions of two distinct, but interacting, circuits: the AFP biases pitch in a context-specific manner to promote specificity of learning, while modifications in the motor pathway (MP) are responsible for the expression of generalized learning (Chapter 2, Figures 2.4, 2.5). By examining correlations between neural activity in LMAN and RA, and how those correlations change during learning, we reported evidence that, at the neural level, AFP pitch bias reflects a premotor instructive signal that influences activity in RA through fast synaptic transmission (Chapter 3).

How does the AFP "learn" a context-specific pitch bias?

In the Discussion for Chapter 2, I outlined a circuit-level model for how the AFP learns a context-specific bias during training. There are a few things to add to that model. One possibility is that plasticity does not actually occur not at HVC-X synapses, as proposed in that model, but somewhere else in the AFP [e.g., related to plasticity mechanisms in

juvenile LMAN (Boettiger and Doupe, 2001)], or downstream at LMAN-RA synapses. There is no direct evidence regarding the locus of plasticity, but there seems to be reasonable evidence suggesting that plasticity occurs in Area X, potentially at HVC-X synapses as originally proposed. First, there is evidence for a general role for reward-mediated plasticity at cortico-striatal synapses[15] in motor learning [e.g., (Wickens et al., 2003; Xiong et al., 2015)]; second, there is evidence for a causal role for dopaminergic inputs to Area X in driving plasticity crucial for pitch modifications (Gadagkar et al., 2016; Hisey et al., 2018; Hoffmann et al., 2016; Xiao et al., 2018), and the possibility of HVC-X synapses undergoing dopamine-mediated plasticity is supported by experiments in brain slices (Ding and Perkel, 2004). Fourth, normal transmission of activity at LMAN-RA synapses is not required for the learning of bias in the AFP (Charlesworth et al., 2012), which suggests that the relevant plasticity is upstream of the LMAN-RA synapses, and potentially within the AFP. On the other hand, there is direct evidence that the synapses from LMAN to RA can undergo plastic changes in slices, that this plasticity may be crucial for pitch modifications (Mehaffey and Doupe, 2015). Taken together, evidence suggests that it is plausible, but not certain, that the relevant plasticity underlying AFP bias occurs within the AFP, potentially at HVC-X synapses.

A second point regards the source of contextual signals in the AFP. We suggested that these signals may be conveyed from HVC neurons that project to Area X (HVC$_X$ neurons); indeed, there is evidence from neural recordings in Bengalese finches that HVC inputs can encode information about sequential context (Fujimoto et al., 2011). This would be consistent with a model in which AFP bias depends on plasticity at these HVC$_X$-X

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[15] The synapses from HVC to spiny neurons in X may be analogous.

synapses, because then changes that drive pitch bias would presumably only be expressed in the appropriate sequential context [similar to ideas proposed in (Fee and Goldberg, 2011; Mooney, 2014)]. This is consistent with the possibility that the physiology of and patterns of afferent connections to striatal spiny neurons in Area X may make them well-suited to detect context in the moment-to-moment configuration of inputs from HVC (Fee and Goldberg, 2011; Houk and Wise, 1995).

However, it is worth explicitly addressing the possibility of some other sources of contextual information: RA projects to DLM, which projects to LMAN. This input could potentially convey information to the AFP regarding the sequence of ongoing song (Goldberg and Fee, 2012; Vates et al., 1997). We think that this is unlikely because RA activity only weakly encodes sequential context (Leonardo and Fee, 2005; Wohlgemuth et al., 2010). Another possibility is that context is encoded not in inputs to the AFP, but as "memory" in the AFP; at any point in song AFP activity may reflect some influence of the last few syllables in the current song bout. This lasting influence may occur, for instance, as a result of recurrent activity in the AFP[16]. However, given the direct evidence for encoding of context in HVC_X neurons and the likelihood that plasticity at HVC_X-X synapses is important for learning (see above), we favor the originally proposed possibility that context relevant for learning is conveyed from HVC[17].

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[16] Either through the LMAN-DLM-Area X loop, or from local connectivity within these regions. Both LMAN and Area X are known to contain recurrent connections.

[17] If it were true that contextual signals conveyed from HVC were crucial for the AFP to generate a biasing signal, then an experimental prediction would be that perturbation of HVC-X activity that "jumbles" this contextual signal would block AFP bias and as a result lead to reversion of learning.

How is learning transferred to the motor pathway?

Our findings indicate that generalization results from modifications in the motor pathway (see Figure 2.4, 2.5 and Discussion for Chapter 2). A potential mechanism for how these modifications occur, and how they lead to generalization, is laid out in the Discussion for Chapter 2. In brief, modifications would occur at HVC-RA synapses, driven by instructive signals from LMAN biasing plasticity in the motor pathway through a process of "systems consolidation" (Dudai, 2004). While it is likely that plasticity in the motor pathway underlies the component of learning that remains when AFP output is blocked, there is no direct evidence for instructive signals from LMAN directing plasticity in the motor pathway[18]. A plausible alternative mechanism posits that plasticity may occur in the MP as a result of feedback signals that arrive directly in the MP, not through the AFP, for instance at HVC-RA synapses. However, an argument against this model is that learning does not seem to occur at all unless LMAN synaptic input to RA is active (Charlesworth et al., 2012), which suggests that plasticity in the AFP must occur first.[19] Moreover, the motor

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[18] Indirect evidence comes from the fact that the learning encoded in the MP is remarkably similar to that encoded by AFP bias [warren; andalman]; moreover there is evidence that plasticity in the MP cannot occur unless there is active synaptic transmission from the AFP [covert].

[19] An argument against this interpretation is that silencing LMAN blocks not only LMAN's synaptically transmitted activity, but also the secretion of neurotrophic factors from LMAN that may be crucial for plasticity in RA. An argument against this argument is that learning is blocked even when LMAN's inputs are silenced by antagonizing LMAN's synaptic input in RA thus, presumably, leaving intact LMAN's ability to secrete neurotrophic factors (Charlesworth et al., 2012).

biasing signal [that we found evidence for in correlations between LMAN and RA neural activity (Chapter 3)] may represent a mechanism that drives plastic changes in the MP. While we currently lack direct evidence for such a serial transfer mechanism, this transfer process accounts reasonably well for the existing data. Strong evidence in support or against the instructive model may come from experiments testing whether systematic stimulation of LMAN (in a manner sufficient to drive consistent, naturalistic, biases of behavior) is sufficient to instruct corresponding learning-related modifications to the motor pathway.

How do modifications in the motor pathway contribute to generalization?

A model for how changes in the MP may underlie generalization is described in the Discussion for Chapter 2. There are a few predictions to be made. First, the occurrence of generalization as a function of plasticity at HVC-RA synapses predicts that the same, or largely overlapping, set of synapses from HVC to RA should be active when the same syllable is sung in different contexts. It is not clear, however, to what extent there is an overlap in the synapses active in different contexts, nor do we know what the activity of neurons in HVC that project to RA (HVC_{RA} neurons) looks like during singing in Bengalese finch. This model predicts that HVC_{RA} activity should be highly overlapping in different contexts[20]. However, if it is the case that those inputs are not highly overlapping [as

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[20] HVC_{RA} single unit activity has been recorded during singing, but in zebra finches, which sing highly stereotyped song with little to no sequence variability (Hahnloser et al., 2002; Kozhevnikov and Fee, 2007). In Bengalese finches, if it were the case that HVC_{RA} activity is more similar across contexts than is HVC_X activity (which we already know to have substantial differences across contexts; see above), then it raises the question of how

predicted by some theoretical models of the motor pathway control of sequencing (Jin and Kozhevnikov, 2011; Katahira et al., 2011)] then we might still account for the role for the motor pathway in generalization by positing that plasticity may alter activity of a given RA neuron in a manner that is not synapse-specific. This may occur through changes to excitability of RA neurons due to either alteration of intrinsic conductances [e.g., (Aizenman and Linden, 2000)] or local inhibitory activity[21] (Miller et al., 2017; Spiro et al., 1999); this may lead to generalization, since it is already known that activity for a given RA neuron is quite similar across contexts (Leonardo and Fee, 2005; Wohlgemuth et al., 2010).

Note that the motor pathway did exhibit some residual capacity to learn separate modifications based on context (Figures 2.4, 2.5). This argues against a strictly hierarchical model in which RA encodes a given syllable identically across contexts; this slight contextual difference is evident in neural recording studies (Wohlgemuth et al., 2010). This may suggest that there are slight differences in the pattern of HVC inputs across contexts, which may allow HVC-RA plasticity some capacity to encode context-dependent pitch differences. Alternatively, RA changes may be identical across contexts, with the separation

contextual differences are present in one cell type but not another in the same local circuit. Slice experiments indicate that a dominant microcircuit motif in HVC is from HVC_{RA} to interneurons to HVC_X neurons (Mooney and Prather, 2005). Interneurons may act to "separate" small differences in patterns of HVC_{RA} activity into larger differences at HVC_X (similar to what has been suggested for some other circuits in striatum, hippocampus, and cerebellar cortex). Alternatively, interneurons may contain a longer memory of sequential activity, which would be conveyed to HVC_X neurons.

[21] In principle excitability could also be modulated by plasticity at local excitatory connections in RA; however, those connections seem to be either very rare or nonexistent in adult RA (Miller et al., 2017).

in pitch reflecting context-dependent differences in activity in the motor periphery (Forssberg et al., 1975; Ostry et al., 1996) that differentially transforms the same RA activity.

At what timescale does transfer of learning to the motor pathway occur? Is this timescale consistent with the timescale of generalization?

We have suggested that it may be the signals conveyed from LMAN to RA during singing that drive modifications in RA. This predicts that MP-dependent learning should exhibit a gradual increase over the course of many song bouts, and, at a finer resolution, potentially even discrete increments locked to song bouts. The lag from each song bout to these increments would depend on the speed with which relevant plasticity occurs. From inspection of example learning trajectories (e.g. Figure 2.1, 2.2) it is clear that generalization occurs with a timecourse grossly similar to learning at the target syllable. Rendition-by-rendition analysis of the timecourse of both consolidation (assessed using electrical stimulation of LMAN by T. Warren and J. Charlesworth, data not shown) and generalization (analysis of behavioral data in Figure 2.1; data not shown) are consistent with consolidation and generalization occurring with a similar timecourse, and in a manner tightly locked to singing. This result is therefore consistent with the possibility that the motor pathway is gradually updated during singing, perhaps in response to instructive premotor signals from the AFP[22].

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[22] This result also rules out the possibility that transfer to the MP occurs entirely, or even mostly, overnight, as has been suggested for some other forms of motor skills [see (Bönstrup et al., 2019) for a critical account of that idea].

Do the mechanisms that allow that AFP to contribute to context-dependent pitch modifications in adults also contribute to the differentiation of syllables in juvenile song learning?

What do these findings suggest regarding the role of the AFP and MP during song learning during development? A differential contribution of the AFP to specificity and MP to generalization may account for one aspect of song development: how birds obtain their adult repertoire of syllables. Longitudinal tracking of the developmental trajectory of song has revealed different "strategies" undertaken by different individual birds (Liu and Nottebohm, 2004; Okubo et al., 2015; Tchernichovski et al., 2001). One strategy involves having different adult syllables arise from the same early stage protosyllable[23] sung in different sequential contexts. For example, the sequence of protosyllables "AAAA" may differentiate early in development into "AADA" and then at a late stage of development to "BCDE" (Liu and Nottebohm, 2004); in this case, four variants of the same protosyllable ("A"), which differed by sequential context, eventually differentiated into four different adult syllables.

In the brain, protosyllables are encoded similarly in HVC but, as protosyllables gradually differentiate into adult syllables, HVC activity concurrently "splits" into different representations for each adult syllable (Okubo et al., 2015). One possible mechanism for this splitting is that different AFP input in each context to RA acts to "push" apart the

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[23] A poorly structured developmental precursor to the adult syllable.

representations in RA for the same syllable. These differences may then be stabilized and eventually crystallize by transfer to HVC-RA synapses[24].

How might this model account for the splitting of activity that occurs in HVC? One possibility is that auditory feedback will differ as the RA representation differentiates - this feedback may drive modifications in HVC to separate its representations across contexts (Mackevicius and Fee, 2018)[25]. One prediction if this idea is correct is that during the protosyllable period neural activity in the AFP should show contextual differences that precede (and predict) contextual differences in HVC.

4.2. Some broader principles for motor skill adaptation in relation to these findings.

A simple model for tuning the balance between generalization and specificity depending on the complexity of feedback signals in a given environment.

The ability to adaptively balance generalization and specificity of motor skills in humans is evident from studies of naturalistic behavior (Adolph and Eppler, 2002; Bernstein, 1996; Clearfield and Thelen, 2001; MacKay, 1982; Wolpert et al., 2001) as well as motor adaption studies; perhaps most pertinent are two classes of studies. One class

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[24] A general role for the AFP in syllable differentiation is supported by the findings that LMAN lesions in late-stage juveniles leads to song prematurely crystallizing in the state it is at when the lesion occurs - it is almost as if normal development including syllable differentiation is suddenly halted (Bottjer et al., 1984; Scharff and Nottebohm, 1991).

[25] This differentiation process may represent a mechanism for a potentially broad principle in motor skill learning, namely that initial learning of a skill may progress by first learning a small set of motor primitives from which more complex motor gestures are derived trough differentiation.

demonstrates that subjects will naturally generalize learning across contexts if training signals are provided in only one context (Houde and Jordan, 1998; Howard and Franklin, 2015; Rochet-Capellan et al., 2012), yet reveal a capacity for learning more specific modifications if training signals differ across contexts (Gandolfo et al., 1996; Howard et al., 2012; Nozaki et al., 2006; Pearson et al., 2010; Rochet-Capellan and Ostry, 2011; Wainscott et al., 2004)[26]. These studies are analogous to the songbird behavioral experiments I described in Chapter 2, and suggest the ability to "tune" the propensity to generalize depending on whether feedback occurs in one or multiple contexts. Another class of studies more directly tries to measure patterns of generalization as they are changing in response to changing environments [(Krakauer et al., 2006; Taylor and Ivry, 2013; Thoroughman and Taylor, 2005); also see (Gandolfo et al., 1996; Rochet-Capellan and Ostry, 2011)[27]]. In these studies, subjects are shown to be able to modify their natural pattern of generalization based on the history of how feedback depends on contextual cues. As a

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[26] Note that these references are biased towards studies of sequential context; it is clear that similar specificity of learning can be enabled by contexts of many sorts of modalities - I reference a few of those studies here, but also in Chapter 1 and the section below specifically addressing the potential general role of basal ganglia circuits in context-specific adaptation.

[27] These two studies are interesting because this tuning of generalization is not of major concern to the authors, but seems to be present by inspection of the example experiments; i.e., in both of these studies subjects receiving feedback that differs across contexts seem to initially generalize, then gradually exhibit more specificity (as if gradually learning that in this environment less generalization is better). Learning about the environment in this way may be commonplace and expressed in experiments where such learning is not directly being tested.

result, the patterns of generalization, in response to the exact same training signal, can generalize more or less broadly depending on whether prior feedback was more or less similar across contexts. In principle, these behavioral effects likely depend on some ability of the nervous system to keep track of the statistics of feedback across contexts, for a given environment, and to use that information to decide whether generalization or specificity is appropriate.

This ability to shape generalization to fit the current environment is thought, in an abstract sense, to reflect the inference of appropriate "internal models" (Wolpert and Kawato, 1998)[28]. These internal models encode the relationship between contextual cues, motor parameters, and behavioral outcome[29]; this relationship would be the one inferred to be good with respect to its ability to account for the history of feedback (especially the correlation between contextual cues, motor output, and feedback) in a given environment (Korenberg and Ghahramani, 2002; Krakauer et al., 2006; Wolpert and Kawato, 1998)[30].

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[28] For example, if prior experience indicates that in this environment feedback tends to differ across contexts, then subjects may exhibit less generalization when retested in this environment. Note that some have argued that in some paradigms, in contrast, generalization shows very little modifiability (Mattar and Ostry, 2007).

[29] These models could influence action in a predictive manner, or alternatively by influencing movement using online feedback (Todorov and Jordan, 2002) or by modulating effector stiffness through muscle co-contraction (Davidson and Wolpert, 2003).

[30] For the behavioral experiments in this dissertation, it is the correlation between context and feedback, over prior renditions, that determines the state of the environment. Indeed there is empirical evidence that people evaluate not only the history of prior contexts, but also the history of prior feedback (errors) (Herzfeld and Shadmehr, 2014), of history of correlation between errors and context (Roemmich and Bastian, 2015; Thoroughman and

However, the ability to learn and utilize an internal model appropriate for a given environment is an abstract one with no obvious or empirically-supported underlying neural mechanism.[31]

The current findings suggest a simple biological mechanism for one version of behavior that involves tuning of patterns of generalization. The bird's "internal model" consists of mappings between each syllable to its appropriate pitch, encoded in the MP, and mappings between each syllable/context combination to an appropriate pitch, encoded in the AFP. Empirically, the relative contributions of these two mappings to behavior are tuned based on the complexity of feedback across contexts (Figure 2.5D). The proposed circuit-level mechanism for this tuning is simple and described above (see Chapter 4.1); essentially, the more similar the inputs from LMAN to RA are across contexts, the lesser the

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Taylor, 2005), and that the resulting knowledge may accelerate future learning (Braun et al., 2009, 2010; Welch et al., 1993). For some other forms of learning, a similar Bayesian statistical learning process may also enable flexibility (Gershman, 2017).

[31] A class of biologically-inspired models accounts for patterns of generalization by similarity of the underlying "tuning" of units in the sensory-motor circuits contributing to different movements. If the sensory cues (i.e., context) and motor outputs for a given action overlap strongly with those of another action, then any adaption for one will generalize spontaneously to the other (Darshan et al., 2014; Nozaki and Scott, 2009; Poggio and Bizzi, 2004; Shadmehr and Mussa-Ivaldi, 1994). To account for cases where patterns of generalization can change depending on the environment, the tuning of units must be adaptable (i.e., broader tuning leading to broader generalization) (Thoroughman and Taylor, 2005). Alternatively, credit assignment (i.e., the determination of which weights to update during training) may be tuned by context (Nozaki and Scott, 2009). It is not clear how either of these possibilities are implemented, but cerebellum has been implicated (see below).

interference of plasticity in RA and thus the stronger the accumulated plastic changes in RA (see Discussion in Chapter 2). A direct result of this interference in RA is that the more simple the environment, the more learning is transferred to update the "core" syllable representation in RA. One may say that the internal model has therefore learned that in this environment generalization is good. A similar idea would play out in more complex environments, but would instead involve greater contributions from the AFP.[32]

Some evidence for "interference" of plasticity in motor cortex

There is indirect evidence for a potentially similar "interference" effect in other motor skills. For example, interference of modifications in motor cortex may occur when learning multiple similar motor tasks that require learning different sensorimotor transformations (Ganguly and Carmena, 2010; Zach et al., 2012)]; moreover, changes in activity in motor cortex can predict patterns of generalization (Arce et al., 2010) and encoding can be similar in motor cortex for similar movements that are performed in different contexts (see Chapter 1). Moreover, there is evidence in mice that overlap in dendrite-specific plasticity in motor cortex can contribute to interference in motor learning[33] (Cichon and Gan, 2015).[34] These findings are consistent with patterns of

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[32] It is however still not fully clear if and how a transfer process from LMAN to RA occurs; therefore, there is no strong empirical evidence that interference in RA plasticity actually occurs as described (see above for weaker evidence in support of this interference).

[33] Interestingly, they also show that activity of SST interneurons may control the level of overlap in representation in these dendrites, suggesting a potential mechanism to tune interference.

generalization normally being dependent on the nature of learning-related changes in motor cortex.[35] More broadly, in addition to motor cortex there may be other plausible brain regions where modifications may be expected to alter representations of motor primitives (see Chapter 1.1) and thus lead to generalization.

Hierarchical representations of motor skills

Since the idea of a hierarchical neural architecture underlies some of the framing of my results, it is worth explicitly clarifying potential confusion regarding the different ways hierarchy may be attributed to motor skills. An underlying idea in this dissertation is that the motor system reuses a smaller set of motor primitives in a larger set of sequential contexts. In this framework, there are at least two broad classes of hierarchies. The first

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[34] This idea of interference of plasticity impairing learning in behavior is potentially related to the problem of credit assignment. Learning partially depends on answering the question: variation at what level of behavior (e.g. selection, sequencing, planning, execution) best predicts variation in feedback/performance? Was failure due to choosing the wrong action or executing the right action incorrectly? For the birdsong studies here, the corresponding question is: are patterns of WN feedback most strongly predicted by the syllable being sung? Or the combination of syllable and context? Assuming that variation in these behavioral features (syllable and context) correspond to variation in neural representation in different "dimensions" (e.g., in a simple case, different brain regions), then determining whether one should modify syllable-related or context-related representations is a particular case of the general problem of credit assignment in reinforcement learning theory (Sutton and Barto, 1998). See (Gulati et al., 2017) for discussion of empirical evidence regarding potential neural mechanisms of credit assignment in motor learning.

[35] Also maybe consistent with this possibility: stimulation of motor cortex can affect patterns of generalization (Orban de Xivry et al., 2011).

class corresponds to the architecture described for context-dependent pitch modification (Figure 2.4, 2.5), with lower levels representing motor gestures (e.g., syllables) and higher levels representing nodes that can impose context-specific modifications to execution of those gestures. This hierarchy can be schematized as multiple biasing inputs converging onto a given motor primitive.

The second is a class that addresses the ability to learn and control variable sequences in the first place. The higher level of the hierarchy represents some understanding of the learned (or unlearned) probabilities of transitioning between individual gestures; these higher nodes can be crudely thought of as representing appropriate "chunks" of actions, or even more abstract sequences of goals (Botvinick, 2008; Diedrichsen and Kornysheva, 2015; Lashley, 1951, 1951; Markowitz et al., 2018)[36]. In contrast to the first hierarchy, here a single higher node would diverge to multiple lower nodes that represent the motor primitives that comprise the action sequence.

These two hierarchies can coexist and work together to control motor skills, but it is not very clear what circuit mechanisms these hierarchies may map onto, or to be fair, to what extent this is a generally accurate and fruitful way (in terms of research progress) of thinking about motor sequences. For birdsong, however, one might loosely map this

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[36] For some types of sequences there seems to be a relatively clear distinction between higher (abstract) and lower (execution) level representations, since a given sequence can be implemented with very different movements. This "motor equivalence" is perhaps most strikingly demonstrated by the ability to write a sequence of words with a pen held in either ones hands or ones toes. Similar is the finding that a sequence of finger presses when learned with one hand can be readily produced with the other (MacKay, 1982; Witt et al., 2010).

hierarchy onto two pathways emanating from HVC that directly and indirectly project to LMAN (Figure 2.3). HVC neurons that project to RA may represent a high-level node in the second hierarchy, to the extent that activity of these neurons are crucial for controlling the sequencing of syllables and seemingly less important for controlling the details of how individual syllables are executed (Hahnloser et al., 2002; Hosino and Okanoya, 2000; Jin and Kozhevnikov, 2011; Katahira et al., 2011; Long and Fee, 2008; Margoliash and Yu, 1996; Vu et al., 1994; Zhang et al., 2017). My experiments suggest that HVC_X neurons that project indirectly to RA through the AFP may correspond to a higher node in the first hierarchy, which acts to bias the execution of a given syllable depending on context, but are not crucial for determining the sequential order of syllables. This framework conveniently suggests that one place were those hierarchies may interact is in HVC[37].

Can associative learning mechanisms account for all features of motor skill flexibility?

In this dissertation, flexibility in birdsong depends on associative learning mechanisms; e.g., context-specificity of modifications depends on the AFP learning the association between context and adaptive pitch bias. Associations require experience to learn. However, some aspects of flexibility require adapting to contexts that have never before been visited. These abilities may involve higher-level cognitive processes, including prospective decision-making [e.g., in infants learning new skills (Adolph and Eppler, 2002)]. While fundamentally different mechanisms may enable this sort of adaptive

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[37] One simple reason interaction is important is that the influence of the hierarchies must be temporally coordinated.

behavior, it is possible that these mechanisms may similarly involve habitual behavior being modified by more rapidly adapting, context-dependent signals.

4.3. The basal ganglia in context-dependent modification of motor skills.

A conserved role for basal ganglia in context-dependent modification of motor skills?

The AFP plays a crucial role in the context-dependent modification of syllable execution, but plays little role in the sequencing of adult song (Bottjer et al., 1984; Hampton et al., 2009; Scharff and Nottebohm, 1991). In seeming contrast, it is thought that basal ganglia in mammals are especially important for selecting and sequencing actions to maximize expected reward resulting from those actions [e.g., (Aldridge and Berridge, 1998; Canavan et al., 1989; Doya, 2000; Graybiel, 1995; Hikosaka et al., 1999; Kim and Hikosaka, 2013; Mannella and Baldassarre, 2015; Wise and Murray, 2000)]. This function is though to depend on an implementation of reinforcement learning algorithms in cortical-basal ganglia circuitry (Doya, 2000; Schultz et al., 1997; Sutton and Barto, 1998) so that the basal ganglia learn the association between context, actions, and reward. However, basal ganglia circuits have well-conserved roles in motor learning and production across vertebrates (Grillner et al., 2013); mammalian basal ganglia and the AFP have striking homologies of cell type, physiology, gene expression, and anatomy (Doupe et al., 2005). In the Discussion in Chapter 2 I briefly summarized evidence that in both mammals and birds a function of these circuits is to integrate contextual cues to enable adaptive, context-dependent learning and execution of motor skills. Below I flesh out this idea.

Partly because context-dependent motor skill adaptation has largely been studied in humans, there is little direct evidence, as far I know, for its underlying neural mechanisms.

There is evidence that motor adaptation depends on cerebellum (Shmuelof and Krakauer, 2011), with one study implicating a specific subregion of cerebellum in context-dependent adaptation of arm movements (Lewis, 2003)[38]. It is less clear whether basal ganglia in mammals play a prominent role in motor skill adaptation, let alone context-specific adaptation.

There are a few lines of evidence consistent with the basal ganglia contributing to the adaptation of motor skills. Some of the earliest experimental lesions of basal ganglia in primates revealed severe disruption in the execution of movements, but often had little to no effect on the ability to select actions or sequence actions appropriately [see reviews in (Dudman and Krakauer, 2016; Turner and Desmurget, 2010)]. Impairment of skilled movements has been shown in recent studies of rodents as well (Dudman and Krakauer, 2016; Paton and Lau, 2015; Rueda-Orozco and Robbe, 2015). Moreover, both fMRI and intra-cranial recordings in humans have revealed signals in basal ganglia that correlate with learning-related variables during motor skill adaptation [e.g., (Doyon et al., 2009; Shadmehr and Holcomb, 1997; Tan et al., 2014)]. Also, behavioral experiments indicate that reward-based learning, often thought to depend strongly on basal ganglia, plays a crucial role in motor skill adaptation (Huang et al., 2011; Izawa and Shadmehr, 2011; Morehead et al., 2015; Shmuelof et al., 2012; Taylor and Ivry, 2014)[39]. Finally, there is evidence that patients with Huntington's and Parkinson's disease, which both disrupt basal

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[38] Although I have not been able to find any follow-up study since then.

[39] Perhaps telling are the findings that reinforcement-learning processes seem to be especially important for long-term savings (see references in text), while Parkinson's disease similarly seems to especially affect savings (see text).

ganglia function, can have impairments in the control and adaptation of movements (Bédard and Sanes, 2011; Maurice et al., 2000)[40].

A behavior for which there is relatively strong neurophysiological evidence for a role for basal ganglia is the context-dependent modification of saccade kinematics (Hikosaka et al., 2006). In these tasks monkeys must make saccades to visual targets to obtain reward. Learning is reflected in the fact that movement kinematics (e.g., velocity) are influenced by the association between contextual cues and expected reward (more expected reward leads to faster saccades). Detailed manipulation, neural recording, and anatomical studies provide relatively strong evidence in support of a model in which striatal plasticity, gated by dopaminergic reward signals, encodes the association between context and appropriate saccade execution (through motor circuits in the superior colliculus)[41]. Moreover, behavioral studies have demonstrated that saccade execution can be modified based on a large variety of contextual cues (Azadi and Harwood, 2014; Herman et al., 2009)[42].

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[40] Evidence suggests that Hungtinton's affects online feedback-dependent control while Parkinson's affects long-term saving of learned adaptation.

[41] Studies of human patients provide strong evidence for involvement of cerebellum in saccade adaptation, but also evidence for cortical-basal ganglia circuits (MacAskill et al., 2002)

[42] However I am not aware of any direct evidence for the neural mechanisms of these behaviors (context-dependent adaptation of saccade gain), including the possibility that they utilize the same mechanisms as the control of vigor of memory-guided saccades in the studies by Hikosaka et al.

Crucially, it is plausible that these neural mechanisms may contribute to movements beyond saccades. The basal ganglia interface a large swath of inputs from cortex and thalamus[43] with outputs to a large swath of motor circuits[44] (Alexander et al., 1986; Houk and Wise, 1995). However, direct evidence is lacking regarding the extent to which basal ganglia contribute to the diverse ways in which context can influence motor skill execution.

Do basal ganglia contribute to both context-dependent action selection and execution?

It is possible that basal ganglia contribute to both action selection and movement kinematics using similar mechanisms. In a hierarchically organization in which different circuits encode behaviors at different time scales - ranging from 10s of milliseconds for fast muscle synergies, to seconds for sequences of actions, to potentially longer for planned sequences of goals - biasing of this system at different levels may selectively influence

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[43] Indeed recordings have revealed that activity of neurons in striatum can correlate with a wide variety of contextual cues, including those reflecting visual and auditory stimuli, cognitive states like task set, and motor context sequential context: (Aldridge and Berridge, 1998; Dudman and Krakauer, 2016; Hikosaka et al., 2006; Kimura, 1990; Lidsky et al., 1985; Mushiake and Strick, 1995; Nakahara et al., 2004; Nieuwenhuis et al., 2005; Samejima et al., 2005; Turner and Anderson, 2005; Turner and Desmurget, 2010). Signals encoding motor contextual cues (e.g., sequential context) have often been interpreted as reflecting a role in selecting actions to form a sequence [e.g. (Aldridge and Berridge, 1998)]; it is possible, as suggested in my results, that in some cases these signals are actually (or also) crucial for allowing basal ganglia to monitor position in sequence so that it may learn sequential-context specific modifications to movements.

[44] Activity in striatal circuits can correlate with a large variety of movements. This activity often peaks during the movement, consistent with a role in modifying its execution.

behavior at different levels (e.g., action selection vs. execution). Basal ganglia output is incredibly diverse and therefore the same learning principles may allow different basal ganglia outputs to bias motor structures at different organizational levels, and therefore contribute to both context-dependent action selection and execution. Indeed, behavioral evidence already suggests action selection and execution are not really driven by dissociable neural processes (Cisek, 2012)[45].

The contribution of basal ganglia to these different levels may differ depending on the stage of learning. This is evident in birdsong, where basal ganglia control both action selection (i.e. sequencing) and structure early on in song development (Aronov et al., 2008; Bottjer et al., 1984; Olveczky et al., 2005; Scharff and Nottebohm, 1991), but later on in adults only influence acoustic structure (Kao et al., 2005). In the case of birdsong, it could be that once sequence is "hard-wired" in the motor pathway, it becomes less labile and thus not affected by LMAN activity. Similarly, there is some evidence that in mammals frontal cortical-BG circuits contribute to sequencing early on in learning, but become dispensable for sequencing once the behavior is well-learned (Hikosaka et al., 1999; Miyachi et al., 1997); separate studies indicate that at this late timepoint these circuits may still control kinematics (Turner and Desmurget, 2010). One reconciliatory possibility is that cortical-basal ganglia circuits play similar roles in birdsong and mammalian arm movement sequences (i.e., being involved in both sequencing and gesture kinematics); discrepancies in experimental findings may simply reflect differences in learning stage and/or other aspects of the tasks.

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[45] For example, action selection seems to involve evaluation of the kinematics of potentially chosen actions.

4.4. Distributed circuits for motor skills.

In addition to key roles for cerebellum and possible roles for basal ganglia in adaptive motor skills, there is separate evidence for involvement of multiple other regions, including sensorimotor cortex in mice (Mathis et al., 2017), and frontal, motor, and parietal cortices in humans [e.g., (Doyon et al., 2009; Makino et al., 2016; Shadmehr and Holcomb, 1997; Shmuelof and Krakauer, 2011; Tanaka et al., 2009)]. The involvement of multiple regions in motor skill adaptation may simply be a result of the incremental nature by which evolutionary changes to the brain build up over time. Alternatively, it may suggest computationally unique and interacting roles for different brain regions (Doya, 2000; Houk and Wise, 1995; Shmuelof and Krakauer, 2011). Future work may seek to elucidate how these regions interact to enable behavior, including the possibility for a mapping between computation and anatomical modularity.

4.5. Some thoughts on adaptive behavior, beyond motor skill execution, inspired by neural mechanisms of birdsong pitch modifications.

Early learning and instruction driven by basal ganglia circuits

A transfer of learning may occur in which the AFP provides instructive signals that act to drive learning-related plasticity in the motor pathway (see above). There is some evidence for a similar "tutoring" role for basal ganglia in mammalian systems. First, maybe the strongest evidence comes from findings that BG circuits seem to be especially important during early stages of learning, but are less important, or even dispensable, for performance later on. This is from a variety of inactivation and neural recording

experiment (Doyon et al., 2009; Dudman and Krakauer, 2016; Miyachi et al., 1997; Pasupathy and Miller, 2005; Sawada and Nishimura, 2015; Turner and Desmurget, 2010; Yin et al., 2009). Moreover, there is some inactivation-based evidence for a serial process by which learning in some parts of cortex requiring modifications to first occur in BG (Atallah et al., 2007).

These findings have led to conceptual and computational models in which the basal ganglia first learns from reward, and then biases activity in order to "tutor" downstream circuits. This tutoring may occur through a, potentially slower, Hebbian mechanism in cortex that effectively extracts regularities in the correlation structure of stimuli, rewards, and other variables. (Ashby et al., 2007; Atallah et al., 2004; Doya, 2000; Hélie et al., 2015; Houk and Wise, 1995; Makino et al., 2016, 2016). However, although there is evidence for interaction in neural signals between cortex and striatum during learning (Koralek et al., 2013; Sawada and Nishimura, 2015), I am not aware of much direct evidence for how neural signals may drive such tutoring.

It is important to note that there is also evidence arguing against a direct tutoring role by frontal-basal ganglia circuits. Lacking direct evidence for neural signals that could implement such tutoring, there are other plausible explanations for why inactivation of a brain region may have a stronger behavioral effect early in learning[46]. Moreover, in some cases learning does seem to occur in parallel in multiple brain regions, but with different learning rates [e.g., (Yin et al., 2004)]. And for some skills early learning may depend on a tutoring role for motor cortex (Kawai et al., 2015). It seems like a simple serial learning

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[46] For example, an early stage contribution may be to enable a state of heightened motivation, as argued in (Sawada and Nishimura, 2015).

model may not provide a general explanation for learning (not least because of the extensive bidirectional interaction that occurs between cortex and basal ganglia). Future experiments may indicate whether the instructive signals that we argue for in birdsong reflect a general mechanism by which cortical-basal ganglia circuits tutor other motor circuits[47].

Are the circuits that contribute to early learning also the circuits that enable context-dependent behavior?

The AFP is crucial for early learning and also for context-specificity of learning. How general is this relationship across different behaviors and brain circuits? A similar relationship has been suggested for cerebellum[48] [(Broussard and Kassardjian, 2004; Medina et al., 2000, 2002); although this simple model does not seem to account for all behavioral findings (Boyden et al., 2004)], hippocampus[49] [e.g., (Atallah et al., 2004)], and frontal-basal ganglia circuits[50] in flexible, rule-based behavior (Hilario et al., 2012; Kim and

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[47] One advantage of birdsong in detecting these sorts of instructive signals is the relatively precise behavioral correlate of learning (pitch), and the relatively simple anatomy (specific projections from LMAN to RA), which facilitate in isolating neural signals that relate to behavioral features of learning.

[48] e.g., in adapting sensorimotor associations; context can refer to many things that may be encoded in parallel fiber input to Purkinje cells, including for instance the precise timing of sensory cues.

[49] e.g., in rapidly learning and adapting rapidly to new environmental contexts.

[50] e.g., in flexible, goal-directed behavior, such as adapting to changes in task set (e.g. the set of behavioral rules for a given environment or block of time).

Hikosaka, 2013; Miyachi et al., 1997)[51]. It has been suggested that the presence of two separate learning systems, one fast and context-dependent, the other slow and prone to extraction of generalities, allows a system to balance the need to continuously monitor rapidly changing features of a situation, while maintaining and gradually updating a repertoire of generally useful automatic responses [e.g., (Atallah et al., 2004; Doyon et al., 2009; Shine and Shine, 2014)]. Delegating automatic responses to more stable circuits may free up more "executive" circuits for the adaptive context-dependent biasing of behavior.

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[51] With varying degree of evidence; my impression is that in none of these cases is there incontrovertible evidence for these conclusions.

Bibliography

Adolph, K., and Eppler, M. (2002). Flexibility and specificity in infant motor skill acquisition. In Progress in Infancy Research,.

Aizenman, C., and Linden, D. (2000). Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. Nat. Neurosci. *3*.

Akutagawa, E., and Konishi, M. (1994). Two separate areas of the brain differentially guide the development of a song control nucleus in the zebra finch. Proc. Natl. Acad. Sci. *91*, 12413–12417.

Akutagawa, E., and Konishi, M. (1998). Transient expression and transport of brain-derived neurotrophic factor in the male zebra finch's song system during vocal development. Proc. Natl. Acad. Sci. *95*, 11429–11434.

Aldridge, J.W., and Berridge, K.C. (1998). Coding of Serial Order by Neostriatal Neurons: A "Natural Action" Approach to Movement Sequence. J. Neurosci. *18*, 2777–2787.

Alexander, G.E., DeLong, M.R., and Strick, P.L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. *9*, 357–381.

Alonso, J.-M., Usrey, W.M., and Reid, R.C. (1996). Precisely correlated firing in cells of the lateral geniculate nucleus. Nature *383*.

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

Ansuini, C., Giosa, L., Turella, L., Altoè, G., and Castiello, U. (2008). An object for an action, the same object for other actions: effects on hand shaping. Exp. Brain Res. *185*, 111–119.

Arce, F., Novick, I., Mandelblat-Cerf, Y., Israel, Z., Ghez, C., and Vaadia, E. (2010). Combined Adaptiveness of Specific Motor Cortical Ensembles Underlies Learning. J. Neurosci. *30*, 5415–5425.

Aronov, D., Andalman, A.S., and Fee, M.S. (2008). A Specialized Forebrain Circuit for Vocal Babbling in the Juvenile Songbird. Science *320*, 630–634.

Ashby, F.G., Ennis, J.M., and Spiering, B.J. (2007). A neurobiological theory of automaticity in perceptual categorization. Psychol. Rev. *114*, 632–656.

Atallah, H.E., Frank, M.J., and O'Reilly, R.C. (2004). Hippocampus, cortex, and basal ganglia: Insights from computational models of complementary learning systems. Neurobiol. Learn. Mem. *82*, 253–267.

Atallah, H.E., Lopez-Paniagua, D., Rudy, J.W., and O'Reilly, R.C. (2007). Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. Nat. Neurosci. *10*, 126–131.

d'Avella, A., Saltiel, P., and Bizzi, E. (2003). Combinations of muscle synergies in the construction of a natural motor behavior. Nat. Neurosci. *6*, 300–308.

d'Avella, A., Giese, M., Ivanenko, Y.P., Schack, T., and Flash, T. (2015). Editorial: Modularity in motor control: from muscle synergies to cognitive action representation. Front. Comput. Neurosci. *9*.

Averbeck, B.B., Chafee, M.V., Crowe, D.A., and Georgopoulos, A.P. (2002). Parallel processing of serial movements in prefrontal cortex. Proc. Natl. Acad. Sci. *99*, 13172–13177.

Ayala, M.N. (2015). Context-Dependent Dual Adaptation to Opposing Visuomotor Rotations.

Azadi, R., and Harwood, M.R. (2014). Visual cues that are effective for contextual saccade adaptation. J. Neurophysiol. *111*, 2307–2319.

Bédard, P., and Sanes, J.N. (2011). Basal ganglia-dependent processes in recalling learned visual-motor adaptations. Exp. Brain Res. *209*, 385–393.

Ben-Shaul, Y., Drori, R., Asher, I., Stark, E., Nadasdy, Z., and Abeles, M. (2004). Neuronal Activity in Motor Cortical Areas Reflects the Sequential Context of Movement. J. Neurophysiol. *91*, 1748–1762.

Berger, D.J., Gentner, R., Edmunds, T., Pai, D.K., and d'Avella, A. (2013). Differences in Adaptation Rates after Virtual Surgeries Provide Direct Evidence for Modularity. J. Neurosci. *33*, 12384–12394.

Bernstein, N. (1996). Dexterity and Its Development (Taylor & Francis).

Boettiger, C.A., and Doupe, A.J. (2001). Developmentally Restricted Synaptic Plasticity in a Songbird Nucleus Required for Song Learning. Neuron *31*, 809–818.

Bönstrup, M., Iturrate, I., Thompson, R., Cruciani, G., Censor, N., and Cohen, L.G. (2019). A Rapid Form of Offline Consolidation in Skill Learning. Curr. Biol. *29*, 1346–1351.e4.

Bottjer, S.W., Miesner, E.A., and Arnold, A.P. (1984). Forebrain Lesions Disrupt Development but not Maintenance of Song in Passerine Birds. Science *224*, 901–903.

Bottjer, S.W., Roselinsky, H., and Tran, N.B. (1997). Sex Differences in Neuropeptide Staining of Song-Control Nuclei in Zebra Finch Brains. Brain. Behav. Evol. *50*, 284–303.

Botvinick, M.M. (2008). Hierarchical models of behavior and prefrontal function. Trends Cogn. Sci. *12*, 201–208.

Bouchard, K.E., and Chang, E.F. (2014). Control of Spoken Vowel Acoustics and the Influence of Phonetic Context in Human Speech Sensorimotor Cortex. J. Neurosci. *34*, 12662–12677.

Boyden, E.S., Katoh, A., and Raymond, J.L. (2004). CEREBELLUM-DEPENDENT LEARNING: The Role of Multiple Plasticity Mechanisms. Annu. Rev. Neurosci. *27*, 581–609.

Brainard, M.S., and Doupe, A.J. (2000). Interruption of a basal ganglia–forebrain circuit prevents plasticity of learned vocalizations. Nature *404*, 762–766.

Braun, D.A., Aertsen, A., Wolpert, D.M., and Mehring, C. (2009). Motor Task Variation Induces Structural Learning. Curr. Biol. *19*, 352–357.

Braun, D.A., Mehring, C., and Wolpert, D.M. (2010). Structure learning in action. Behav. Brain Res. *206*, 157–165.

Broussard, D.M., and Kassardjian, C.D. (2004). Learning in a simple motor system. Learn. Mem. *11*, 127–136.

Canavan, A.G.M., Nixon, P.D., and Passingham, R.E. (1989). Motor learning in monkeys (Macaca fascicularis) with lesions in motor thalamus. Exp. Brain Res. *77*, 113–126.

Caudrelier, T., Perrier, P., Schwartz, J.-L., and Rochet-Capellan, A. (2016). Does Auditory-

    Motor Learning of Speech Transfer from the CV Syllable to the CVCV Word? Interspeech

    2016 Proc. 2095–2099.

Charlesworth, J.D., Tumer, E.C., Warren, T.L., and Brainard, M.S. (2011). Learning the

    microstructure of successful behavior. Nat. Neurosci. *14*, 373–380.

Charlesworth, J.D., Warren, T.L., and Brainard, M.S. (2012). Covert skill learning in a

    cortical-basal ganglia circuit. Nature *486*, 251–255.

Chaure, F.J., Rey, H.G., and Quian Quiroga, R. (2018). A novel and fully automatic spike-

    sorting implementation with variable number of features. J. Neurophysiol. *120*, 1859–

    1871.

Cichon, J., and Gan, W.-B. (2015). Branch-specific dendritic Ca2+ spikes cause persistent

    synaptic plasticity. Nature *520*, 180–185.

Cisek, P. (2012). Making decisions through a distributed consensus. Curr. Opin. Neurobiol.

    22, 927–936.

Clearfield, M., and Thelen, E. (2001). Stability and Flexibility in the Acquisition of Skilled

    Movement. In Handbook of Developmental Cognitive Neuroscience, (MIT Press),.

Cothros, N., Wong, J., and Gribble, P.L. (2009). Visual Cues Signaling Object Grasp Reduce

    Interference in Motor Learning. J. Neurophysiol. *102*, 2112–2120.

Cromwell, H.C., and Berridge, K.C. (1996). Implementation of action sequences by a

    neostriatal site: a lesion mapping study of grooming syntax. J. Neurosci. *16*, 3444–3458.

Darshan, R., Leblois, A., and Hansel, D. (2014). Interference and Shaping in Sensorimotor Adaptations with Rewards. PLoS Comput Biol *10*, e1003377.

Davidson, P.R., and Wolpert, D.M. (2003). Motor learning and prediction in a variable environment. Curr. Opin. Neurobiol. *13*, 232–237.

Diedrichsen, J., and Kornysheva, K. (2015). Motor skill learning between selection and execution. Trends Cogn. Sci. *19*, 227–233.

Ding, L., and Perkel, D.J. (2004). Long-term potentiation in an avian basal ganglia nucleus essential for vocal learning. J. Neurosci. Off. J. Soc. Neurosci. *24*, 488–494.

Dominici, N., and Lacquaniti, F. (2011). Locomotor Primitives in Newborn Babies and Their Development. Science *334*.

Doupe, A.J., and Konishi, M. (1991). Song-selective auditory circuits in the vocal control system of the zebra finch. Proc. Natl. Acad. Sci. *88*, 11339–11343.

Doupe, A.J., and Kuhl, P.K. (1999). Birdsong and human speech: common themes and mechanisms. Annu. Rev. Neurosci. *22*, 567–631.

Doupe, A., Perkel, D., Reiner, A., and Stern, E. (2005). Birdbrains could teach basal ganglia research a new song. Trends Neurosci. *28*, 353–363.

Doya, K. (2000). Complementary roles of basal ganglia and cerebellum in learning and motor control. Curr. Opin. Neurobiol. *10*, 732–739.

Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., Lehéricy, S., and Benali, H. (2009). Contributions of the basal ganglia and functionally related brain structures to motor learning. Behav. Brain Res. *199*, 61–75.

Duan, C.A., Erlich, J.C., and Brody, C.D. (2015). Requirement of Prefrontal and Midbrain

    Regions for Rapid Executive Control of Behavior in the Rat. Neuron *86*, 1491–1503.

Dudai, Y. (2004). The Neurobiology of Consolidations, Or, How Stable is the Engram? Annu.

    Rev. Psychol. *55*, 51–86.

Dudman, J.T., and Krakauer, J.W. (2016). The basal ganglia: from motor commands to the

    control of vigor. Curr. Opin. Neurobiol. *37*, 158–166.

Engel, K.C., Flanders, M., and Soechting, J.F. (1997). Anticipatory and sequential motor

    control in piano playing. Exp. Brain Res. *113*, 189–199.

Fee, M.S., and Goldberg, J.H. (2011). A hypothesis for basal ganglia-dependent

    reinforcement learning in the songbird. Neuroscience *198*, 152–170.

Forssberg, H., GRILLNER, S., and ROSSIGNOL, S. (1975). Phase dependent reflex reversal

    during walking in chronic spinal cats. Brain Res. *85*, 103–107.

Fujimoto, H., Hasegawa, T., and Watanabe, D. (2011). Neural Coding of Syntactic Structure

    in Learned Vocalizations in the Songbird. J. Neurosci. *31*, 10023–10033.

Fusi, S., Drew, P.J., and Abbott, L.F. (2005). Cascade Models of Synaptically Stored

    Memories. Neuron *45*, 599–611.

Gadagkar, V., Puzerey, P.A., Chen, R., Baird-Daniel, E., Farhang, A.R., and Goldberg, J.H.

    (2016). Dopamine neurons encode performance error in singing birds. Science *354*,

    1278–1282.

Gandolfo, F., Mussa-Ivaldi, F.A., and Bizzi, E. (1996). Motor learning by field approximation.

    Proc. Natl. Acad. Sci. *93*, 3843–3846.

Ganguly, K., and Carmena, J.M. (2010). Neural Correlates of Skill Acquisition with a Cortical Brain–Machine Interface. J. Mot. Behav. *42*, 355–360.

Garcia, J., and Koelling, R.A. (1966). Relation of cue to consequence in avoidance learning. Psychon. Sci. *4*, 123–124.

Gershman, S.J. (2017). Context-dependent learning and causal structure. Psychon. Bull. Rev. *24*, 557–565.

Goldberg, J.H., and Fee, M.S. (2012). A cortical motor nucleus drives the basal ganglia-recipient thalamus in singing birds. Nat. Neurosci. *15*, 620–627.

Graybiel, A.M. (1995). Building action repertoires: memory and learning functions of the basal ganglia. Curr. Opin. Neurobiol. *5*, 733–741.

Grillner, S., Robertson, B., and Stephenson-Jones, M. (2013). The evolutionary origin of the vertebrate basal ganglia and its role in action selection: The selection of motor programmes. J. Physiol. *591*, 5425–5431.

Gulati, T., Guo, L., Ramanathan, D.S., Bodepudi, A., and Ganguly, K. (2017). Neural reactivations during sleep determine network credit assignment. Nat. Neurosci. *20*, 1277–1284.

Hahnloser, R.H.R., Kozhevnikov, A.A., and Fee, M.S. (2002). An ultra-sparse code underliesthe generation of neural sequences in a songbird. Nature *419*, 65–70.

Hahnloser, R.H.R., Kozhevnikov, A.A., and Fee, M.S. (2006). Sleep-Related Neural Activity in a Premotor and a Basal-Ganglia Pathway of the Songbird. J. Neurophysiol. *96*, 794–812.

Hampton, C.M., Sakata, J.T., and Brainard, M.S. (2009). An Avian Basal Ganglia-Forebrain Circuit Contributes Differentially to Syllable Versus Sequence Variability of Adult Bengalese Finch Song. J. Neurophysiol. *101*, 3235–3245.

Harris-Warrick, R.M., and Marder, E. (1991). Modulation of neural networks for behavior. Annu. Rev. Neurosci. *14*, 39–57.

Hélie, S., Ell, S.W., and Ashby, F.G. (2015). Learning robust cortico-cortical associations with the basal ganglia: An integrative review. Cortex *64*, 123–135.

Herman, J.P., Harwood, M.R., and Wallman, J. (2009). Saccade Adaptation Specific to Visual Context. J. Neurophysiol. *101*, 1713–1721.

Herzfeld, D., and Shadmehr, R. (2014). A memory of errors in sensorimotor learning. Science *345*.

Hessler, N.A., and Doupe, A.J. (1999). Social context modulates singing-related neural activity in the songbird forebrain. Nat. Neurosci. *2*, 209–211.

Hickok, G. (2012). Computational neuroanatomy of speech production. Nat. Rev. Neurosci. *13*, 135.

Hikosaka, O., Nakahara, H., Rand, M.K., Sakai, K., Lu, X., Nakamura, K., Miyachi, S., and Doya, K. (1999). Parallel neural networks for learning sequential procedures. Trends Neurosci. *22*, 464–471.

Hikosaka, O., Nakamura, K., and Nakahara, H. (2006). Basal Ganglia Orient Eyes to Reward. J. Neurophysiol. *95*, 567–584.

Hilario, M., Holloway, T., Jin, X., and Costa, R.M. (2012). Different dorsal striatum circuits mediate action discrimination and action generalization. Eur. J. Neurosci. *35*, 1105–1114.

Hisey, E., Kearney, M.G., and Mooney, R. (2018). A common neural circuit mechanism for internally guided and externally reinforced forms of motor learning. Nat. Neurosci. *21*, 589–597.

Hoffmann, L.A., and Sober, S.J. (2014). Vocal Generalization Depends on Gesture Identity and Sequence. J. Neurosci. *34*, 5564–5574.

Hoffmann, L.A., Saravanan, V., Wood, A.N., He, L., and Sober, S.J. (2016). Dopaminergic Contributions to Vocal Learning. J. Neurosci. *36*, 2176–2189.

Hosino, T., and Okanoya, K. (2000). Lesion of a higher-order song nucleus disrupts phrase level complexity in Bengalese finches. Neuroreport *11*, 2091–2095.

Houde, J.F., and Jordan, M.I. (1998). Sensorimotor Adaptation in Speech Production. Science *279*, 1213–1216.

Houk, J.C., and Wise, S.P. (1995). Distributed modular architectures linking basal ganglia, cerebellum, and cerebral cortex: their role in planning and controlling action. Cereb. Cortex *5*, 95–110.

Howard, I.S., and Franklin, D.W. (2015). Neural Tuning Functions Underlie Both Generalization and Interference. PLOS ONE *10*, e0131268.

Howard, I.S., Ingram, J.N., and Wolpert, D.M. (2011). Separate representations of dynamics in rhythmic and discrete movements: evidence from motor learning. J. Neurophysiol. *105*, 1722–1731.

Howard, I.S., Ingram, J.N., Franklin, D.W., and Wolpert, D.M. (2012). Gone in 0.6 Seconds: The Encoding of Motor Memories Depends on Recent Sensorimotor States. J. Neurosci. *32*, 12756–12768.

Howard, I.S., Wolpert, D.M., and Franklin, D.W. (2013). The effect of contextual cues on the encoding of motor memories. J. Neurophysiol. *109*, 2632–2644.

Huang, V.S., Haith, A., Mazzoni, P., and Krakauer, J.W. (2011). Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. Neuron *70*, 787–801.

Ikegami, T., Hirashima, M., Taga, G., and Nozaki, D. (2010). Asymmetric Transfer of Visuomotor Learning between Discrete and Rhythmic Movements. J. Neurosci. *30*, 4515–4521.

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

Izawa, J., and Shadmehr, R. (2011). Learning from Sensory and Reward Prediction Errors during Motor Adaptation. PLOS Comput Biol *7*, e1002012.

Jerde, T.E., Soechting, J.F., and Flanders, M. (2003). Coarticulation in fluent fingerspelling. J. Neurosci. *23*, 2383–2393.

Jin, D.Z., and Kozhevnikov, A.A. (2011). A Compact Statistical Model of the Song Syntax in Bengalese Finch. PLoS Comput Biol *7*, e1001108.

Johnson, F., and Bottjer, S.W. (1994). Afferent influences on cell death and birth during development of a cortical nucleus necessary for learned vocal behavior in zebra finches. Development *120*, 13–24.

Johnson, F., Hohmann, S.E., DiStefano, P.S., and Bottjer, S.W. (1997). Neurotrophins suppress apoptosis induced by deafferentation of an avian motor-cortical region. J. Neurosci. *17*, 2101–2111.

Jordan, M. (1986). Serial order: A parallel distributed processing approach. ICS Rep. 8604.

Kao, M.H., Doupe, A.J., and Brainard, M.S. (2005). Contributions of an avian basal ganglia–forebrain circuit to real-time modulation of song. Nature *433*, 638–643.

Kao, M.H., Wright, B.D., and Doupe, A.J. (2008). Neurons in a Forebrain Nucleus Required for Vocal Plasticity Rapidly Switch between Precise Firing and Variable Bursting Depending on Social Context. J. Neurosci. *28*, 13232–13247.

Katahira, K., Suzuki, K., Okanoya, K., and Okada, M. (2011). Complex Sequencing Rules of Birdsong Can be Explained by Simple Hidden Markov Processes. PLoS ONE *6*, e24516.

Kawai, R., Markman, T., Poddar, R., Ko, R., Fantana, A.L., Dhawale, A.K., Kampff, A.R., and Ölveczky, B.P. (2015). Motor Cortex Is Required for Learning but Not for Executing a Motor Skill. Neuron *86*, 800–812.

Kim, H.F., and Hikosaka, O. (2013). Distinct Basal Ganglia Circuits Controlling Behaviors Guided by Flexible and Stable Values. Neuron *79*, 1001–1010.

Kimpo, R.R., Theunissen, F.E., and Doupe, A.J. (2003). Propagation of correlated activity through multiple stages of a neural circuit. J. Neurosci. *23*, 5750–5761.

Kimura, M. (1990). Behaviorally contingent property of movement-related activity of the primate putamen. J. Neurophysiol. *63*, 1277–1296.

Kittelberger, J.M., and Mooney, R. (1999). Lesions of an Avian Forebrain Nucleus That Disrupt Song Development Alter Synaptic Connectivity and Transmission in the Vocal Premotor Pathway. J. Neurosci. *19*, 9385–9398.

Kittelberger, J.M., and Mooney, R. (2005). Acute injections of brain-derived neurotrophic factor in a vocal premotor nucleus reversibly disrupt adult birdsong stability and trigger syllable deletion. J. Neurobiol. *62*, 406–424.

Kojima, S., Kao, M.H., Doupe, A.J., and Brainard, M.S. (2018). The Avian Basal Ganglia Are a Source of Rapid Behavioral Variation That Enables Vocal Motor Exploration. J. Neurosci. *38*, 9635–9647.

Konishi, M. (1965). The Role of Auditory Feedback in the Control of Vocalization in the White-Crowned Sparrow 1. Z. Für Tierpsychol. *22*, 770–783.

Koralek, A.C., Costa, R.M., and Carmena, J.M. (2013). Temporally Precise Cell-Specific Coherence Develops in Corticostriatal Networks during Learning. Neuron *79*, 865–872.

Korenberg, A., and Ghahramani, Z. (2002). A Bayesian view of motor adaptation. Curr. Psychol. Cogn. *21*.

Kozhevnikov, A.A., and Fee, M.S. (2007). Singing-Related Activity of Identified HVC Neurons in the Zebra Finch. J. Neurophysiol. *97*, 4271–4283.

Krakauer, J.W., Mazzoni, P., Ghazizadeh, A., Ravindran, R., and Shadmehr, R. (2006). Generalization of Motor Learning Depends on the History of Prior Action. PLoS Biol *4*, e316.

Kumar, A., Rotter, S., and Aertsen, A. (2010). Spiking activity propagation in neuronal networks: reconciling different perspectives on neural coding. Nat. Rev. Neurosci. *11*, 615–627.

Lashley, K.S. (1951). The problem of serial order in behavior (Bobbs-Merrill).

Leonardo, A., and Fee, M.S. (2005). Ensemble Coding of Vocal Control in Birdsong. J. Neurosci. *25*, 652–661.

Leonardo, A., and Konishi, M. (1999). Decrystallization of adult birdsong by perturbation of auditory feedback. Nature *399*, 466–470.

Lewis, R.F. (2003). Context-dependent adaptation of visually-guided arm movements and vestibular eye movements: role of the cerebellum. The Cerebellum *2*, 123–130.

Lidsky, T.I., Manetto, C., and Schneider, J.S. (1985). A consideration of sensory factors involved in motor functions of the basal ganglia. Brain Res. Rev. *9*, 133–146.

Lindefors, N., Amberg, G., and Ungerstedt, U. (1989). Intracerebral Microdialysis: I. Experimental Studies of Diffusion Kinetics. J. Pharmacol. Methods *22*, 141–156.

Liu, W., and Nottebohm, F. (2004). Juvenile zebra finches can use multiple strategies to learn the same song. PNAS *101*.

Long, M.A., and Fee, M.S. (2008). Using temperature to analyse temporal dynamics in the songbird motor pathway. Nature *456*, 189–194.

Long, M.A., Katlowitz, K.A., Svirsky, M.A., Clary, R.C., Byun, T.M., Majaj, N., Oya, H., Howard, M.A., and Greenlee, J.D.W. (2016). Functional Segregation of Cortical Regions Underlying Speech Timing and Articulation. Neuron *89*, 1187–1193.

Lu, X., and Ashe, J. (2005). Anticipatory Activity in Primary Motor Cortex Codes Memorized Movement Sequences. Neuron *45*, 967–973.

MacAskill, M.R., Anderson, T.J., and Jones, R.D. (2002). Adaptive modification of saccade amplitude in Parkinson's disease. Brain *125*, 1570–1582.

MacKay, D.G. (1982). The problems of flexibility, fluency, and speed–accuracy trade-off in skilled behavior. Psychol. Rev. *89*, 483–506.

Mackevicius, E.L., and Fee, M.S. (2018). Building a state space for song learning. Curr. Opin. Neurobiol. *49*, 59–68.

Makino, H., Hwang, E.J., Hedrick, N.G., and Komiyama, T. (2016). Circuit Mechanisms of Sensorimotor Learning. Neuron *92*, 705–721.

Mannella, F., and Baldassarre, G. (2015). Selection of cortical dynamics for motor behaviour by the basal ganglia. Biol. Cybern. *109*, 575–595.

Margoliash, D., and Yu, A.C. (1996). Temporal Heirarchical Control of Singing in Birds.

Markowitz, J.E., Gillis, W.F., Beron, C.C., Neufeld, S.Q., Robertson, K., Bhagat, N.D., Peterson, R.E., Peterson, E., Hyun, M., Linderman, S.W., et al. (2018). The Striatum Organizes 3D Behavior via Moment-to-Moment Action Selection. Cell *174*, 44–58.e17.

Marler, P. A comparative approach to vocal learning: Song development in white-crowned sparrows. J. Comp. Physiol. Psychol. 1970.

Mathis, M.W., Mathis, A., and Uchida, N. (2017). Somatosensory Cortex Plays an Essential
Role in Forelimb Motor Adaptation in Mice. Neuron *93*, 1493–1503.e6.

Mattar, A.A.G., and Ostry, D.J. (2007). Modifiability of Generalization in Dynamics Learning.
J. Neurophysiol. *98*, 3321–3329.

Maurice, S., Brandt, J., and Shadmehr, R. (2000). Motor disorder in Huntington's disease
begins as a dysfunction in error feedback control. Nature *403*.

Medina, J.F., Nores, W.L., Ohyama, T., and Mauk, M.D. (2000). Mechanisms of cerebellar
learning suggested by eyelid conditioning. Curr. Opin. Neurobiol. *10*, 717–724.

Medina, J.F., Christopher Repa, J., Mauk, M.D., and LeDoux, J.E. (2002). Parallels between
cerebellum- and amygdala-dependent conditioning. Nat. Rev. Neurosci. *3*, 122–131.

Mehaffey, W.H., and Doupe, A.J. (2015). Naturalistic stimulation drives opposing
heterosynaptic plasticity at two inputs to songbird cortex. Nat. Neurosci. *18*, 1272–
1280.

Mello, G.B.M., Soares, S., and Paton, J.J. (2015). A Scalable Population Code for Time in the
Striatum. Curr. Biol. *25*, 1113–1122.

Miller, E.K., and Cohen, J.D. (2001). An integrative theory of prefrontal cortex function.
Annu. Rev. Neurosci. *24*, 167–202.

Miller, M.N., Cheung, C.Y.J., and Brainard, M.S. (2017). Vocal learning promotes patterned
inhibitory connectivity. Nat. Commun. *8*.

Miyachi, S., Hikosaka, O., Miyashita, K., Kárádi, Z., and Rand, M.K. (1997). Differential roles
of monkey striatum in learning of sequential hand movement. Exp. Brain Res. *115*, 1–5.

Mooney, R. (2014). Auditory-vocal mirroring in songbirds. Philos. Trans. R. Soc. B Biol. Sci. *369*, 20130179–20130179.

Mooney, R. (2018). The Song Remains the Same. Trends Neurosci. *41*.

Mooney, R., and Konishi, M. (1991). Two distinct inputs to an avian song nucleus activate different glutamate receptor subtypes on individual neurons. Proc. Natl. Acad. Sci. *88*, 4075–4079.

Mooney, R., and Prather, J.F. (2005). The HVC microcircuit: the synaptic basis for interactions between song motor and vocal plasticity pathways. J. Neurosci. Off. J. Soc. Neurosci. *25*, 1952–1964.

Morehead, J.R., Qasim, S.E., Crossley, M.J., and Ivry, R. (2015). Savings upon Re-Aiming in Visuomotor Adaptation. J. Neurosci. *35*, 14386–14396.

Morrison, R.G., and Nottebohm, F. (1993). Role of a telencephalic nucleus in the delayed song learning of socially isolated zebra finches. J. Neurobiol. *24*, 1045–1064.

Mowrey, R.A., and MacKay, I.R.A. (1990). Phonological primitives: Electromyographic speech error evidence. J. Acoust. Soc. Am. *88*, 1299–1312.

Mushiake, H., and Strick, P.L. (1995). Pallidal neuron activity during sequential arm movements. J. Neurophysiol. *74*, 2754–2758.

Nakahara, H., Itoh, H., Kawagoe, R., Takikawa, Y., and Hikosaka, O. (2004). Dopamine Neurons Can Represent Context-Dependent Prediction Error. Neuron *41*, 269–280.

Narayanan, N.S., and Laubach, M. (2006). Top-Down Control of Motor Cortex Ensembles by Dorsomedial Prefrontal Cortex. Neuron *52*, 921–931.

Nieuwenhuis, S., Heslenfeld, D.J., Alting von Geusau, N.J., Mars, R.B., Holroyd, C.B., and
Yeung, N. (2005). Activity in human reward-sensitive brain areas is strongly context
dependent. NeuroImage *25*, 1302–1309.

Nordeen, K.W., and Nordeen, E.J. (1992). Auditory feedback is necessary for the
maintenance of stereotyped song in adult zebra finches. Behav. Neural Biol. *57*, 58–66.

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

Nozaki, D., and Scott, S.H. (2009). Multi-compartment model can explain partial transfer of
learning within the same limb between unimanual and bimanual reaching. Exp. Brain
Res. *194*, 451–463.

Nozaki, D., Kurtzer, I., and Scott, S.H. (2006). Limited transfer of learning between
unimanual and bimanual skills within the same limb. Nat. Neurosci. *9*, 1364–1366.

Okanoya, K. (2004). The Bengalese Finch: A Window on the Behavioral Neurobiology of
Birdsong Syntax. Ann. N. Y. Acad. Sci. *1016*, 724–735.

Okanoya, K., and Yamaguchi, A. (1997). Adult bengalese finches (Lonchura striata var.
domestica) require real-time auditory feedback to produce normal song syntax. J.
Neurobiol. *33*, 343–356.

Okubo, T.S., Mackevicius, E.L., Payne, H.L., Lynch, G.F., and Fee, M.S. (2015). Growth and
splitting of neural sequences in songbird vocal development. Nature *528*, 352–357.

Olveczky, B.P., Andalman, A.S., and Fee, M.S. (2005). Vocal experimentation in the juvenile
songbird requires a basal ganglia circuit. PLoS Biol. *3*, e153.

Orban de Xivry, J.-J., Marko, M.K., Pekny, S.E., Pastor, D., Izawa, J., Celnik, P., and Shadmehr, R. (2011). Stimulation of the Human Motor Cortex Alters Generalization Patterns of Motor Learning. J. Neurosci. *31*, 7102–7110.

Ostry, D.J., Gribble, P.L., and Gracco, V.L. (1996). Coarticulation of jaw movements in speech production: is context sensitivity in speech kinematics centrally planned? J. Neurosci. *16*, 1570–1579.

Osu, R., Hirai, S., Yoshioka, T., and Kawato, M. (2004). Random presentation enables subjects to adapt to two opposing forces on the hand. Nat. Neurosci. *7*, 111–112.

Pasupathy, A., and Miller, E.K. (2005). Different time courses of learning-related activity in the prefrontal cortex and striatum. Nature *433*, 873–876.

Paton, J.J., and Lau, B. (2015). Tread softly and carry a clock's tick. Nat. Neurosci. *18*, 329–330.

Pearson, T.S., Krakauer, J.W., and Mazzoni, P. (2010). Learning Not to Generalize: Modular Adaptation of Visuomotor Gain. J. Neurophysiol. *103*, 2938–2952.

Poggio, T., and Bizzi, E. (2004). Generalization in vision and motor control. Nature *431*, 768.

Price, P.H. (1979). Developmental determinants of structure in zebra finch song. J. Comp. Physiol. Psychol. *93*, 260.

Rochet-Capellan, A., and Ostry, D.J. (2011). Simultaneous Acquisition of Multiple Auditory–Motor Transformations in Speech. J. Neurosci. *31*, 2657–2662.

Rochet-Capellan, A., Richer, L., and Ostry, D.J. (2012). Nonhomogeneous transfer reveals specificity in speech motor learning. J. Neurophysiol. *107*, 1711–1717.

Roemmich, R.T., and Bastian, A.J. (2015). Two ways to save a newly learned motor pattern. J. Neurophysiol. *113*, 3519–3530.

Rueda-Orozco, P.E., and Robbe, D. (2015). The striatum multiplexes contextual and kinematic information to constrain motor habits execution. Nat. Neurosci. *18*, 453–460.

Sakata, J.T., and Brainard, M.S. (2006). Real-Time Contributions of Auditory Feedback to Avian Vocal Motor Control. J. Neurosci. *26*, 9619–9628.

Samejima, K., Ueda, Y., Doya, K., and Kimura, M. (2005). Representation of Action-Specific Reward Values in the Striatum. Science *310*, 1337–1340.

Sawada, M., and Nishimura, Y. (2015). Function of the nucleus accumbens in motor control during recovery after spinal cord injury. Science *350*.

Scharff, C., and Nottebohm, F. (1991). A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. J. Neurosci. *11*, 2896–2913.

Schmidt, M.F., and Wild, J.M. (2014). The respiratory-vocal system of songbirds: Anatomy, physiology, and neural control. Prog. Brain Res. *212*, 297–335.

Schultz, W., Dayan, P., and Montague, P.R. (1997). A Neural Substrate of Prediction and Reward. Science *275*.

Shadmehr, R., and Holcomb, H.H. (1997). Neural correlates of motor memory consolidation. Science *277*, 821–825.

Shadmehr, R., and Mussa-Ivaldi, F.A. (1994). Adaptive representation of dynamics during learning of a motor task. J. Neurosci. *14*, 3208–3224.

Shah, A., Barto, A.G., and Fagg, A.H. (2013). A dual process account of coarticulation in motor skill acquisition. J. Mot. Behav. *45*, 531–549.

Shallice, T.I.M., and Burgess, P.W. (1991). Deficits in strategy application following frontal lobe damage in man. Brain *114*, 727–741.

Shine, J.M., and Shine, R. (2014). Delegation to automaticity: the driving force for cognitive evolution? Front. Neurosci. *8*.

Shmuelof, L., and Krakauer, J.W. (2011). Are We Ready for a Natural History of Motor Learning? Neuron *72*, 469–476.

Shmuelof, L., Huang, V.S., Haith, A.M., Delnicki, R.J., Mazzoni, P., and Krakauer, J.W. (2012). Overcoming Motor "Forgetting" Through Reinforcement Of Learned Actions. J. Neurosci. *32*, 14617–14621a.

Simpson, H.B., and Vicario, D.S. (1990). Brain pathways for learned and unlearned vocalizations differ in zebra finches. J. Neurosci. *10*, 1541–1556.

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

Sober, S.J., Wohlgemuth, M.J., and Brainard, M.S. (2008). Central Contributions to Acoustic Variation in Birdsong. J. Neurosci. *28*, 10370–10379.

Sohrabji, F., Nordeen, E.J., and Nordeen, K.W. (1990). Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav. Neural Biol. *53*, 51–63.

Sosnik, R., Hauptmann, B., Karni, A., and Flash, T. (2004). When practice leads to co-articulation: the evolution of geometrically defined movement primitives. Exp. Brain Res. *156*, 422–438.

Spiro, J.E., Dalva, M.B., and Mooney, R. (1999). Long-range inhibition within the zebra finch song nucleus RA can coordinate the firing of multiple projection neurons. J. Neurophysiol. *81*, 3007–3020.

Sternberg, S., and Wright, C. (1978). The Latency and Duration of Rapid Movement Sequences: Comparisons of Speech and Typewriting.

Sutton, R., and Barto, A. (1998). Reinforcement Learning: An Introduction (MIT Press).

Tan, H., Zavala, B., Pogosyan, A., Ashkan, K., Zrinzo, L., Foltynie, T., Limousin, P., and Brown, P. (2014). Human Subthalamic Nucleus in Movement Error Detection and Its Evaluation during Visuomotor Adaptation. J. Neurosci. *34*, 16744–16754.

Tanaka, H., Sejnowski, T.J., and Krakauer, J.W. (2009). Adaptation to Visuomotor Rotation Through Interaction Between Posterior Parietal and Motor Cortical Areas. J. Neurophysiol. *102*, 2921–2932.

Tanji, J., and Shima, K. (1994). Role for supplementary motor area cells in planning several movements ahead. Nature *371*, 413–416.

Taylor, J.A., and Ivry, R.B. (2013). Context-dependent generalization. Front. Hum. Neurosci. *7*.

Taylor, J.A., and Ivry, R.B. (2014). Cerebellar and Prefrontal Cortex Contributions to

    Adaptation, Strategies, and Reinforcement Learning. In Progress in Brain Research,

    (Elsevier), pp. 217–253.

Tchernichovski, O., Mitra, P.P., Lints, T., and Nottebohm, F. (2001). Dynamics of the Vocal

    Imitation Process: How a Zebra Finch Learns Its Song. Science *291*, 2564–2569.

Thoroughman, K.A., and Taylor, J.A. (2005). Rapid Reshaping of Human Motor

    Generalization. J. Neurosci. *25*, 8948–8953.

Thorpe, W.H. (1958). The learning of song patterns by birds, with especial reference to the

    song of the chaffinch Fringilla coelebs. Ibis *100*, 535–570.

Todorov, E., and Jordan, M.I. (2002). Optimal feedback control as a theory of motor

    coordination. Nat. Neurosci. *5*, 1226–1235.

Tumer, E.C., and Brainard, M.S. (2007). Performance variability enables adaptive plasticity

    of "crystallized" adult birdsong. Nature *450*, 1240–1244.

Turner, R.S., and Anderson, M.E. (2005). Context-Dependent Modulation of Movement-

    Related Discharge in the Primate Globus Pallidus. J. Neurosci. *25*, 2965–2976.

Turner, R.S., and Desmurget, M. (2010). Basal ganglia contributions to motor control: a

    vigorous tutor. Curr. Opin. Neurobiol. *20*, 704–716.

Vandecasteele, M., M., S., Royer, S., Belluscio, M., Berényi, A., Diba, K., Fujisawa, S., Grosmark,

    A., Mao, D., Mizuseki, K., et al. (2012). Large-scale Recording of Neurons by Movable

    Silicon Probes in Behaving Rodents. J. Vis. Exp.

Vates, G.E., Vicario, D.S., and Nottebohm, F. (1997). Reafferent thalamo-"cortical" loops in

    the song system of oscine songbirds. J. Comp. Neurol. *380*, 275–290.

Vu, E.T., Mazurek, M.E., and Kuo, Y.C. (1994). Identification of a forebrain motor

    programming network for the learned song of zebra finches. J. Neurosci. *14*, 6924–6934.

Wainscott, S.K., Donchin, O., and Shadmehr, R. (2004). Internal Models and Contextual Cues:

    Encoding Serial Order and Direction of Movement. J. Neurophysiol. *93*, 786–800.

Warren, T.L., Tumer, E.C., Charlesworth, J.D., and Brainard, M.S. (2011). Mechanisms and

    time course of vocal learning and consolidation in the adult songbird. J. Neurophysiol.

    106, 1806–1821.

Warren, T.L., Charlesworth, J.D., Tumer, E.C., and Brainard, M.S. (2012). Variable

    Sequencing Is Actively Maintained in a Well Learned Motor Skill. J. Neurosci. *32*, 15414–

    15425.

Welch, R.B., Bridgeman, B., Anand, S., and Browman, K.E. (1993). Alternating prism

    exposure causes dual adaptation and generalization to a novel displacement. Percept.

    Psychophys. *54*, 195–204.

Wickens, J.R., Reynolds, J.N., and Hyland, B.I. (2003). Neural mechanisms of reward-related

    motor learning. Curr. Opin. Neurobiol. *13*, 685–690.

Williams, H., and Mehta, N. (1999). Changes in adult zebra finch song require a forebrain

    nucleus that is not necessary for song production. J. Neurobiol. *39*, 14–28.

Wise, S.P., and Murray, E.A. (2000). Arbitrary associations between antecedents and

    actions. Trends Neurosci. *23*, 271–276.

Witt, K., Margraf, N., Bieber, C., Born, J., and Deuschl, G. (2010). Sleep consolidates the effector-independent representation of a motor skill. Neuroscience *171*, 227–234.

Wohlgemuth, M.J., Sober, S.J., and Brainard, M.S. (2010). Linked Control of Syllable Sequence and Phonology in Birdsong. J. Neurosci. *30*, 12936–12949.

Wolpert, D.M., and Kawato, M. (1998). Multiple paired forward and inverse models for motor control. Neural Netw. *11*, 1317–1329.

Wolpert, D.M., Ghahramani, Z., and Flanagan, J.R. (2001). Perspectives and problems in motor learning. Trends Cogn. Sci. *5*, 487–494.

Woolley, S.M., and Rubel, E.W. (1997). Bengalese finches Lonchura striata domestica depend upon auditory feedback for the maintenance of adult song. J. Neurosci. *17*, 6380–6390.

Woolley, S.C., Rajan, R., Joshua, M., and Doupe, A.J. (2014). Emergence of Context-Dependent Variability across a Basal Ganglia Network. Neuron *82*, 208–223.

Xiao, L., Chattree, G., Oscos, F.G., Cao, M., Wanat, M.J., and Roberts, T.F. (2018). A Basal Ganglia Circuit Sufficient to Guide Birdsong Learning. Neuron *98*, 208–221.e5.

Xiong, Q., Znamenskiy, P., and Zador, A.M. (2015). Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature *521*, 348–351.

Yin, H.H., Knowlton, B.J., and Balleine, B.W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur. J. Neurosci. *19*, 181–189.

Yin, H.H., Mulcare, S.P., Hilário, M.R.F., Clouse, E., Holloway, T., Davis, M.I., Hansson, A.C., Lovinger, D.M., and Costa, R.M. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat. Neurosci. *12*, 333–341.

Zach, N., Inbar, D., Grinvald, Y., and Vaadia, E. (2012). Single Neurons in M1 and Premotor Cortex Directly Reflect Behavioral Interference. PLoS ONE *7*, e32986.

Zandvakili, A., and Kohn, A. (2015). Coordinated Neuronal Activity Enhances Corticocortical Communication. Neuron *87*, 827–839.

Zhang, Y.S., Wittenbach, J.D., Jin, D.Z., and Kozhevnikov, A.A. (2017). Temperature Manipulation in Songbird Brain Implicates the Premotor Nucleus HVC in Birdsong Syntax. J. Neurosci. *37*, 2600–2611.

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