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Learning-dependent processing of natural communication sounds in single neurons and neural populations

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Neurosciences
with Specialization in Computational Neurosciences

by

James McClure Jeanne

Committee in charge:

Professor Timothy Q. Gentner, Chair
Professor Tatyana O. Sharpee, Co-Chair
Professor William B. Kristan, Jr.
Professor Pamela Reinagel
Professor John H. Reynolds

2012

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Co-Chair

Chair

University of California, San Diego

2012

DEDICATION

I dedicate this dissertation to my family and friends. Thank you for your support and encouragement.

EPIGRAPH

Nothing in biology makes sense
except in the light of evolution

Theodosius Dobzhansky

It is not the critic who counts; not the man who points out how the strong man stumbles, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly; who errs, who comes short again and again, because there is no effort without error and shortcoming; but who does actually strive to do the deeds; who knows great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and who at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who neither know victory nor defeat.

Theodore Roosevelt
Speech at the Sorbonne
Paris, France
April 23, 1910

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PUBLICATIONS

- Jeanne, J.M., Sharpee, T.O., Gentner, T.Q., Learning alters neural encoding of natural auditory signals in relation to their informativeness for behavior. In preparation.
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FIELDS OF STUDY

Major Field: Neurosciences (specialization in Computational Neurosciences)

ABSTRACT OF THE DISSERTATION

Learning-dependent processing of natural communication sounds in single neurons and neural populations

by

James McClure Jeanne

Doctor of Philosophy in Neurosciences, with Specialization in Computational Neuroscience

University of California, San Diego, 2012

Professor Timothy Q. Gentner, Chair
Professor Tatyana O. Sharpee, Co-Chair

The ability to learn to recognize new sensory signals such as voices or faces is an important cognitive function in many species. This ability is thought to involve the plasticity of neural representations in high-level sensory cortical areas, but this plasticity is poorly understood. Using European starlings (a species of songbird) trained to recognize natural conspecific song segments, I investigated the emergence of neural representations for learned signals across two auditory forebrain regions: the caudolateral mesopallium (CLM) and the caudomedial mesopallium (CMM). In both CLM and CMM, neurons encoded more information about the motifs (short, stereotyped segments of song) that make up songs paired with reward during training than the motifs that make up novel songs. This shows that behavioral experience is an important modulator of neural encoding in the songbird auditory forebrain.

In the natural world, individuals learn which signals convey relevant information for particular behaviors. However, it is unknown how this behavioral information influences neural encoding in the brain. I explored this by training starlings on a paired-motif recognition task where one motif was informative about the behavior required to obtain reward and the other motif was not informative. Following training, single neurons in CLM responded more strongly to informative motifs than to uninformative or novel

motifs, whereas single neurons in CMM responded strongly to both informative and uninformative motifs. This suggests that encoding in CLM may serve to emphasize those signals that are particularly behaviorally relevant.

Sensory encoding in cortical areas is distributed across many neurons. But how learning alters these neural population representations remains unexplored. To explore this question, I analyzed the correlated activity of simultaneously recorded neurons within CLM. When processing informative motifs, the correlations led to enhanced population discriminability, relative to the correlations when processing uninformative or novel motifs. Thus, the information that a sensory signal conveys about behavior modulates neural encoding in both single neurons and in neural populations. Collectively, these studies demonstrate that behavioral relevance substantially influences neural processing by both single neurons and larger populations in cortical brain regions.

I. Introduction

Learning and the brain

Behavior is flexible. Perhaps one of the most important survival strategies is the ability of individuals to adapt to changing conditions. Many animals are capable of gathering information about the world, organizing it in a meaningful way, and calling upon it to change their behavior, a process known as learning. For instance, the marine mollusk *Aplysia californica* exhibits a gill-withdrawal reflex triggered by touching the siphon (a tube-like appendage used for feeding). With repeated stimulation of the siphon, however, the magnitude of the gill withdrawal decreases in a form of learning known as habituation (Pinsker et al., 1970). American Crows (*Corvus brachyrhynchos*) crack nuts open by dropping them from the air, and can learn that harder walnut species need to be dropped from higher altitude than softer walnut species and harder ground surfaces require shorter drops than softer ground surfaces (Cristol and Switzer, 1999). Humans (*Homo sapiens*) learn a large set of vocalizations known as speech that enable a remarkably complex ability to communicate (Kuhl, 2004). This ability of organisms to learn to alter their behavior in meaningful ways requires changes in the structure and function of the nervous system, but the nature of these changes are only just beginning to be elucidated.

Learning to make associations between sensory signals and behavioral outcomes leads to functional changes in multiple areas of the brain. In owl monkeys trained to distinguish between tones of similar frequency, the region of auditory cortex that best encodes the training frequencies expands in proportion with discrimination ability (Recanzone et al., 1993). This large-scale effect of learning on the topographic organization of the auditory cortex (known as a tonotopic map) must result from changes in the frequency encoding properties of individual neurons. Indeed, after less than an hour of training to associate a tone of a particular frequency with a noxious stimulus such as a foot shock alters the neural responses of single auditory cortical neurons specifically at the learned frequency (Diamond and Weinberger, 1986; Bakin and Weinberger, 1990). Similar findings have been reported across many sensory modalities, including somatosensation (Jenkins et al., 1990; Recanzone et al., 1992), vision (Rainer and Miller, 2000;

Schoups et al., 2001; Rainer et al., 2004), and olfaction (Brennan and Keverne, 1997). These studies demonstrate that neural processing of sensory signals in the cortex is highly dynamic and corresponds directly to the animal's recent behavioral experience. Importantly, these changes in the cortical representation are not merely corollaries to learning: they in fact have a causal influence on behavior. After inducing expansions of the tonotopic representation of certain frequencies in the auditory cortex in rats, their ability to learn to discriminate nearby frequencies increased substantially, compared to control rats with no tonotopic map expansions (Reed et al., 2011). The plasticity of neural representations in the cortex is therefore an important feature of the brain that subserves an animal's ability to learn new associations and subsequently change its behavior.

Investigations into the neurochemical basis of cortical plasticity have revealed that neuromodulators play an important role in reshaping neural receptive fields. In particular, acetylcholine (ACh) can alter the responses of single cortical neurons in primary auditory cortex (Metherate and Weinberger, 1989), and lesions to the cholinergic nucleus basalis disrupt rats' ability to learn a discrimination task (Butt and Hodge, 1995). Consistently, pairing acoustic stimuli with stimulation of the nucleus basalis (resulting in ACh release into the cortex) leads to an expansion of the cortical representation of the paired frequencies, suggesting a critical role for ACh in shaping experience-dependent organization of cortical function (Kilgard and Merzenich, 1998). Similarly, dopamine release from the ventral tegmental area (VTA), which is associated with discrepancies in the prediction of reward, is thought to play an important role in cortical plasticity since errors in reward prediction are an important signal in learning (Hollerman and Schultz, 1998). In parallel with the effects of stimulating nucleus basalis, when stimulation of the VTA is paired with presentation of an acoustic tone, the area of cortical representation for that tone in the auditory cortex increases (Bao et al., 2001). Collectively, these results suggest that the cortical plasticity that underlies learning depends not only on behavioral experience, but also on the coordinated action of multiple neuromodulatory signals from multiple subcortical brain regions.

In addition to the role of neuromodulators, several cellular and sub-cellular mechanisms are thought to underlie this functional plasticity of cortical circuitry. Synaptic plasticity, including long-term potentiation (LTP, an increase in synaptic strength) and long-term depression (LTD, a decrease in synaptic

strength), in the amygdala and hippocampus is required for certain forms of learning (e.g. fear memories) (Maren, 2005; Feldman, 2009). Similar mechanisms appear to mediate the learning-driven changes to receptive fields of primary visual cortical neurons (Dan and Poo, 2006), and likely occur in other sensory cortical areas as well (Feldman, 2009). Excitatory synaptic transmission typically occurs at specialized protrusions along the dendrite known as dendritic spines. A recent study in juvenile zebra finches demonstrates that learning to sing is accompanied by an increase in the number and size of dendritic spines in the sensorimotor cortical area HVC, a region known to be involved in the production of song (Roberts et al., 2010). Importantly, both acetylcholine (Patil et al., 1998) and dopamine (Otani et al., 2003) have a powerful influence on long-term synaptic plasticity, meaning that synaptic mechanisms are well-poised to subserve the ability of animals to learn new associations and behaviors.

Learning in Songbirds

The songbirds (suborder Passeri of the order Passeriformes) form the most diverse group of birds, with more than 4500 known species around the world (Perrins, 2009). Songbirds are particularly notable in that their vocalizations are learned rather than innate, a trait shared only with cetaceans, bats, parrots, hummingbirds, and humans (Doupe and Kuhl, 1999). Vocal learning in songbirds has been explored extensively since early observations that juvenile chaffinches (*Fringilla coelebs*) raised in isolation did not develop normal songs (Thorpe, 1954, 1958). The process of song learning is highly variable from species to species, but tends to proceed through several stages. During the first stage, known as the memorization stage, juvenile birds actively listen to the songs of their tutor (often their father) and memorize this song into what is called a “template.” During the second stage, known as the sensorimotor stage, juveniles first produce rambling, inconsistently structured, and highly variable vocalizations known as “subsong.” Gradually, the juvenile begins to imitate the tutor’s song, and produces progressively more stable and consistent vocalizations known as “plastic song”. The final stage, known as crystallization, occurs when the variability of the plastic song decreases and the song becomes highly stable and stereotyped (Hultsch and Todt, 2008). In some species, such as the zebra finch (*Taeniopygia guttata*) and white-crowned sparrow (*Zonotrichia leucophrys*), the song remains crystallized throughout life; these species are known as closed-

ended learners. In other species, such as the European starling (*Sturnus vulgaris*) studied here, new song material can be added in subsequent years; these species are known as open-ended learners (Beecher and Brenowitz, 2005). To date, song learning in juvenile songbirds remains one of the most remarkable non-human examples of learning in the natural world, and has become a particularly valuable model for speech and language learning in humans (Doupe and Kuhl, 1999).

In addition to learning to sing their own songs, songbirds also learn to recognize the songs of other individuals. Song playback experiments in the field show that white-throated sparrows (*Zonotrichia albicollis*) make more flight and singing responses to playback of a stranger's song in a neighbor's territory than to playback of the true neighbor's song, demonstrating the ability to distinguish between different individuals based on song (Falls and Brooks, 1975). Furthermore, songbirds form long-lasting memories of their neighbors. In the male hooded warbler (*Wilsonia citrina*), a migratory songbird, specific behavioral responses to neighboring individuals' songs persisted even after 8 months during which birds ceased singing, migrated to, and returned from Central America (Godard, 1991). This recognition ability likely subserves the functions of both mate attraction and territory defense. When broadcasting male songs from a speaker in the field, the number of conspecific males that visit the territory around the speaker is smaller than during control sounds (Yasukawa, 1981) and the number of females that visit the territory is greater than during control sounds (Johnson and Searcy, 1996). These data support the hypothesis that learning to recognize songs of other individuals is an important natural behavior in songbirds that relates to reproductive goals.

Vocal behavior in the European starling

European starlings are a species of songbird known for their complex and variable songs, and their vocal mimicry (Eens, 1997). An early account of the song of the starling described it as "a lively rambling melody of throaty warbling, chirruping, clicking and gurgling notes interspersed with musical whistles and pervaded by a peculiar creaking quality" (Witherby et al., 1943). However, the application of modern spectrographic methods reveals that the song has a remarkably hierarchical structure. A full song lasts from 30 to 90 seconds, and is composed of stereotyped acoustic units called motifs, each lasting about 0.5 to 1.5

seconds. Each motif consists of a combination of individual sounds called notes (Figure 1.1). Starling motifs are classified into four types, whistle motifs, variable motifs, rattle motifs, and high-frequency motifs. Starlings have multiple motifs of each type in their repertoire, and a typical song progresses through singing several motifs from each of the four motif classes in order, with each motif repeated 1-4 times (Adret-Hausberger and Jenkins, 1988; Eens, 1997). Individual starlings have a repertoire consisting of 15 – 70 motifs (Eens et al., 1991). Starlings also mimic the sounds of other birds and environmental sounds, and can even produce utterances that resemble human speech (Eens, 1997). Like other songbirds, starlings raised in isolation produce songs that lack the organization and repertoire size of normally reared individuals (Chaiken et al., 1993). Given the choice, female starlings prefer naturally ordered male songs over reverse ordered songs and longer male songs over shorter songs (Gentner and Hulse, 2000b). Thus it is in a male starling's best interest to learn the natural song organization and to continue to expand his vocal repertoire. Because starlings are open-ended learners (Hultsch and Todt, 2008), expanding the vocal repertoire can occur throughout life and the vocal repertoire size may be an indicator of age and fitness (Chaiken et al., 1993). The ability to learn new sounds is thus a very important aspect of natural starling behavior, both for learning to produce song, and in learning to recognize the songs of other individuals.

Bringing starlings into the laboratory and using operant conditioning procedures enables the investigation of specific perceptual mechanisms that underlie the ability to learn to distinguish between songs of multiple conspecific individuals. For example, in European starlings, this ability derives, in part, from learning repertoire of motifs sung by an individual, as well as learning the particular sequences in which these components are combined (Gentner and Hulse, 1998). Furthermore, the classification performance of individuals based on song is proportional to the fraction of that individual's motifs that are actually present in the song (Gentner and Hulse, 2000a). These experiments demonstrate that the structure of the starling's song is important not just for sexual selection, but for individual recognition as well.

The songbird auditory forebrain

Understanding how learning changes the songbird brain requires an understanding of its functional anatomy. Figure 1.2 shows a schematic view of the major forebrain regions involved in processing auditory signals. Auditory information from the mesencephalicus lateralis dorsalis (MLd), a brainstem nucleus

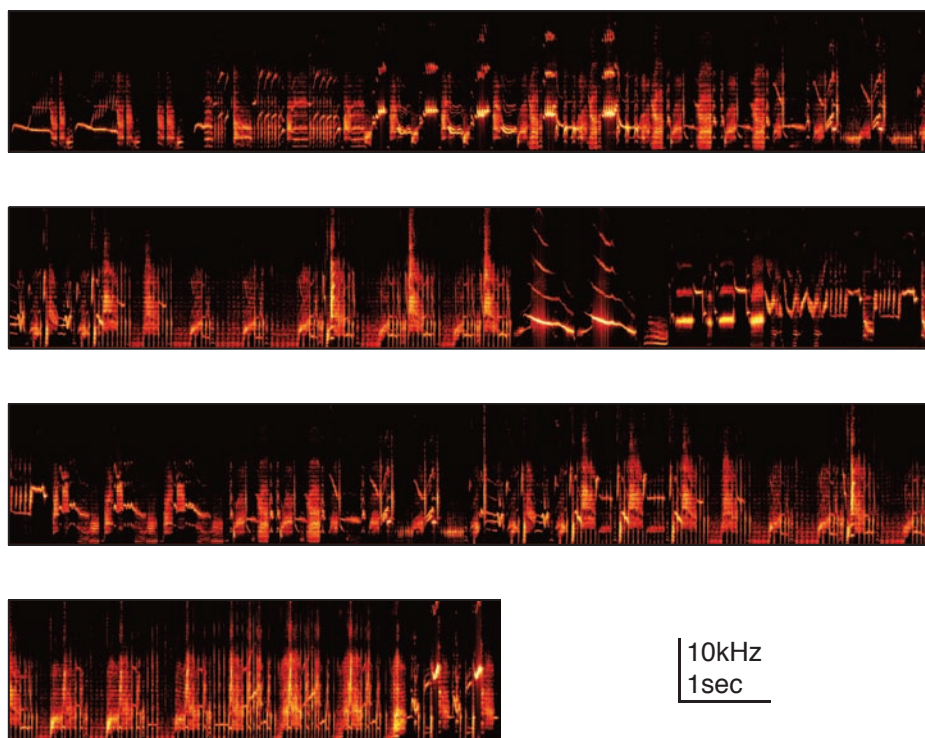


Figure 1.1 Spectrogram of a full starling song. The x-axis is time, and y-axis is frequency. The four panels connect end-to-end.

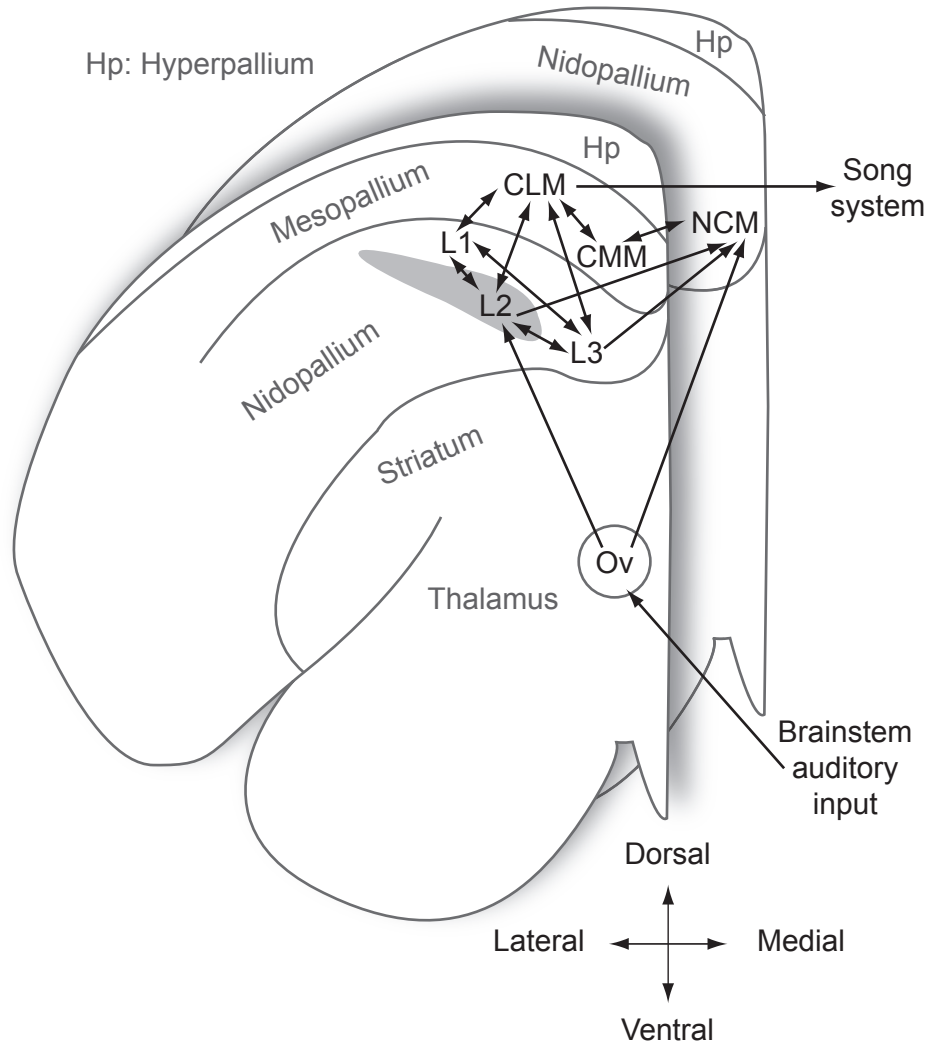


Figure 1.2. Schematic of two coronal sections through starling forebrain auditory areas. Midline is to the right. Arrows denote connectivity. Hp: Hyperpallium; CLM: Caudolateral Mesopallium; CMM: Caudomedial Mesopallium; NCM: Caudomedial Nidopallium; L1, L2, L3: Subregions of Field L; Ov: Ovoidalis.

analogous to inferior colliculus, projects primarily to the nucleus ovoidalis (Ov), the avian equivalent of the medial geniculate body. Ov, in turn, projects to the forebrain region known as field L, analogous to primary auditory cortex (Vates et al., 1996). Field L is divided into several subregions. Field L2a is the primary thalamorecipient region, contains many small granular neurons, and is analogous to layer 4 of mammalian cortex (Wang et al., 2010). L2a projects to both Field L1 and L3, which correspond anatomically to supragranular and subgranular layers, respectively (Wang et al., 2010). All subregions of Field L project to the caudomedial nidopallium (NCM) and the caudolateral mesopallium (CLM), two areas analogous to secondary auditory cortex. Finally, both NCM and CLM project to the caudomedial mesopallium (CMM), another secondary auditory area. No obvious anatomical boundary exists between CLM and CMM, except for the different projection patterns to and from Field L and NCM (Vates et al., 1996). CLM and CMM are collectively referred to as CM. Importantly, nearly all the connectivity in the forebrain (all subregions of field L, CLM, CMM, and NCM) are bidirectional, creating a highly recurrent network of auditory information pathways. One advantage of the songbird auditory system over mammalian systems is that at least some of the projections to non-auditory forebrain regions are well-described and functionally constrained. In particular, CLM projects to HVC, a sensorimotor area known to be involved in the production of song (Bauer et al., 2008). Similarly, Field L1 and L3 both project to the HVC shelf, the area surrounding HVC, and appear to make indirect connections into HVC (Vates et al., 1996). CLM, in particular, may provide auditory feedback into HVC and the song motor system that is critical for juvenile song learning and adult song maintenance (Lei and Mooney, 2010).

The neural encoding of song gets progressively more complex as one ascends this auditory pathway. As noted above, much of the connectivity in the auditory forebrain is reciprocal, yet it remains useful to follow the changes in auditory processing as one ascends from the periphery. In MLd and Ov, neural responses are tightly time-locked to changes in the stimulus, and linear spectrotemporal receptive field (STRF) models predict nearly all the variance in the response, whereas in field L this linearity is reduced, indicating greater complexity of the neural encoding (Woolley et al., 2009; Amin et al., 2010). The responses of CM neurons are even less well modeled by STRF models than most field L neurons (Sen et al., 2001) and show tuning that more strongly matches the spectrotemporal statistics of song (Hsu et al.,

2004). Furthermore, neurons in CMM are tuned specifically to those songs that are familiar (Gentner and Margoliash, 2003). The auditory system of songbirds is thus loosely organized into an ascending hierarchy, along which neurons become increasingly specialized for processing song.

The remarkable behavioral abilities, adaptability to laboratory environments, richness of acoustic repertoire, and well-understood forebrain circuitry collectively make songbirds (and in particular, the European starling) an excellent model for studying the brain mechanisms that underlie complex cognitive functions such as learning. The remaining three sections of this introductory chapter will briefly introduce the specific experiments presented in chapters 2-4 that advance our understanding of how learning modifies the function of cortical neural circuitry.

Learning-Dependent Plasticity of neural encoding in CLM and CMM

Neural encoding is the means by which external sensory signals, such as sounds, are transformed into patterns of action potentials (spikes) in neurons in the nervous system. For neurons to encode aspects of a sensory stimulus, their spiking response must vary in a way that correlates with the stimulus. Sometimes, this variability is imminently obvious. For example, neurons in primary visual cortex are tuned to orientation, meaning that bars of light at certain angles elicit high firing rates, while bars of light at other angles elicit low firing rates (Hubel and Wiesel, 1962). However, in some neurons, the variability can be subtle. In these instances, a measure known as mutual information can be useful because it formalizes the notion of sensory encoding by neurons as the reduction in uncertainty about a stimulus that results by observing a neural response (Nelken and Chechik, 2007). Uncertainty is measured as entropy, a quantification of variability similar to variance. Mutual information is then the difference between the total entropy across all neural responses and the noise entropy, which is the uncertainty in neural responses when the stimulus is known. Mutual information can therefore be thought of as simply the variability in the neural response that directly relates to the sensory stimulus, and none of the remaining variability. Because neural responses can often be noisy and variable from trial to trial, mutual information is a useful way to quantify neural encoding, even when it is rather subtle.

How does learning affect neural encoding of natural birdsong stimuli in CLM, and how does this differ with neural encoding in CMM? In starlings trained to recognize segments of conspecific song,

neurons in CMM respond to trained songs with higher firing rates than to unfamiliar songs (Gentner and Margoliash, 2003). However, the degree to which learning modifies neural encoding in CLM has not been explored, and is particularly relevant to understand because CLM provides auditory input into CMM (Vates et al., 1996) and may be a principal brain region for providing auditory feedback information into the song production system (Bauer et al., 2008). Furthermore, because starling songs consist of sequences of spectrally and temporally complex motifs, neural encoding in individual neurons may vary from motif to motif. In chapter 2 of this thesis, I describe experiments conducted to compare the effects of learning (and of association with reward) on encoding between CLM and CMM, using methods from information theory to capture more precisely the amount of neural variability that corresponds to differences in motifs.

The role of behavioral information on plasticity in CLM and CMM

In the natural world, many sensory signals have the potential to become informative for an animal's behavior. Consistent with this, one role for learning is to associate particular signals with particular behaviors that lead to a desired outcome. For example, when learning to drive, we associate red, yellow, and green lights with distinct behaviors that help to ensure our safety on the road. For starlings learning to recognize the songs of their neighbors or potential mates, the complexity of the songs means that multiple different components could be used for recognition, while some components may be shared between individuals and therefore ambiguous. Are those song components that convey more information about a singer's identity encoded differently in CLM and CMM? In chapter 3 of this thesis, I describe experiments conducted to explore this question. Through a carefully controlled behavioral paradigm, these experiments specifically control the information that motifs convey about the motor response required by the bird to receive a food reward. This design allows an independent analysis of the role of information in modifying neural encoding, separate from the previously explored effects of familiarity and reward.

Learning-dependent plasticity of neural population coding in CLM

The brain is made up of billions of neurons which function together to support perception and cognition. However, most studies of neural function rely on sampling the activity of single neurons recorded independently. With such techniques, many aspects of the function of the larger population cannot be explored. For instance, because the neural encoding of most sensory signals is distributed across

multiple neurons, one way to improve the signal-to-noise ratio of these representations would be to form an average of the responses of similarly tuned neurons (Averbeck et al., 2006). Previous studies have shown, however, that the noise in the neural response is not independent between neurons; rather, this noise tends to be positively correlated (Zohary et al., 1994). Consequently, averaging across such a neural population will not remove all the noise in the response. Positively correlated trial-to-trial variability in neural responses (known as “noise correlations”) is not, however, necessarily detrimental to the fidelity of a neural population code. For instance, the responses of two neurons that encode opposite features but have positively correlated noise could be subtracted to remove common noise, while enhancing signal (Romo et al., 2003). The tuning similarity of multiple neurons (known as “signal correlations” because they are stimulus-driven) is thus as important for measuring the information encoded in a neural population as the noise correlations. In diverse neural populations, therefore, it is the particular relationship between signal and noise correlations—and not simply the noise correlations themselves—that directly impacts the population code (Averbeck and Lee, 2006; Gu et al., 2011). While a positive relationship between signal and noise correlations is detrimental, a negative relationship is beneficial (Figure 1.3). However, previous studies find only positive (or flat) relationships (Lee et al., 1998; Bair et al., 2001; Constantinidis and Goldman-Rakic, 2002; Kohn and Smith, 2005; Cohen and Maunsell, 2009; Gu et al., 2011), thought to be due to common input providing both correlated signal and noise. Despite its theoretical benefit, the existence of negative relationships between signal and noise correlations has yet to be observed.

How does recognition learning alter neural population coding? Despite extensive work on both population coding and learning-related plasticity, the two have rarely been investigated in conjunction (c.f. Gu et al., 2011). In chapter 4 of this thesis, I describe experiments conducted to study how learning alters the structure of neural population representations. Using the same behavioral paradigm as described in chapter 3, but now taking advantage of the simultaneous recording of multiple well-isolated single neurons, I investigate the influence of learning (and behavioral information) on the relationship between signal and noise correlations. Using a simple model of neural discriminability, the results are related directly to population coding.

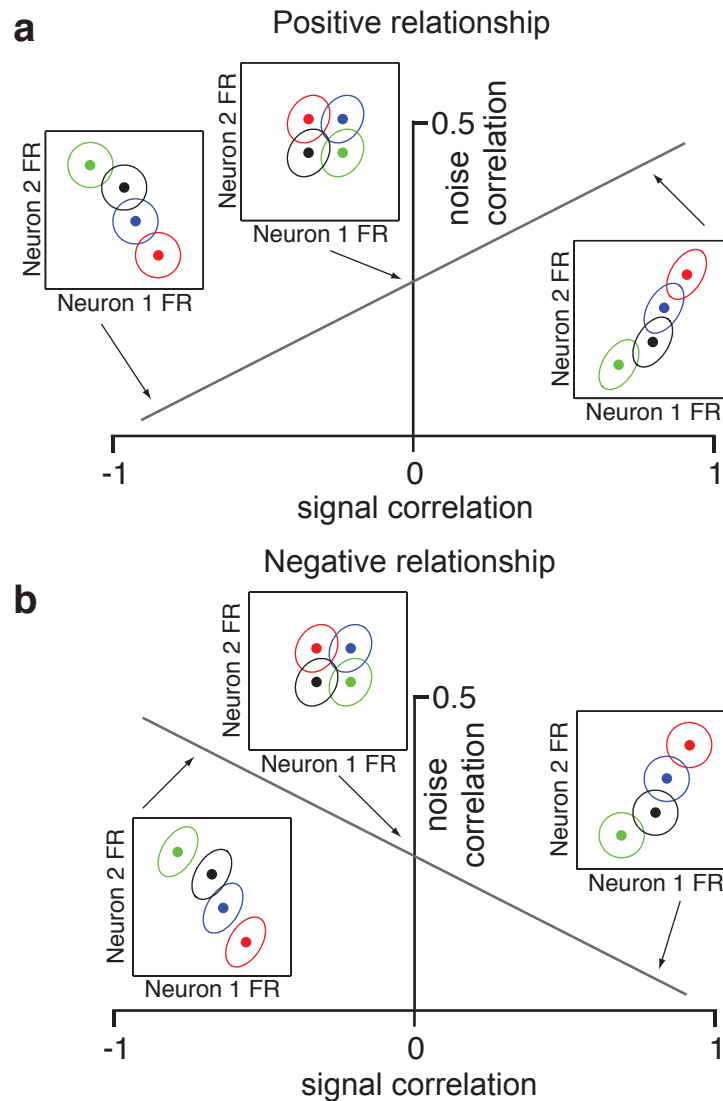


Figure 1.3. Schematic of theoretical relationships between signal and noise correlations. Each colored dot denotes the mean response for two neurons to each of four stimuli. Each colored ellipse denotes the standard deviation of the two-dimensional response distribution for each stimulus. For the positive relationship in (a), neuron pairs with positive signal correlation and large noise correlation have substantial overlap in their responses (inset right), while pairs with negative signal correlation and small noise correlation have less overlap (inset left). Center inset depicts pairs with zero signal correlation but moderate noise correlation. For the negative relationship in (b), neuron pairs with positive signal correlation and small noise correlation have some overlap in their responses (inset right), while neuron pairs with negative signal correlation and large noise correlation have very little overlap (inset left). The negative relationship thus yields neural populations that discriminate between stimuli better than the positive relationship.

Collectively, the experiments described in chapters 2-4 provide a coherent picture of how learning to recognize songs changes the neural encoding of those songs by both single neurons and larger neural populations in the auditory forebrain of the European starling. The observations reported here likely reflect the general operating principles of learning-related plasticity in cortical circuits across diverse taxa, including humans.

References

- Adret-Hausberger M, Jenkins PF (1988) Complex Organization of the Warbling Song in the European Starling *Sturnus Vulgaris*. *Behaviour* 107:138-156.
- Amin N, Gill P, Theunissen FE (2010) Role of the zebra finch auditory thalamus in generating complex representations for natural sounds. *J Neurophysiol* 104:784-798.
- Averbeck BB, Lee D (2006) Effects of noise correlations on information encoding and decoding. *J Neurophysiol* 95:3633-3644.
- Averbeck BB, Latham PE, Pouget A (2006) Neural correlations, population coding and computation. *Nature Reviews Neuroscience* 7:358-366.
- Bair W, Zohary E, Newsome WT (2001) Correlated firing in macaque visual area MT: time scales and relationship to behavior. *J Neurosci* 21:1676-1697.
- Bakin JS, Weinberger NM (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res* 536:271-286.
- Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412:79-83.
- Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R (2008) A synaptic basis for auditory-vocal integration in the songbird. *J Neurosci* 28:1509-1522.
- Beecher MD, Brenowitz EA (2005) Functional aspects of song learning in songbirds. *Trends Ecol Evol* 20:143-149.
- Brennan PA, Keiverne EB (1997) Neural mechanisms of mammalian olfactory learning. *Prog Neurobiol* 51:457-481.
- Butt AE, Hodge GK (1995) Acquisition, retention, and extinction of operant discriminations in rats with nucleus basalis magnocellularis lesions. *Behav Neurosci* 109:699-713.
- Chaiken M, Bohner J, Marler P (1993) Song Acquisition in European Starlings, *Sturnus vulgaris* - a comparison of the songs of live-tutored, tape-tutored, untutored, and wild-caught males. *Anim Behav* 46:1079-1090.
- Cohen M, Maunsell J (2009) Attention improves performance primarily by reducing interneuronal correlations. *Nat Neurosci*.
- Constantinidis C, Goldman-Rakic PS (2002) Correlated discharges among putative pyramidal neurons and interneurons in the primate prefrontal cortex. *J Neurophysiol* 88:3487-3497.
- Cristol DA, Switzer PV (1999) Avian prey-dropping behavior. II. American crows and walnuts. *Behav Ecol* 10:220-226.
- Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. *Physiol Rev* 86:1033-1048.
- Diamond DM, Weinberger NM (1986) Classical conditioning rapidly induces specific changes in frequency receptive fields of single neurons in secondary and ventral ectosylvian auditory cortical fields. *Brain Res* 372:357-360.

- Doupe AJ, Kuhl PK (1999) Birdsong and human speech: common themes and mechanisms. *Annual Review of Neuroscience* 22:567-631.
- Eens M (1997) Understanding the Complex Song of the European Starling: An Integrated Ethological Approach. *Adv Study Behav* 26:355-434.
- Eens M, Pinxten R, Verheyen RF (1991) Organization of Song in the European Starling - Species-Specificity and Individual-Differences. *Belg J Zool* 121:257-278.
- Falls JB, Brooks RJ (1975) Individual Recognition by Song in White-Throated Sparrows .2. Effects of Location. *Can J Zool* 53:1412-1420.
- Feldman DE (2009) Synaptic mechanisms for plasticity in neocortex. *Annu Rev Neurosci* 32:33-55.
- Gentner TQ, Hulse SH (1998) Perceptual mechanisms for individual vocal recognition in European starlings, *Sturnus vulgaris*. *Anim Behav* 56:579-594.
- Gentner TQ, Hulse SH (2000a) Perceptual classification based on the component structure of song in European starlings. *J Acoust Soc Am* 107:3369-3381.
- Gentner TQ, Hulse SH (2000b) Female European starling preference and choice for variation in conspecific male song. *Anim Behav* 59:443-458.
- Gentner TQ, Margoliash D (2003) Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669-674.
- Godard R (1991) Long-Term-Memory of Individual Neighbors in a Migratory Songbird. *Nature* 350:228-229.
- Gu Y, Liu S, Fetsch CR, Yang Y, Fok S, Sunkara A, Deangelis GC, Angelaki DE (2011) Perceptual learning reduces interneuronal correlations in macaque visual cortex. *Neuron* 71:750-761.
- Hollerman JR, Schultz W (1998) Dopamine neurons report an error in the temporal prediction of reward during learning. *Nat Neurosci* 1:304-309.
- Hsu A, Woolley SM, Fremouw TE, Theunissen FE (2004) Modulation power and phase spectrum of natural sounds enhance neural encoding performed by single auditory neurons. *J Neurosci* 24:9201-9211.
- Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J Physiol* 160:106-154.
- Hultsch H, Todt D (2008) Comparative aspects of song learning. In: *Neuroscience of Birdsong* (Zeigler HP, Marler P, eds), pp 204-239. Cambridge, UK: Cambridge University Press.
- Jenkins WM, Merzenich MM, Ochs MT, Allard T, Guic-Robles E (1990) Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. *J Neurophysiol* 63:82-104.
- Johnson LS, Searcy WA (1996) Female attraction to male song in house wrens (*Troglodytes aedon*). *Behaviour* 133:357-366.
- Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. *Science* 279:1714-1718.
- Kohn A, Smith M (2005) Stimulus Dependence of Neuronal Correlation in Primary Visual Cortex of the Macaque. *Journal of Neuroscience* 25:3661.

- Kuhl PK (2004) Early language acquisition: cracking the speech code. *Nat Rev Neurosci* 5:831-843.
- Lee D, Port NL, Kruse W, Georgopoulos AP (1998) Variability and correlated noise in the discharge of neurons in motor and parietal areas of the primate cortex. *J Neurosci* 18:1161-1170.
- Lei H, Mooney R (2010) Manipulation of a central auditory representation shapes learned vocal output. *Neuron* 65:122-134.
- Maren S (2005) Synaptic mechanisms of associative memory in the amygdala. *Neuron* 47:783-786.
- Metherate R, Weinberger NM (1989) Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex. *Brain Res* 480:372-377.
- Nelken I, Chechik G (2007) Information theory in auditory research. *Hear Res* 229:94-105.
- Otani S, Daniel H, Roisin MP, Crepel F (2003) Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. *Cereb Cortex* 13:1251-1256.
- Patil MM, Linster C, Lubenov E, Hasselmo ME (1998) Cholinergic agonist carbachol enables associative long-term potentiation in piriform cortex slices. *J Neurophysiol* 80:2467-2474.
- Perrins C, ed (2009) *Princeton Encyclopedia of Birds*. Princeton, NJ: Princeton University Press.
- Pinsker H, Kupfermann I, Castellucci V, Kandel E (1970) Habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. *Science* 167:1740-1742.
- Rainer G, Miller EK (2000) Effects of visual experience on the representation of objects in the prefrontal cortex. *Neuron* 27:179-189.
- Rainer G, Lee H, Logothetis NK (2004) The effect of learning on the function of monkey extrastriate visual cortex. *PLoS Biol* 2:275-283.
- Recanzone GH, Merzenich MM, Schreiner CE (1992) Changes in the distributed temporal response properties of SI cortical neurons reflect improvements in performance on a temporally based tactile discrimination task. *J Neurophysiol* 67:1071-1091.
- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13:87-103.
- Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron* 70:121-131.
- Roberts TF, Tschida KA, Klein ME, Mooney R (2010) Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. *Nature* 463:948-952.
- Romo R, Hernández A, Zainos A, Salinas E (2003) Correlated neuronal discharges that increase coding efficiency during perceptual discrimination. *Neuron* 38:649-657.
- Schoups A, Vogels R, Qian N, Orban G (2001) Practising orientation identification improves orientation coding in V1 neurons. *Nature* 412:549-553.
- Sen K, Theunissen FE, Doupe AJ (2001) Feature analysis of natural sounds in the songbird auditory forebrain. *J Neurophysiol* 86:1445-1458.
- Thorpe WH (1954) The Process of Song-Learning in the Chaffinch as Studied by Means of the Sound Spectrograph. *Nature* 173:465-469.

- Thorpe WH (1958) Further Studies on the Process of Song Learning in the Chaffinch (*Fringilla-Coelebs-Gengleri*). *Nature* 182:554-557.
- Vates GE, Broome BM, Mello CV, Nottebohm F (1996) Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366:613-642.
- Wang Y, Brzozowska-Prechtl A, Karten HJ (2010) Laminar and columnar auditory cortex in avian brain. *Proc Natl Acad Sci U S A* 107:12676-12681.
- Witherby HF, Jourdain FCR, Ticehurst NF, Tucker BW (1943) *The Handbook of British Birds*. London: H. F. & G. Whiterby Ltd.
- Woolley SM, Gill PR, Fremouw T, Theunissen FE (2009) Functional groups in the avian auditory system. *J Neurosci* 29:2780-2793.
- Yasukawa K (1981) Song and Territory Defense in the Red-Winged Blackbird. *Auk* 98:185-187.
- Zohary E, Shadlen MN, Newsome WT (1994) Correlated neuronal discharge rate and its implications for psychophysical performance. *Nature* 370:140-143.

II. Emergence of learned categorical representations within an auditory forebrain circuit

Abstract

Many learned behaviors are thought to require the activity of high-level neurons that represent categories of complex signals, such as familiar faces or native speech sounds. How these complex, experience-dependent neural responses emerge within the brain's circuitry is not well understood. The caudomedial mesopallium (CMM), a secondary auditory region in the songbird brain, contains neurons that respond to specific combinations of song components and respond preferentially to the songs that birds have learned to recognize. Here, we examine the transformation of these learned responses across a broader forebrain circuit that includes the caudolateral mesopallium (CLM), an auditory region that provides input to CMM. We recorded extracellular single-unit activity in CLM and CMM in European starlings trained to recognize sets of conspecific songs and compared multiple encoding properties of neurons between these regions. We find that the responses of CMM neurons are more selective between song components, convey more information about song components, and are more variable over repeated components than the responses of CLM neurons. While learning enhances neural encoding of song components in both regions, CMM neurons encode more information about the learned categories associated with songs than CLM neurons. Collectively, these data suggest that CLM and CMM are part of a functional sensory hierarchy that is modified by learning to yield representations of natural vocal signals that are increasingly informative with respect to behavior.

Introduction

Faced with an immense quantity of sensory input, individuals learn to identify sensory features relevant to behavioral goals and ignore others that are irrelevant. The representation of objects by high-level sensory neurons depends heavily on this form of learning (e.g. Rolls et al., 1989; Sigala and Logothetis, 2002; Gentner and Margoliash, 2003) and some neurons represent learned categories of objects rather than the objects themselves (Freedman et al., 2001; Sigala and Logothetis, 2002; Freedman and Assad, 2006). These kinds of complex representations are commonly understood to result from processing

pathways in which higher-order neurons integrate convergent input from lower-order neurons (Felleman and Van Essen, 1991; Binder et al., 2000; Kaas and Hackett, 2000; Riesenhuber and Poggio, 2000; Chechik et al., 2006; Rauschecker and Scott, 2009). However, very few studies have examined changes in the encoding of natural stimuli along these processing pathways (Chechik et al., 2006; Rust and DiCarlo, 2010) or how learning mediates this encoding (Freedman et al., 2003; Freedman and Assad, 2006), particularly in the auditory domain.

Songbirds serve as an excellent model system to study the learning-dependent neural encoding of natural signals because they easily learn to identify conspecific songs (Gentner and Margoliash, 2003) and have well-defined neural circuitry specialized for processing songs (Hsu et al., 2004; Woolley et al., 2005; Woolley et al., 2009). The caudomedial mesopallium (CMM), a secondary auditory area in the songbird, contains some of the most complex neurons in the avian auditory system (Meliza et al., 2010), which elicit stronger responses to learned songs than to novel songs (Gentner and Margoliash, 2003). As with high-level brain regions in mammals, however, the circuitry that produces the experience-dependent responses in CMM are not well understood. CMM receives indirect input from the Field L complex (the analogue of mammalian primary auditory cortex) by way of bidirectional connectivity with the adjacent caudolateral mesopallium (CLM) and the caudomedial nidopallium (NCM; Fig 2.1a; Vates et al., 1996). The responses of neurons in CLM are less well predicted by linear receptive field models (Sen et al., 2001) and are more sharply tuned for the statistical properties of song (Hsu et al., 2004) than many neurons in Field L. Although it is possible that neural processing in CLM contributes to the learning-dependent representations of song in CMM, it is unknown whether the neural encoding of songs in CLM differs from CMM or whether CLM encodes learning-dependent information.

To understand the functional relationship between CLM and CMM, we compare their neural encoding properties in European starlings (*Sturnus vulgaris*) that have learned to recognize sets of conspecific songs and ask whether learning modifies the responses of neurons differently between CLM and CMM. We show that the responses of neurons in CMM are more selective between song components, convey more information about song components, and respond with greater variability to repeated song components. In both regions, learning increases the information encoded about song components, but

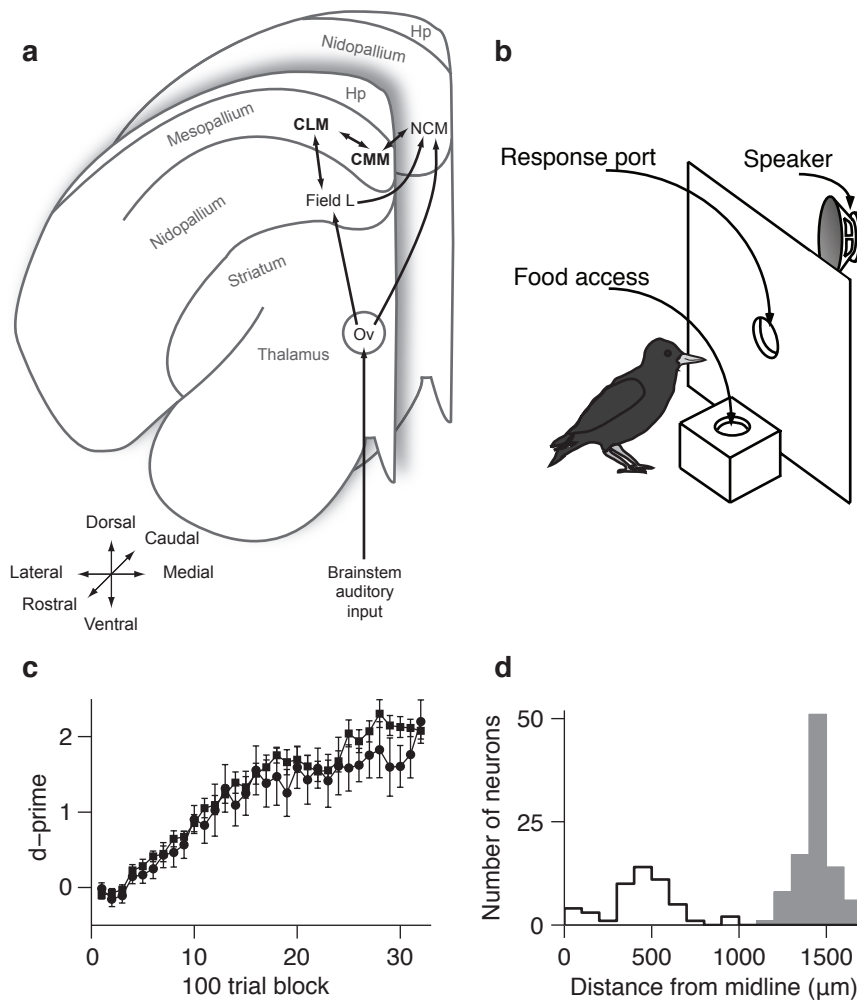


Figure 2.1. Behavioral training and starling neuroanatomy. **(a)** Schematic diagram of the connectivity of the songbird auditory system across two coronal planes of one hemisphere. **(b)** Schematic of operant apparatus used for behavioral training. **(c)** Mean (\pm SEM) behavioral performance (d-prime) during learning for subjects used in CLM experiments (squares) and subjects used in CMM experiments (circles). Error bars are across subjects. **(d)** Distributions of recording locations for CLM neurons (black) and CMM neurons (white). Hp, Hyperpallium; CLM, Caudolateral Mesopallium; CMM, Caudomedial Mesopallium; NCM, Caudomedial Nidopallium; Ov, Ovoidalis.

CMM neurons encode more information about behaviorally defined song categories. These results are consistent with a model of neural processing in which information about natural vocal signals flows through CLM to CMM, giving rise to complex representations of acoustic signals and their behavioral relevance.

Materials and Methods

All experiments were performed in accordance with the Institutional Animal Care and Use Committee of the University of California, San Diego.

Subjects

Nineteen adult European Starlings (*Sturnus vulgaris*) were wild-caught in southern California, and housed in aviaries with free access to food and water until the commencement of behavioral training. At the start of training, subjects were naïve to all experimental procedures and stimuli. Thirteen starlings were used for CLM experiments, five starlings were used for CMM experiments, and one starling was used for both CLM and CMM experiments. Data from CMM experiments were combined with a subset of previously published data (Gentner and Margoliash, 2003) from an additional four starlings, yielding a total of 23 subjects. Very few differences in behavioral training were observed between the two sets of CMM data (supplemental table 1). 28 of the 48 CMM neurons reported here were from this previously published data.

Stimuli

Six starling song stimuli were created from a collection of songs previously recorded from four adult male European starlings. Each stimulus was a unique section of continuous song (durations ranging from 9.1s – 10.7s) from a single male and shared no motifs with any other stimulus. The six song stimuli were divided into three sets of two song stimuli each. For each experimental subject, the three stimulus sets were assigned as “rewarded,” “unrewarded,” and “novel” stimuli to reflect the subject’s experience with those stimuli during behavioral training. Across subjects, this assignment was counterbalanced such that the same stimuli were used for different conditions in different birds. Nearly identical stimuli were used for

CLM and CMM experiments.

Behavioral training

After acclimation to individual housing in a sound-attenuating chamber (Acoustic Systems, ETS-Lindgren, Austin, TX), each subject was trained on a standard go/no-go operant-conditioning procedure to classify two of the song stimulus sets (Gentner and Margoliash, 2003). For each bird, the rewarded songs were assigned as the “go” stimulus set and the unrewarded songs were assigned as the “no-go” stimulus set. A subject started a trial by inserting its beak into a small hole on a response panel inside the sound-attenuating chamber (Fig 2.1b), which initiated the playback of one of the four stimuli, chosen at random. After stimulus playback ended, a subject had two seconds to report its classification decision by either inserting its beak again (a “go” response) or by refraining from inserting its beak (a “no-go” response). Go responses to the go stimulus set were rewarded with two-second access to food and go responses to the no-go stimulus set were punished with variable-duration darkness (range, 10s – 90s) during which no food was available and trials could not be initiated. In all cases, no-go responses were neither rewarded nor punished. Water was provided *ad libitum*, but food was only available from correct go responses.

Neurophysiology

After achieving satisfactory classification performance (see results), each subject underwent surgery under isoflurane anesthesia (1.5-2% concentration) to prepare for recording. The top layer of skull was removed from the region above CLM or CMM and a small metal pin was affixed to the skull just caudal to the opening. Each subject recovered for 12-24 hours before neurophysiological recordings began. On the morning of the recording day, the subject was anesthetized with urethane (20% by volume, 7mL/kg), and head-fixed in a stereotactic apparatus inside a sound-attenuating chamber. The subject was situated 30 cm from a speaker through which the song stimuli were presented (all normalized to 95dB peak SPL).

Extracellular electrical activity of single neurons in CLM or CMM in response to 5-50 repetitions of all six song stimuli (presented in random order) was recorded using glass-coated platinum-iridium wire

electrodes (1-3M Ω impedance) inserted through a small craniotomy directly dorsal to CLM or CMM. The extracellular waveform was amplified (5,000X – 50,000X gain), filtered (high pass 300Hz, low pass 3kHz), sampled (25kHz), and stored for offline analysis (Cambridge Electronic Design, Cambridge, UK). At the end of the recording session, one to three fiduciary electrolytic lesions (10-20 μ A, 10-20s) were made to facilitate recording site localization using standard histological techniques.

All recording sites were confirmed to be within CLM or CMM (Fig. 2.S1c). All CLM neurons were located between 1200 μ m and 1650 μ m from the midline; all CMM neurons were located between 0 μ m and 1000 μ m from the midline (Fig. 2.1d). Putative action potentials from single neurons were identified by amplitude and sorted offline using principal component analysis on waveform shape (Cambridge Electronic Design, Cambridge, UK). Only action potential waveforms with a single obvious cluster in principal component-space and with very few refractory-period violations (Fig. 2.S1a,b) were considered to be from a single neuron. Only 4.8% (7/145) of all neurons exhibited any inter-spike intervals (ISIs) shorter than 1ms, and within that subset of neurons, ISIs shorter than 1ms accounted for less than 0.1% of all ISIs in each neuron.

Only neurons that responded significantly to any part of our stimulus set were included in our data analysis. Response significance was determined quantitatively, following previously described methods (Gentner and Margoliash, 2003). Briefly, the mean response to each song was divided into 500ms segments, and the variance of the mean response over each segment was computed. The variance for each song was normalized by the variance of spontaneous firing. To be considered auditory, the largest normalized variance value needed to be greater than $(1.96 \times 1 \text{ S.E.}) + 1$, where S.E. is the standard error of the normalized variance values for the remaining songs, and 1 is the normalized variance that would be expected for a non-auditory neuron.

Data Analysis

Analyses were performed using custom-written MATLAB (Natick, MA) software. Behavioral performance was evaluated using d prime (Macmillan and Creelman, 2005),

$$d' = zscore(Hit\ rate) - zscore(False\ alarm\ rate) \quad (1)$$

a measure of discriminability between two distributions. Values of d' were computed in non-overlapping blocks of 100 trials.

For most analyses of neural activity, responses to full song stimuli were segmented into the responses to each constituent motif (Fig. 2.2). The starting time for a motif was defined as the onset of power for that motif, and the ending time for a motif was defined as the onset of power for the following motif. Thus, a neuron's response to a motif consisted of the neural activity both during that motif and during the subsequent silent period following that motif. Each individual song stimulus contained multiple renditions of some motifs, but because of subtle acoustical variability between renditions, each rendition was considered to be distinct for all analyses except the variability analysis of repeated motifs (Fig. 2.4).

Motif selectivity analysis The non-parametric selectivity of each neuron was calculated over all motifs (rewarded, unrewarded, and novel) collectively for each neuron (Rolls and Tovee, 1995; Vinje and Gallant, 2000):

$$S = \left(1 - \frac{(\sum r_i/n)^2}{\sum (r_i^2/n)} \right) / \left(1 - \frac{1}{n} \right) \quad (2)$$

where r_i is the mean firing rate in response to the i^{th} motif, and n is the total number of motifs. This measure ranges from 0 to 1, with 0 representing the minimum motif selectivity (responses to all motifs are identical) and 1 representing the maximum motif selectivity (response to only one motif). Although this measure includes all responses from each neuron, it emphasizes the larger values in the response distribution. Therefore, we also used the entropy method (Lehky et al., 2005), which equally considers selectivity of both excitatory and suppressive responses. Both measures yielded the same result (Fig. 2.S2). Mean spontaneous firing rates did not differ significantly between CMM (3.24 ± 0.59 Hz) and CLM neurons (3.37 ± 0.34 Hz; Wilcoxon rank sum test, $p = 0.41$).

Information analysis Firing rates for each neuron's evoked response to each motif were divided into six equally spaced bins ranging from the lowest firing rate to the highest firing rate elicited by that

neuron. Six bins were chosen to balance the need to capture the dynamic range of responses for each neuron with the need to appropriately sample the conditional probabilities. For each neuron, identical firing rate bins were used for all information calculations. Control analyses in which the number of bins varied from 2-10 yielded changes in the absolute number of bits but did not alter the effect of learning on information or the differences between CLM and CMM (Fig. 2.S3). In all cases, mutual information was calculated as

$$I(s;r) = \sum_{s,r} p(s,r) \log_2 \left(\frac{p(s,r)}{p(s)p(r)} \right). \quad (3)$$

Where s indexes the stimulus and r indexes the bin of the firing rate response (Brenner et al., 2000; Cover and Thomas, 2006).

For mutual information between motif identity and motif firing rate, $p(r,s)$ represented the empirical joint probability distribution of motif firing rates and motif identities. Because multiple renditions of single motifs were considered to be distinct, the distribution of motif identities, $p(s)$, was always uniform. The distribution of firing rates, $p(r)$, was computed by averaging across the conditional distributions for each motif. Information about motif identity was computed in two ways: across responses to all motifs regardless of stimulus class (rewarded, unrewarded, and novel), and separately for the motifs in each stimulus class. For information encoded about all motifs (Figure 2.3), $p(r)$ was found by averaging the conditional distributions across all motifs presented to each neuron. For the information encoded about motifs within each stimulus class (Figures 2.5, 2.6), the conditional distributions were averaged across motifs within each class to obtain separate $p(r)$ distributions for each class.

For mutual information between motif class (rewarded, unrewarded, novel) and motif firing rate, $p(r,s)$ represented the empirical joint probability distribution of motif firing rates and each motif's associated stimulus class. This distribution was determined by averaging the response distributions for all motifs within each stimulus class. The distribution of stimulus classes, $p(s)$, was uniform and the distribution of firing rates, $p(r)$, was found by averaging the class-conditional distributions.

For mutual information between song class and song firing rate, calculations were exactly analogous to calculations for motif class, except that firing rate responses were averaged over the entire

duration of the presented song.

The significance of information encoded about learned motif and song categories was evaluated relative to information about randomly shuffled categories. For shuffled motif category information, each motif was randomly assigned to one of three categories such that the behavioral meaning of the categories was lost but the association between motif identity and firing rate was preserved (because single trials were not shuffled). This procedure was repeated for 100 random permutations to generate a distribution of shuffled information values, providing an estimate of the information encoded about arbitrary categories. The distribution of shuffled values was then used to determine the p-value for the category information for each neuron. Significance was evaluated at $p < 0.05$. For song category information, shuffling was conducted similarly. However, because there are only two songs per category, there are only eight distinct permutations of category assignment that disrupt all three original category boundaries. Thus, information about learned categories was considered significant when it was greater than the information about all eight shuffled information values for each neuron. Importantly, because neurons could encode any arbitrarily-defined categories, positive information about shuffled categories does not imply the presence of residual bias in the estimation of information about behaviorally-defined categories. Rather, neurons may “categorically” encode other features of song, such as a particular spectrotemporal pattern that only appears in some motifs. Making a comparison with the shuffled categories thus provides a test of whether the information encoded about the learned categories is greater than would be expected by chance.

Two control analyses for the mutual information were carried out. First, to ensure that mutual information values were not solely dependent on variations in a neuron’s dynamic range, we limited responses by the maximum and minimum single-trial firing rate elicited by a novel motif (Fig. 2.S4). In this case, all firing rates from rewarded or unrewarded motifs that were outside this range were ignored (i.e. considered never to have occurred). Second, to test our assumption that responses to multiple renditions of acoustically similar motifs are independent, mutual information was recomputed with multiple renditions considered as a single motif. Both controls yielded qualitatively similar results to those reported in the text (Fig. 2.S5)

In addition, because estimates of information from limited samples are inherently biased upwards

(Treves and Panzeri, 1995), bias was corrected by extrapolating the information estimate for each neuron to an infinite data size (Strong et al., 1998; Brenner et al., 2000). Variability in the estimate of mutual information was determined by a jackknife resampling of the data. The standard deviations of the information estimations were very low (median for estimates over all motifs: 0.014 bits, median for estimates within motif classes: 0.022 bits), which indicates highly stable estimations. Subtracting analytical estimates of the bias (Panzeri et al., 2007) instead yielded qualitatively similar results to the extrapolation method.

Entropy values were computed using the same bins as were used for information calculations.

Total entropy was calculated as

$$H_{Total}(r) = -\sum p(r) \log_2 p(r). \quad (4)$$

Noise entropy was calculated as

$$H_{Noise}(r) = -\sum_s p(s) \sum_r p(r|s) \log_2 p(r|s). \quad (5)$$

Because estimates of the entropy are subject to similar biases as estimates of the mutual information, bias in the computed entropy was corrected by using methods analogous to those described for mutual information.

Repeated motif analysis Four independent observers classified motifs based on visual inspection of the spectrogram and listening to the waveform. Repeated motifs were judged to be renditions of the same type only when all four observers agreed on the classification. Motifs of the same type were then considered to be identical for the purposes of the repeated motif analyses. Variability of responses within sequences of repeated motifs was measured by computing the coefficient of variation (CV) of trial-averaged firing rates in response to each repeated motif sequence presented to each neuron. The CV values for all sequences for a given neuron were averaged to find the mean variability for that neuron.

Statistics All data were tested for normality using the Lilliefors test with $p < 0.05$. Non-parametric

tests were applied when data were not normal. Central tendencies are reported as means \pm standard errors, unless otherwise noted.

Results

To compare how learning affects neural encoding in CLM and CMM, we began all experiments by training European starlings (*Sturnus vulgaris*) to recognize four different conspecific songs using an established operant procedure (Fig. 2.1b) (Gentner and Margoliash, 2003). Birds learned to peck in response to one pair of songs (“rewarded” songs) to obtain a food reward and to withhold pecks to the other pair (“unrewarded” songs) to avoid a mild punishment (Materials and Methods). After birds learned this task (Fig. 2.1c), we analyzed the activity of single neurons (supplemental Fig. 1) in either CLM ($n = 97$ neurons) or CMM ($n = 48$ neurons) (Fig. 2.1d) in response to the rewarded and unrewarded training songs and to two songs with which the birds had no prior experience (“novel” songs; Fig. 2.2; Materials and Methods).

The birds used for CLM recordings learned at similar rates and ultimately reached similar levels of performance as the birds used for CMM recordings. Song recognition performance (measured by d' ; Materials and Methods) exceeded chance by a significant margin ($p < 0.01$) after a mean of 814 ± 100 trials in CLM birds and a mean of 900 ± 87 trials in CMM birds (Fig. 1c; t-test, $p = 0.55$). CLM birds performed a mean of $22,639 \pm 8,713$ trials and CMM birds performed a mean of $28,987 \pm 10,913$ trials (t-test, $p = 0.65$). By the end of training, both sets of birds recognized the training songs with high accuracy (mean d' over the last 500 trials: 2.68 ± 0.20 in the CLM birds and 2.90 ± 0.36 in the CMM birds, t-test, $p = 0.57$). Thus all birds had learned to recognize all the training songs with high proficiency prior to the neural recording.

Motif Selectivity in CLM and CMM

We first sought to characterize functional differences between neurons in CLM and CMM. Because starlings compose their songs from stereotyped clusters of notes called motifs (Chaiken et al., 1993) that are thought to be perceived as discrete auditory objects (Gentner and Hulse, 2000; Gentner, 2008; Seeba and Klump, 2009), we analyzed the neural responses to each motif. In some CMM neurons, small subsets of motifs elicit high firing rates, while many other motifs elicit low firing rates (Gentner and

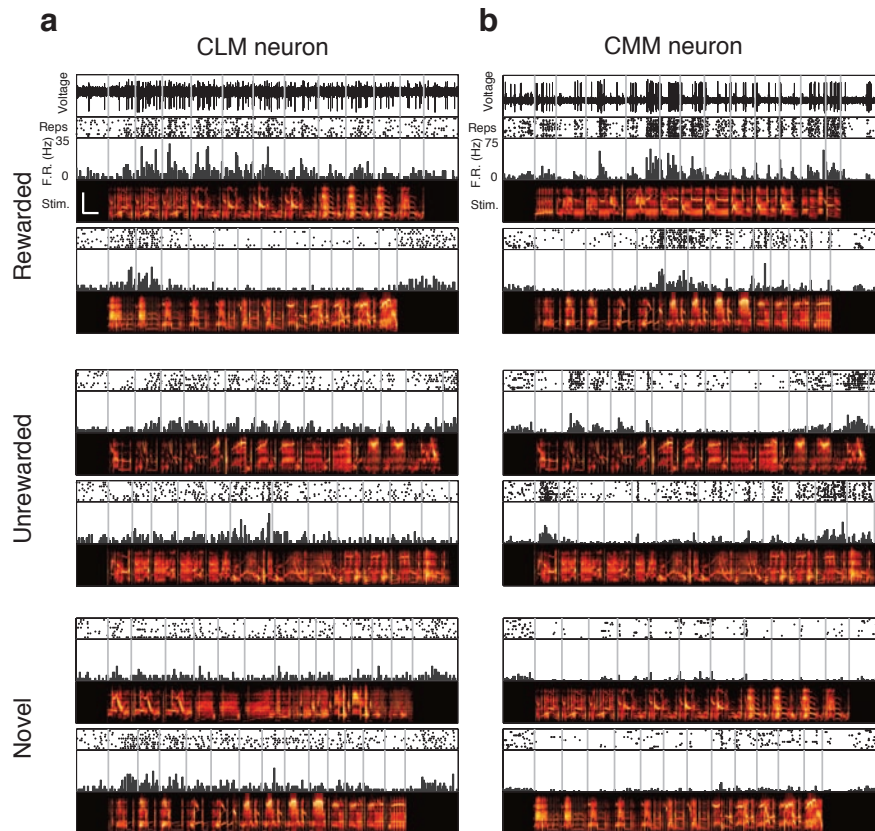


Figure 2.2. Responses from two example neurons. Extracellular electrical activity recorded from a sample CLM neuron (a) and CMM neuron (b) in response to rewarded motifs (top), unrewarded motifs (middle), and novel motifs (bottom). Scale bar in upper left spectrogram denotes 0.5s and 5kHz. Scales are identical for all spectrograms.

Margoliash, 2003; Meliza et al., 2010), a characteristic known as lifetime sparseness or non-parametric selectivity (Willmore and Tolhurst, 2001; Lehky et al., 2005). Here we refer to this characteristic as “motif selectivity”, and compare this measure between the responses of neurons in CMM and CLM to the motifs that made up all four training and the two novel songs. In the representative CLM neuron shown in figure 2.2a, a large number of motifs elicited high firing rates. In the representative CMM neuron (Fig 2.2b), however, a smaller number of motifs elicited high firing rates, while most motifs elicited low firing rates. Differences in the distribution of each neuron’s firing rate response can be directly compared between the sample CLM and CMM neurons by rank-ordering the responses for each motif (Fig 2.3a).

For each neuron in CLM and CMM, we computed the non-parametric motif selectivity (Vinje and Gallant, 2000; Materials and Methods). This measure quantifies the relative extent of the positive tail of the distribution of mean firing rates in response to motifs (Franco et al., 2007). If all motifs elicited the same firing rate, the motif selectivity would be 0; if only one motif elicited a positive firing rate, the motif selectivity would be 1. The CLM neuron in Fig 2.2a had a motif selectivity of 0.24, whereas the CMM neuron in Fig 2.2b had a motif selectivity of 0.49. Although both regions exhibited a large range of motif selectivity values, we found that, on average, neurons in CMM had higher motif selectivity values (0.40 ± 0.04) than neurons in CLM (0.26 ± 0.02 ; Wilcoxon rank sum test, $p = 1.6 \times 10^{-4}$; Fig 2.3b). These motif selectivity differences (and those observed using other measures of selectivity; Fig. 2.S2) reflect the observations that CMM neurons responded with higher firing rates to a smaller subset of motifs than CLM neurons.

Motif information encoding in CLM and CMM

Responding selectively to a small subset of motifs is one effective way to encode information, but responding to many motifs can also encode a substantial amount of information, provided the response to each motif is distinct. Mutual information captures all the differences between the responses to different motifs (Cover and Thomas, 2006) and is thus agnostic to the actual method of encoding. We computed mutual information between firing rate and motif identity from the probability distribution of firing rate conditioned on motif identity (Materials and Methods). The conditional firing rate distributions of the

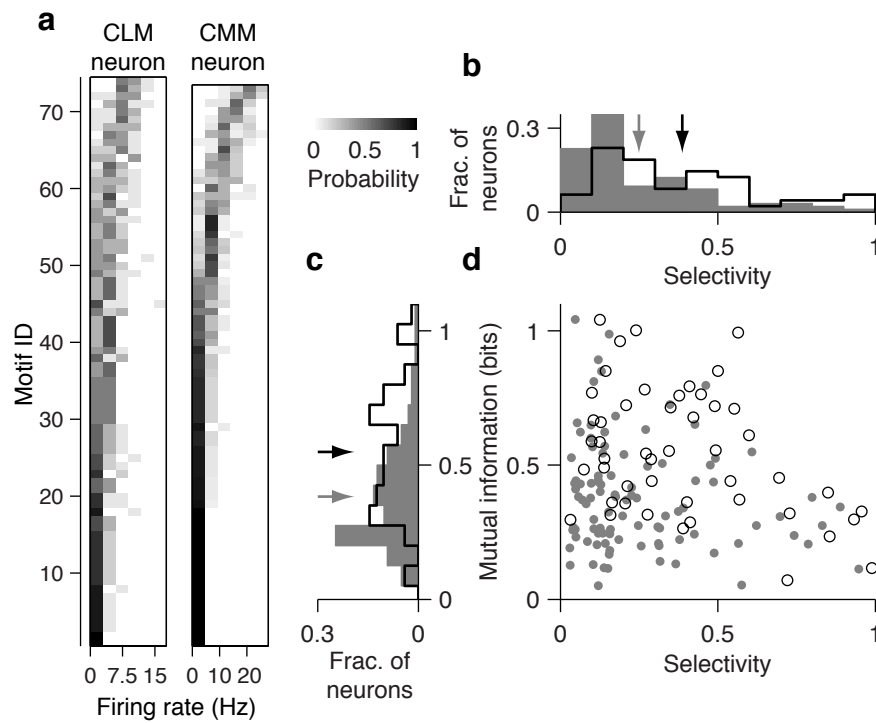


Figure 2.3. Motif selectivity and information encoding in CLM and CMM neurons. **(a)** Distributions of firing rates conditional on motif identity for the CLM neuron (left panel) and CMM neuron (right panel) shown in Fig 2.2a and 2.2b, respectively. Each row in each panel shows the firing rate distribution for a single motif. Motifs are arranged in order of ascending mean firing rates and the conditional probability is encoded in grayscale. **(b)** Distribution of motif selectivity values for CLM (grey bars) and CMM (black line) neurons. Grey arrow denotes mean for CLM neurons, black arrow denotes mean for CMM neurons. **(c)** Distribution of motif information values for CLM (grey bars) and CMM (black line) neurons. Arrows are as in (b). **(d)** Relation between mutual information and motif selectivity in CLM (dots) and CMM (circles) neurons. CMM neurons tend to have either low motif selectivity or low mutual information, but not both. CLM neurons can have both low motif selectivity and low mutual information.

representative neurons from Fig 2.2 show that the CMM neuron exhibited a greater diversity of firing rates than the CLM neuron, and that this diversity was more closely tied to motif identity for the CMM neuron than for the CLM neuron (Fig 2.3a). Accordingly, the CMM neuron encoded 0.76 bits of information about motif identity, whereas the CLM neuron encoded only 0.38 bits of information. Across all neurons, we observed a broad distribution of information values in both CLM and CMM (Fig 2.3c). On average, neurons in CMM encoded significantly more information (0.55 ± 0.03 bits) about motif identity than neurons in CLM (0.38 ± 0.02 bits; Wilcoxon rank sum test, $p = 3.5 \times 10^{-5}$; Fig 2.3c).

In both CLM and CMM, high motif selectivity and high motif information did not coexist in the same neurons (Fig 2.3d). This is expected because each of these measures mutually constrains the other. Motif selectivity measures the distinctness of a neuron's response to a small number of motifs, while mutual information measures the diversity of a neuron's response to many motifs. The response of a highly selective neuron can effectively distinguish between a small number of motifs, yet has little ability to distinguish between the majority of motifs. High motif selectivity thus constrains the amount of information that can be conveyed about the whole set of motifs. Similarly, neurons that encode a large amount of information must necessarily respond to many motifs, but in a manner that maps different responses to different motifs. The populations of neurons from both CLM and CMM range from low motif selectivity but high information, to high motif selectivity but low information (Fig 2.3d). This pattern suggests that neurons in both regions possess a continuum of sensory encoding properties. In addition, because CMM contains fewer neurons with both lower motif selectivity and lower information values than CLM (Fig. 2.3d), CMM neurons encode motifs in a manner that is closer to the constraints set by information and selectivity.

Responses to repeated motifs in CLM and CMM

Over the course of a song, starlings typically sing multiple renditions of one type of motif before switching to a different type of motif (Eens, 1997). We examined whether CLM and CMM neurons elicited variable responses to repeated motifs of the same type. Evidence of such variable responses was found in both regions. The responses of the representative CLM and CMM neurons in Fig 2.4a and b show change

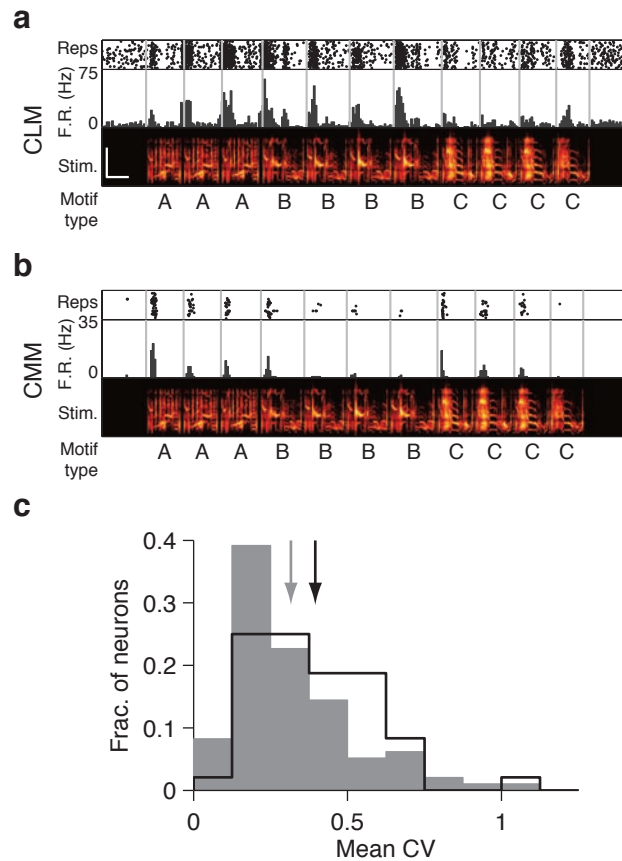


Figure. 2.4. Response variability of CLM and CMM responses to repeated motifs. **(a)** Response of an example CLM neuron to a single song illustrating response variability to repeated motifs. Motif types are denoted as letters below the spectrograms. Motifs of the same type were judged to be acoustically similar for the purposes of the response variability analysis (Materials and Methods). **(b)** Response of an example CMM neuron to the same song, as in (a). **(c)** Distribution of mean coefficient of variation (CV) across repeated motifs for all CLM and CMM neurons. The CV is computed from the firing rates across each sequence of repeated motifs and averaged for each neuron. Higher CV values indicate greater response variability. Scale bar in upper spectrogram denotes 0.5s and 5kHz. Scales are identical for both spectrograms.

across each rendition of the repeated motif types in the sequence. To quantify this observation, we computed the coefficient of variation (CV) of mean firing rates in response to each motif within a repeated sequence. For the sample CLM neuron in Fig 2.4a, CV values were 0.40, 0.22, and 0.30 for motif types A, B, and C, respectively. For the sample CMM neuron in Fig 4b, CV values were 0.54, 1.15, and 0.65, for motif types A, B, and C, respectively. We observed instances of firing rates increasing over motif repetitions (e.g. motif type A in Fig 2.4a), as well as instances of firing rates decreasing over motif repetitions (e.g. motif type C in Fig 2.4b). We computed a measure of each neuron's overall variability to repeated motifs by averaging all the CV values for each sequence of repeated motifs presented to that neuron. On average, the neurons in CMM had higher mean CV values (0.40 ± 0.03) than the neurons in CLM (0.32 ± 0.02 ; Wilcoxon rank-sum test, $p = 0.0040$; Fig 2.4c). These results suggest that neurons in CMM are more variable within a motif type than are neurons in CLM. Together, the results of motif selectivity, mutual information, and variability analyses indicate that song-evoked neuronal responses increase in complexity between CLM and CMM.

Learning increases information encoded about motifs in CLM

One way in which learning might act on CLM neurons is to modify their encoding of individual motifs. To test this idea, we compared the responses of CLM neurons to the motifs that were paired with reward during training (“rewarded motifs”), the motifs not paired with reward during training (“unrewarded motifs”), and the motifs not used for training (“novel motifs”). In the representative CLM neuron in Fig 2.2a, neural activity was more variable among the rewarded motifs than among the unrewarded or novel motifs. We quantified these differences by computing the mutual information between firing rate and motif identity separately for each class of motifs (Materials and Methods). The strength of the association between firing rates and motif identity within each stimulus class corresponds directly to the amount of information each neuron encodes about the motifs in that class. The conditional probability distributions of firing rates for each set of motifs presented to the example neuron (Fig 2.5a) shows that the firing rate diversity was more closely tied to motif identity for familiar motifs than between the unrewarded or novel motifs. Accordingly, this neuron encoded more information about rewarded motifs (0.66 bits) than about

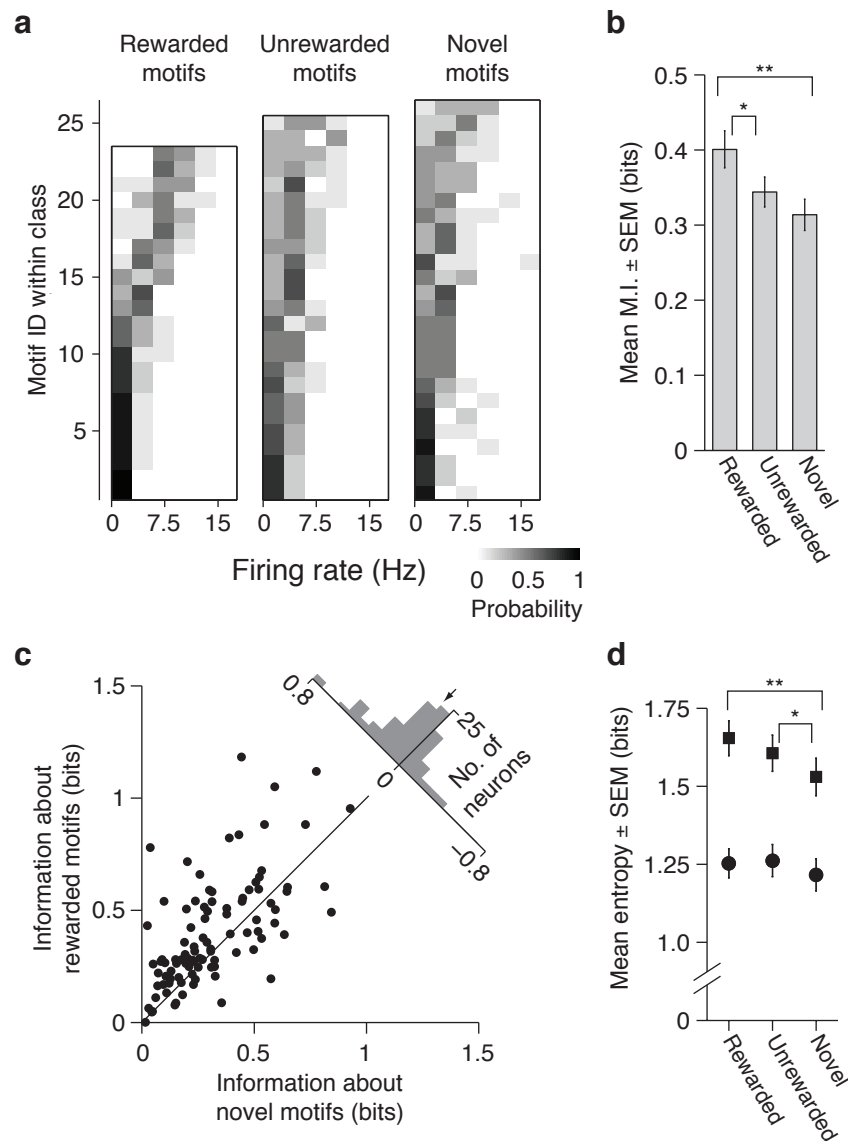


Figure 2.5. Effects of learning on encoding of motif identity in CLM. **(a)** Distributions of firing rates (F.R.) conditional on motif identity for rewarded (left), unrewarded (middle) and novel (right) motifs for the neuron shown in Fig. 2.2a. Each row in each panel shows the firing rate distribution for a single motif. Motifs are arranged in order of ascending mean firing rates and the conditional probability is encoded in grayscale. The firing rates of this neuron allow greater disambiguation of motif identity for rewarded motifs than for novel motifs. **(b)** Comparison of mean (\pm SEM) mutual information across all CLM neurons for rewarded, unrewarded, and novel motifs. Wilcoxon signed-rank test: * $p < 0.01$; ** $p < 10^{-5}$. **(c)** Scatter plot illustrating distributions of information values for novel motifs and rewarded motifs. Each point represents a single neuron. Upper right, histogram of differences between mutual information values for rewarded vs. novel motifs for all neurons. The arrow denotes the mean. **(d)** Mean (\pm SEM) total entropy (squares) and noise entropy (circles) values over all CLM neurons. Paired t-test: * $p < 0.01$; ** $p < 0.005$.

unrewarded motifs (0.16 bits) and novel motifs (0.26 bits).

Across the population of CLM neurons, learning significantly increased the amount of information encoded by individual neurons (Friedman test, $p = 1.7 \times 10^{-4}$). The mean information encoded about rewarded motifs was 34.5% higher than that for novel motifs (Wilcoxon signed-rank test, $p = 1.6 \times 10^{-5}$; Fig 2.5b,c). This learning effect was observed in most neurons: 69 of 97 neurons (71%) encoded more information about rewarded motifs than novel motifs (χ^2 test: $p = 0.0001$; Fig. 2.5c). The mean information encoded about unrewarded motifs was comparable to that for novel motifs (Wilcoxon signed-rank test, $p = 0.091$; Fig. 2.5c). The proportion of neurons encoding more information about unrewarded motifs than about novel motifs was not greater than that expected by chance (56/97; χ^2 test, $p = 0.13$). Thus, the association of songs with reward was necessary to induce significant changes in encoding by single CLM neurons.

The observed effects of learning on information encoding could arise from two forms of firing rate variability. First, the diversity of responses to all motifs (“the total entropy”) could increase, which could potentially allow for a greater number of motifs to be represented. Second, the diversity of responses to repeated presentations of the same motif (“the noise entropy”) could decrease, which could allow for greater discriminatory power between responses to different motifs (Strong et al., 1998). Across all CLM neurons, learning increased the total entropy (repeated measures ANOVA, $p = 2.1 \times 10^{-4}$), but had no effect on the noise entropy (repeated measures ANOVA, $p = 0.13$; Fig 2.5d). Learned motifs thus elicited a greater diversity of responses than novel motifs without compromising the reliability of responses, which increased the capacity of CLM neurons to convey information about learned stimuli.

We then asked what drives the increase in the total entropy of the neural response distribution. In principle, this change may be due to an increase in the total range of responses or to an increase in the number of distinct spike rates observed within a fixed range. Across all CLM neurons, we observed a slightly larger range of firing rates for rewarded motifs (16.5 ± 0.9 Hz) than for unrewarded (16.2 ± 0.8 Hz) or novel motifs (15.2 ± 0.9 Hz; Friedman test: $p = 0.005$). This increased range, however, did not fully account for the increased information encoding. The main effect of learning was unaltered in a control analysis where we omitted any response that fell outside the range of firing rates elicited by the novel motifs (Fig.

2.S4). Learning, therefore, increased the amount of information encoded by CLM neurons primarily by increasing the effectiveness with which this range was used.

Learning increases information encoded about motifs in CMM

Because of the substantial effects of learning on motif encoding in CLM, we next investigated whether learning also modified information encoding about individual motifs in CMM. Fig 2.6a shows the conditional probability distributions of firing rates for each set of motifs presented to the CMM neuron illustrated in Fig 2.2b. As with most CLM neurons, the diversity in this CMM neuron's firing rates was more closely tied to rewarded motifs than to unrewarded or novel motifs. Accordingly, this neuron encoded more information about rewarded motifs (0.91 bits) than about unrewarded motifs (0.60 bits) and novel motifs (0.09 bits). Over our entire sample, learning significantly modulated the information encoded by CMM neurons (repeated measures ANOVA, $p = 0.016$; Fig 2.6b,c). The mean amount of information encoded about rewarded motifs was 27.7% higher than that for novel motifs (paired t-test, $p = 0.012$; Fig 2.6c). Like CLM, the information encoded about unrewarded motifs was comparable to that encoded about novel motifs (paired t-test, $p = 0.41$). Although CMM encoded more information than CLM on average (Fig 2.3c), the effects of learning on neural encoding in both regions were comparable (mixed model ANOVA, interaction term, $p = 0.65$).

Across all CMM neurons, learning increased the total entropy (repeated measures ANOVA, $p = 0.019$), but had no significant effect on the noise entropy (Friedman test, $p = 0.09$; Fig 2.6d). Therefore, as in CLM neurons, learned motifs elicited a greater diversity of responses than novel motifs in CMM neurons, but this greater diversity did not substantially decrease the reliability of responses. We observed no significant learning-dependent increase in the range of firing rates evoked by single motifs (repeated measures ANOVA, $p = 0.08$). Thus, learning and reward enhanced neural encoding of motifs in similar ways in both CLM and CMM. In both regions, the effect of learning on information encoding was not dependent on our assumption that responses to repeated motifs are independent (Materials and Methods); similar differences were observed when repeated motifs are considered to be identical in the mutual information analysis (Fig. 2.S5).

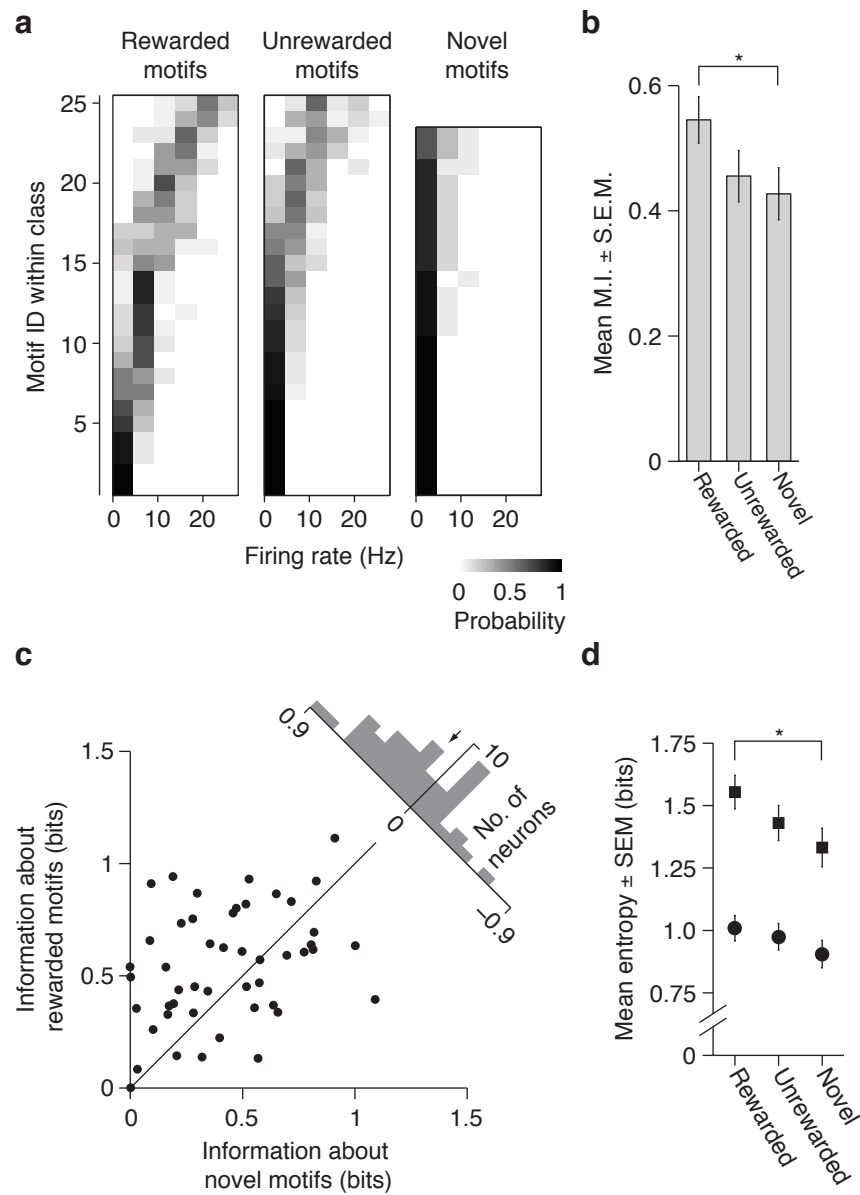


Figure 2.6. Effects of learning on encoding of motif identity in CMM. **(a)** Distributions of firing rates (F.R.) conditional on motif identity for rewarded (left), unrewarded (middle) and novel (right) motifs for the neuron shown in Fig. 2.2b. Conventions are the same as in Fig 2.5a. **(b)** Comparison of mean (\pm SEM) mutual information across all CMM neurons for rewarded, unrewarded, and novel motifs. Wilcoxon signed-rank test: * $p < 0.05$. **(c)** Scatter plot illustrating distributions of information values for novel motifs and rewarded motifs. Each point represents a single neuron. Upper right, histogram of differences between mutual information values for rewarded vs. novel motifs for all neurons. The arrow denotes the mean. **(d)** Mean (\pm SEM) total entropy (squares) and noise entropy (circles) values for all CMM neurons. Paired t-test: * $p < 0.05$.

Learning increases information encoded about motif categories

In addition to encoding more information about the identity of learned motifs than about novel motifs, CLM and CMM might also specifically encode information about the behaviorally relevant categories for motifs acquired through training (i.e rewarded, unrewarded, and novel). Such encoding could appear as any consistent difference in responses to motifs from different categories and thus is distinct from the foregoing analysis of information about motif identity. To explore this possibility, we formed firing rate distributions from responses to all motifs from these three categories (Fig 2.7a). From these distributions, we computed the information encoded by single neurons in CLM and CMM about the behaviorally-defined category of each motif. Because the behaviorally-defined categories are just one way that groups of motifs might be represented, we compared the information about learned categories to the distribution of information values when the category membership of each motif was randomly shuffled into other groupings (Materials and Methods). The example CMM neuron depicted in Fig 2.7a,b encoded 0.22 bits of information about motif category and only 0.03 ± 0.03 (mean \pm s.d.) bits about the randomly shuffled categories. Because different motifs can elicit very different firing rates in the same neuron, the information about motif category is small relative to information about motif identity. Nonetheless, the information about learned categories was significantly greater than the information about shuffled categories (evaluated at $p < 0.05$) in 45.8% (22/48) of CMM neurons and in 28.9% (28/97) of CLM neurons. These proportions are larger than would be expected by chance. On average, the information about behaviorally relevant categories of motifs was larger than the mean information about shuffled categories for neurons in both CLM (0.023 ± 0.002 bits vs. 0.014 ± 0.001 bits; Wilcoxon signed rank test, CLM: $p = 7.3 \times 10^{-8}$; Fig. 2.7b) and CMM (0.072 ± 0.009 bits vs. 0.023 ± 0.002 bits; Wilcoxon signed rank test, $p = 1.2 \times 10^{-8}$; Fig. 2.7c). Correspondingly, neurons in CMM encoded significantly more information about behaviorally relevant categories than neurons in CLM on average (Wilcoxon rank sum test: $p = 1.95 \times 10^{-7}$; Fig 2.7d). Thus, while both CLM and CMM encode information about the learned motif categories, neurons in CMM encode significantly more of this information than neurons in CLM.

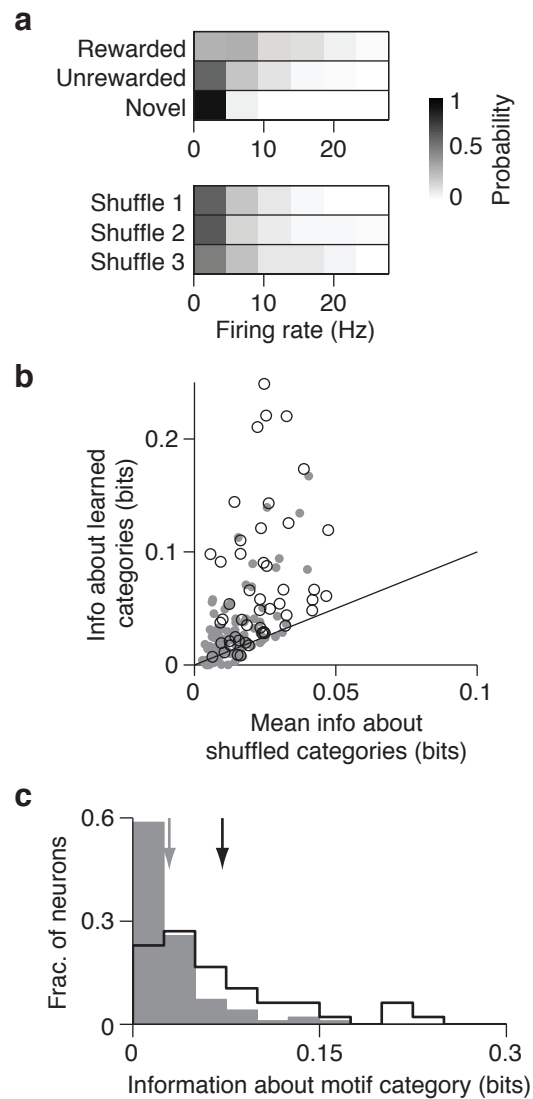


Figure 2.7. Effects of learning on encoding of motif category in CLM and CMM. **(a)** Probability distribution for the sample CMM neuron depicted in Fig 2b of firing rates in response to motifs, conditional on behavioral category. **(b)** Probability distribution of the same CMM neuron conditional on a randomly shuffled set of categories. **(c)** Comparison of information encoded about the learned motif categories (rewarded, unrewarded, novel), and the mean information encoded about 100 permutations of randomly shuffled categories for all CLM (gray dots) and CMM (open circles) neurons. Black line is the unity line. **(d)** Distributions of amounts of information about motif category encoded by CLM neurons (gray bars) and CMM neurons (black outline). Arrows denote means for CLM (gray) and CMM (black).

Learning increases information encoded about song categories

Because the firing rate of CLM and CMM neurons typically varies substantially over the motifs within a song (e.g. Fig 2.2) while the behavioral category (i.e. rewarded, unrewarded, or novel) remains unchanged, we reasoned that the firing rate averaged over the course of the song may better represent the behavioral category. As for motifs, we formed firing rate distributions from responses to each song from the three behavioral categories as well as for all permutations of shuffled categories (Fig 2.8a; Materials and Methods). From these distributions, we computed the information encoded by single neurons in CLM and CMM about the learned categories and shuffled categories. Because there are only two songs per category, there are only eight distinct permutations in which all categories are shuffled. Thus, we compared the information about the learned categories with the information about the shuffled set of categories that encoded the maximum information. The example CMM neuron depicted in Fig 2.8a encoded 0.99 bits of information about song category and a maximum of 0.68 bits about randomly shuffled categories. We found that the information about learned categories was significantly greater than the maximum information about shuffled categories in 37.5% (18/48) of CMM neurons but in only 22.7% (22/97) of CLM neurons. These percentages reflect significant differences between the populations of CLM and CMM neurons. In CMM, on average, information about the song category (0.42 ± 0.04 bits) was significantly greater than the mean information about shuffled categories (0.28 ± 0.03 bits; paired t-test: 4.7×10^{-4} ; Fig 2.8c) In CLM, in contrast, information about the song category (0.19 ± 0.03 bits) was similar to the mean information about shuffled categories (0.17 ± 0.02 bits; paired t-test: $p = 0.11$; Fig 2.8c). Correspondingly, neurons in CMM encoded more category information than neurons in CLM on average (Wilcoxon rank sum test: $p = 7.4 \times 10^{-7}$; Fig 2.8d). This difference can also be observed by comparing the mean firing rates between the rewarded and novel songs (Fig 2.8e) and between the rewarded and unrewarded songs (Fig 2.8f). The average firing rate differences were slightly greater in CMM than in CLM, but variance of these differences was much greater in CMM than in CLM (chi-square variance test: rewarded vs. novel, $p = 2.1 \times 10^{-11}$; rewarded vs. unrewarded, $p = 2.3 \times 10^{-4}$). Learning therefore strongly modulates (by either increasing or decreasing) the average firing rate responses of CMM neurons to

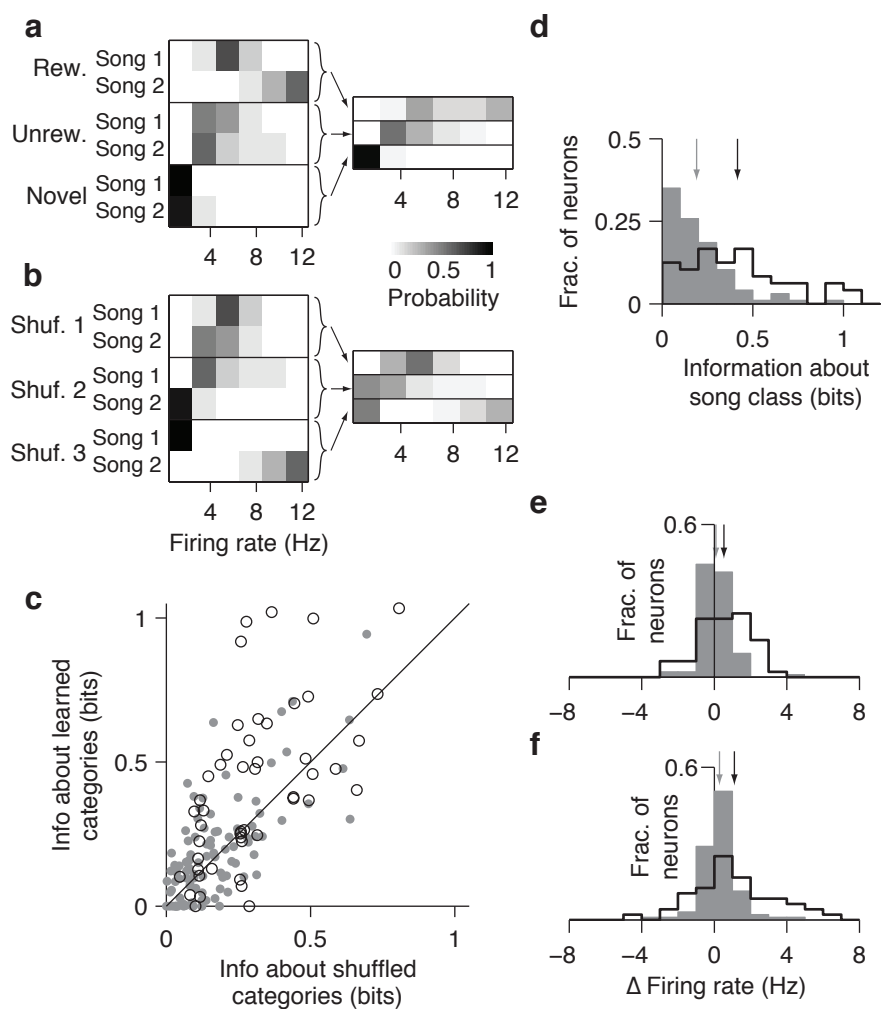


Figure 2.8. Effects of learning on encoding of song category in CLM and CMM. **(a)** Probability distributions of firing rates in response to songs for the sample CMM neuron depicted in Fig 2.2b, conditional on song identity (left) and behavioral category (right). Arrows depict the construction of category-conditional distributions. **(b)** Probability distributions as in (a) but for the randomly shuffled category with the highest information. **(c)** Comparison of information encoded about the learned categories (rewarded, unrewarded, novel), and the mean information about randomly shuffled categories for all CLM (gray dots) and CMM (open circles) neurons. Black line is the unity line. **(d)** Distributions of category information values for CLM neurons (gray bars) and CMM neurons (black outline). Arrows denote means for CLM (gray) and CMM (black). **(e)** Distribution of the change in average firing rate between novel songs and rewarded songs for CLM neurons (gray bars) and CMM neurons (black outline). Positive values indicate higher firing rates for rewarded songs. Arrows denote means for CLM (gray) and CMM (black). **(f)** Distribution of the change in average firing rate between unrewarded songs and rewarded songs for CLM neurons (gray bars) and CMM neurons (black outline). Positive values indicate higher firing rates for rewarded songs. Arrows are as in (e).

enhance the encoding of song category, but such modulation is much less pronounced in CLM neurons.

Discussion

The complex (Meliza et al., 2010), learning-dependent encoding of song by CMM neurons (Gentner and Margoliash, 2003) suggests that these representations are the product of an extensive neural processing network. Our results reveal some of the functional characteristics of that network by highlighting multiple differences between the encoding properties of individual CLM and CMM neurons, and by demonstrating that learning modifies these encoding properties. Together, these results suggest that CLM and CMM are part of a functional sensory circuit across which representations of natural vocal signals become increasingly informative with respect to behavior.

Coding along the avian auditory processing pathway

CLM and CMM sit near the top of a sensory processing pathway along which neural responses get progressively more complex. Within Field L, neurons in the L1 and L3 sub-regions selectively encode species-specific vocalizations more than neurons in the thalamorecipient Field L2 (Bonke et al., 1979; Langner et al., 1981). Linear spectrotemporal receptive field (STRF) models of neurons in Field L2a predict neural responses substantially better than the same models for neurons in CLM, indicating that response nonlinearities increase from L2a to CLM (Sen et al., 2001). Nonlinear stimulus transformations, such as the spectrotemporal “surprise,” substantially improve the predictive power of STRF models for CLM neurons, but only moderately for Field L neurons, again highlighting the increase in nonlinear processing between CLM and Field L (Gill et al., 2008). In addition, some neurons in CLM show a moderate preference to respond to the bird’s own song over other conspecific songs (Bauer et al., 2008), a hallmark of neural complexity (Margoliash, 1983) not observed in Field L (Amin et al., 2004; Shaevitz and Theunissen, 2007).

The differences in neural processing between CLM and CMM resemble those within known hierarchical circuits. First, neurons in CMM have higher motif selectivity than neurons in CLM. Selectivity often increases along ascending hierarchical circuits, including pathways in the visual (Maunsell and Newsome, 1987; Rust and DiCarlo, 2010) and auditory (Janata and Margoliash, 1999; Kikuchi et al., 2010)

systems. Second, neurons in CMM encode more information about motif identity than neurons in CLM. In many sensory processing pathways, neurons at higher levels encode abstract concepts such as object identity whereas neurons at lower levels process the physical components of those objects (Nelken, 2004; Winer et al., 2005; Chechik et al., 2006; Nahum et al., 2008; Russ et al., 2008). Like visual objects, motifs are high-level concepts that are abstracted from the physical combinations of sounds from which they are composed (Gentner and Hulse, 2000; Gentner, 2008; Seeba and Klump, 2009). Third, neurons in CMM exhibit more variability in their responses to repeated motifs of the same type than CLM neurons. Because these repeated motifs have subtle acoustic differences and different positions within the song, we cannot attribute the increased sensitivity of CMM neurons exclusively to either feature. Nonetheless, sensitivity to subtle differences in complex stimuli, such as faces, is a hallmark of responses at high levels in known hierarchical circuits (Desimone et al., 1984), and temporal context-sensitivity increases between the auditory thalamus and auditory cortex in mammals (Asari and Zador, 2009). Collectively, all three of these coding differences—motif selectivity, information, and variability across motif renditions—suggest that neural representations in CMM are more complex than in CLM, and thus support the hypothesis that CLM and CMM are a part of functional hierarchical neural circuit.

Even with the evidence provided here, there are several reasons to be cautious of drawing too strict a conclusion about hierarchical processing across CLM and CMM. First, connectivity between the two regions is reciprocal (Vates et al., 1996), which could make the precise flow of information multifaceted and complex (but does not necessarily preclude hierarchical processing; Van Essen et al., 1992). Second, CMM shares a strong reciprocal connection with the caudomedial nidopallium (NCM), another secondary auditory forebrain region that receives input from Field L (Vates et al., 1996). Responses of NCM neurons are also modified by song-recognition learning (Thompson and Gentner, 2010), and thus may also contribute directly to the emergence of complex, learning-dependent responses in CMM. Finally, the response properties of neurons in both CLM and CMM are heterogeneous and partially overlapping, suggesting that multiple pathways of information flow may be present. Regardless of the specific underlying architecture, however, our data show significant functional differences between CLM and CMM.

Learning modifies information encoding in CLM and CMM

Our results suggest that learning acts on CLM and CMM neurons in at least two ways: by increasing the information about motif identity and by increasing the information about behaviorally defined song categories. We found that both CLM and CMM neurons encoded more information about the identity of the learned motifs than about the identity of novel motifs. Behavioral experiments suggest that starlings recognize conspecifics by memorizing the motifs that compose their repertoires (Gentner and Hulse, 2000). The preferential encoding of the learned motifs by neurons in CLM and CMM may be a part of this stored memory. Alternatively, because of the rich acoustical structure of starling songs, identification could be achieved by learning a subset of the motifs that the bird finds particularly useful for recognition. The additional information encoded by CLM and CMM neurons about the learned motifs may therefore reflect changes in the representations of the most useful motifs. Consistent with this, the strongest effects of learning occur for the motifs paired with reward during training (Figs. 2.5-2.6; Gentner and Margoliash, 2003), pointing to a role for positive reinforcement in shaping the neural codes in both regions. Similar effects have been reported in primary cortical areas in mammals (Blake et al., 2006; Polley et al., 2006). Because learning increases the information encoded about motifs similarly in CLM and CMM, at least some of the learning-dependent representations in CMM (Gentner and Margoliash, 2003) may be inherited from responses in CLM.

Neurons in CLM and CMM also encoded information about the learned behavioral categories and this information was substantially larger in CMM than in CLM. Given that each bird's task was to distinguish between rewarded and unrewarded songs, we hypothesize that neural activity in both regions contributes to this cognitive process and supports categorical processing in post-synaptic targets (Prather et al., 2009). Phenomenologically, our results are similar to the processing of learned categories along the primate dorsal and ventral visual pathways (Freedman et al., 2001, 2003; Freedman and Assad, 2006). The neural encoding of behaviorally relevant categories (i.e. the grouping of signals that share behavioral meanings with similar neural representations) may be a general adaptive principle of cortical sensory processing to organize the complexity of sensory input (Merzenich and deCharms, 1996; Freedman and

Miller, 2008; Hoffman and Logothetis, 2009; Seger and Miller, 2010). To date, however, the behavioral modulation of categorical processing has been studied primarily in the primate visual system and the underlying circuitry remains poorly understood. The emergence of categorical representations between CLM and CMM provides an excellent opportunity to study the encoding of natural acoustic categories at the cellular and circuit level.

Multiple pathways are likely to be involved in the transformation of information between CLM and CMM. For example, because CLM neurons encode relatively small amounts of information about learned categories, a single CMM neuron that processes convergent input from many CLM neurons could amplify this effect substantially. Recent results that the responses of some CMM neurons to whole motifs are well-modeled by a combination of the responses to motif components (Meliza et al., 2010) are consistent with a general pattern of convergence into CMM. Furthermore, synaptic input from NCM neurons, which elicit weaker responses to learned songs than to novel songs (Thompson and Gentner, 2010), likely contributes to the encoding of learned categories in CMM. Because CMM contains large numbers of GABA_A-positive neurons (Pinaud et al., 2004), signals from NCM may specifically suppress the activity in CMM for novel songs. Additional studies will be necessary to compare the roles of CLM and NCM in shaping CMM responses.

This circuit will also be highly valuable for tracking changes in neural encoding over the course of learning, which is extremely difficult to do in primate models because of the large amounts of time required to train monkeys (Hoffman and Logothetis, 2009; c.f. Messinger et al., 2001). In contrast, starlings can learn to recognize songs very quickly (unpublished observations). With awake, behaving recording techniques, the modification of neural responses to encode newly learned categories could be readily observed. Thus, our identification of the emergence of behaviorally relevant information about songs along the CLM to CMM pathway highlights it as an especially valuable model for studying the circuit and plasticity mechanisms that underlie the selective neural processing of learned signals.

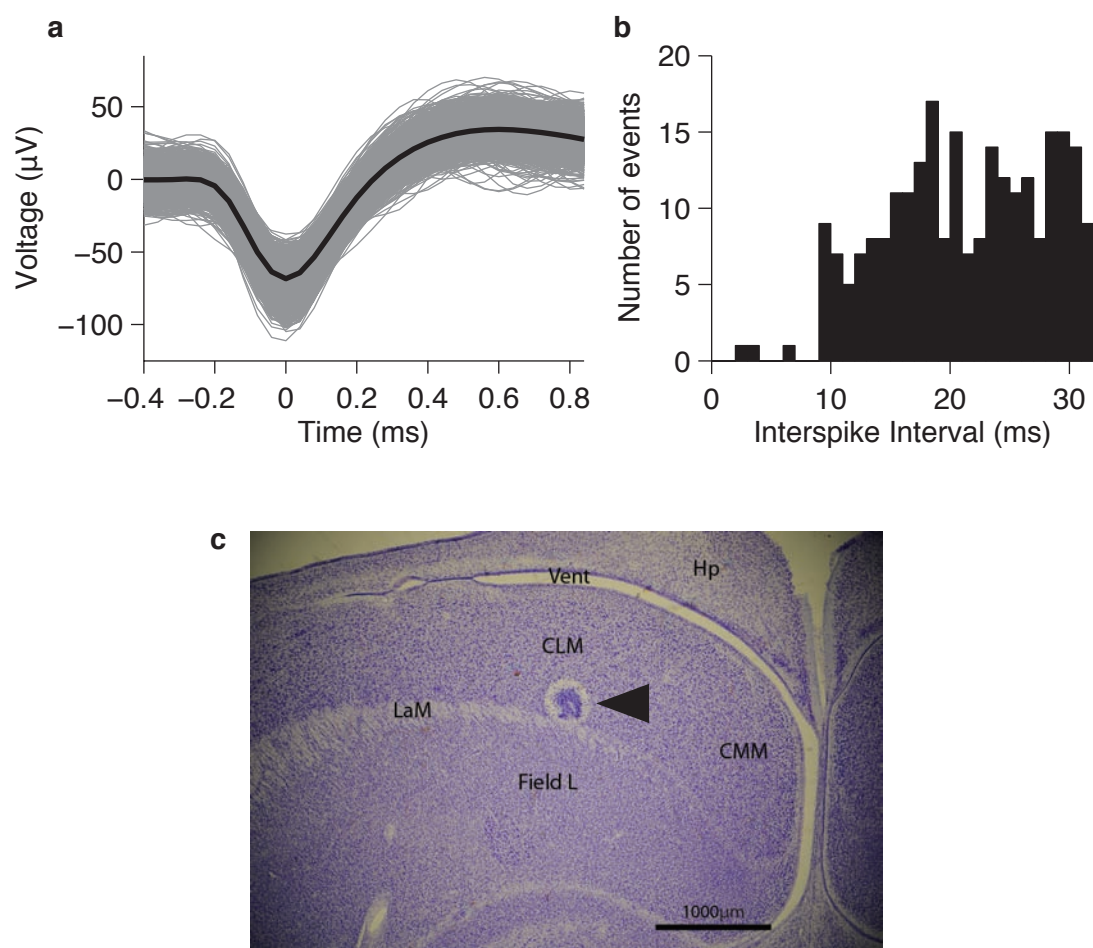


Figure 2.S1. Spike sorting and histology. **(a)** Superimposed plots of extracellular action potential shape for the neuron shown in figure 2.2a. Light grey lines are individual action potential waveforms, black line is mean action potential shape. **(b)** Interspike interval histogram for the same neuron showing very few refractory period violations. **(c)** Photomicrograph of Nissl-stained coronal tissue section showing fiduciary electrolytic lesion in CLM (denoted by arrowhead) at the bottom of a recording penetration. Scale bar = 1000 μm . LaM: Mesopallial Lamina.

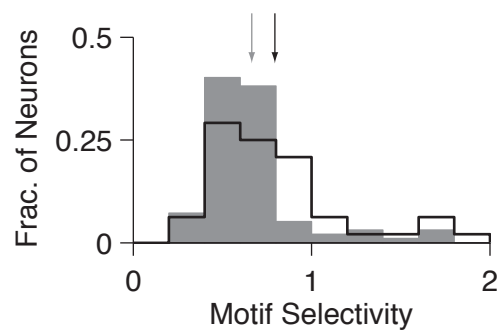


Figure 2.S2. Comparison of selectivity values for CLM and CMM neurons using the entropy method (Materials and Methods). Gray bars denote CLM distribution and black outlines denote CMM distribution. On average, selectivity in CLM was 0.66 ± 0.03 and selectivity in CMM was 0.79 ± 0.05 (Wilcoxon rank sum test, $p = 0.038$).

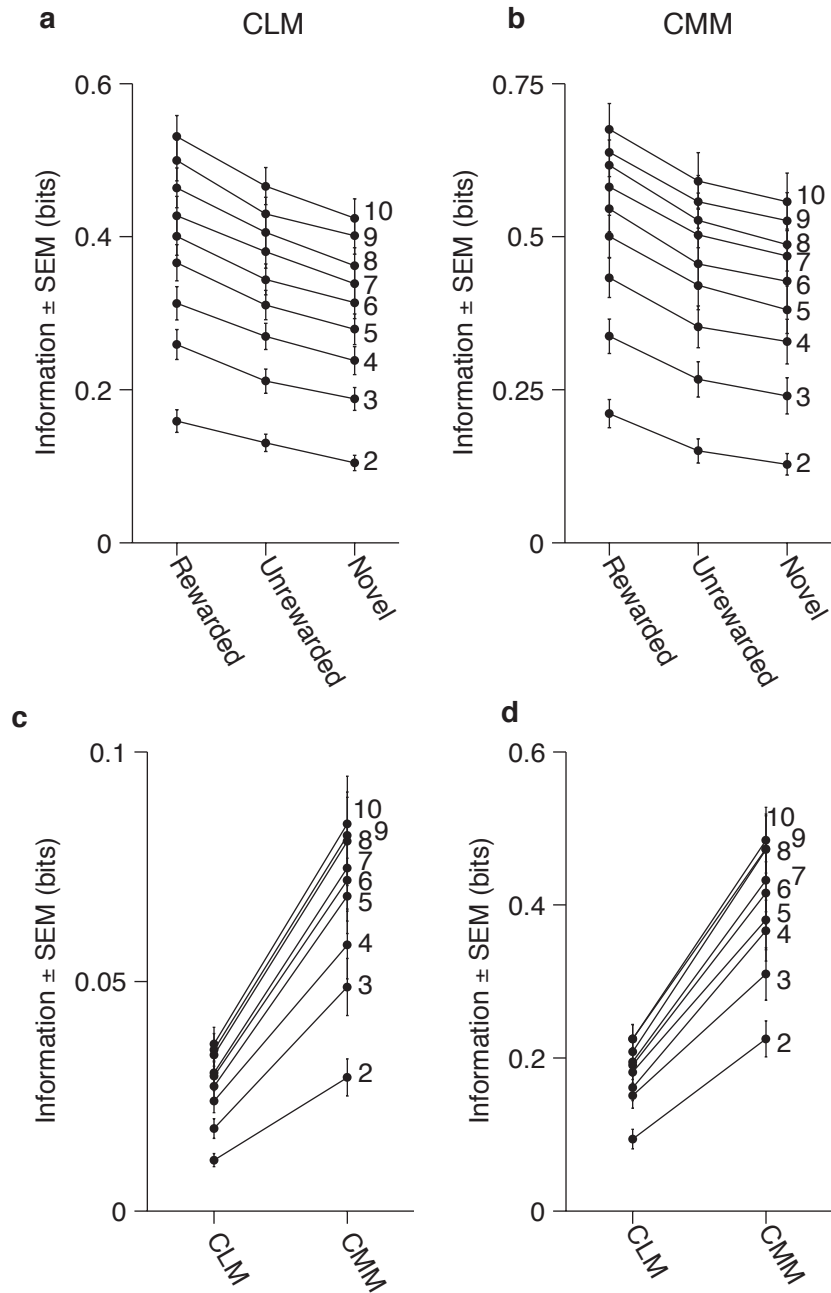


Figure 2.S3. Effects of varying the number of bins in information calculations. **(a)** Information about motif identity in CLM. **(b)** Information about motif identity in CMM. **(c)** Information in motifs about category. **(d)** Information in songs about category. In all plots, the numbers to the right of each set of connected points denotes the number of bins used for that calculation. Increasing the number of bins increases the number of bits, but the overall effects of learning are not affected.

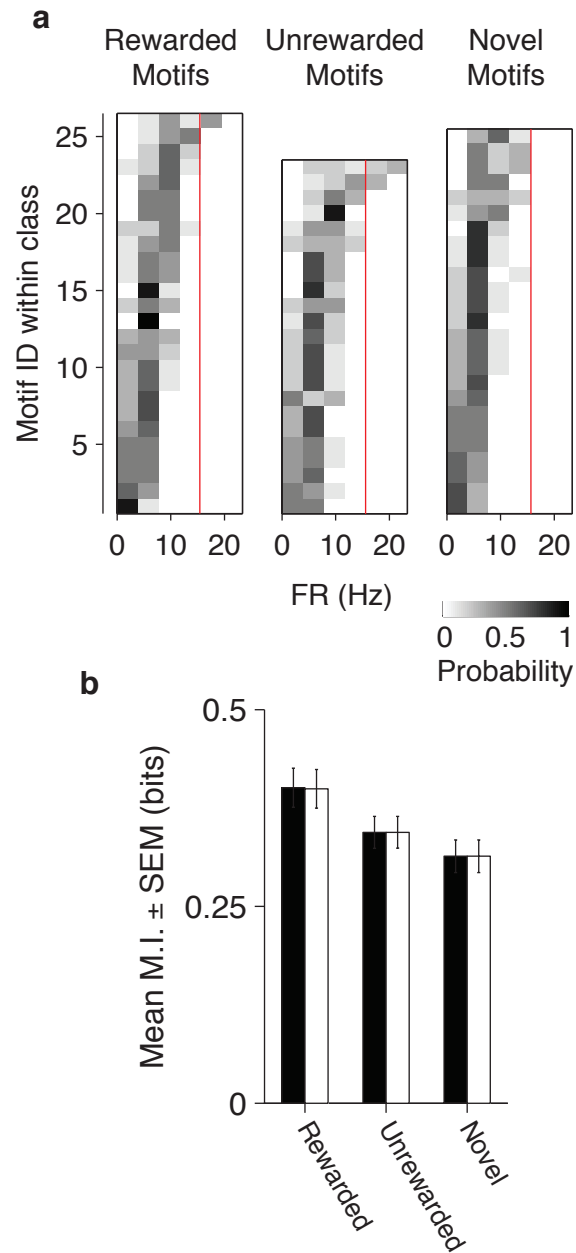


Figure 2.S4. Effects of limiting firing rate range on single neuron information encoding in CLM. **(a)** Conditional probability distributions of a sample CLM neuron. Red bars indicate the maximum firing rate elicited by a novel motif. Information was computed using six bins linearly spaced from the minimum novel firing rate to the maximum novel firing rate. Responses outside this range were ignored in this analysis. **(b)** Comparison of mean (\pm SEM) information encoded under this restricted firing rate range (grey bars; Friedman test, $p = 1.7 \times 10^{-4}$) with the mean information encoded under the full firing rate range (black bars; Friedman test, $p = 1.7 \times 10^{-4}$). No difference is observed between the effects of learning under the two conditions (two-way repeated measures ANOVA interaction term: $p = 0.19$).

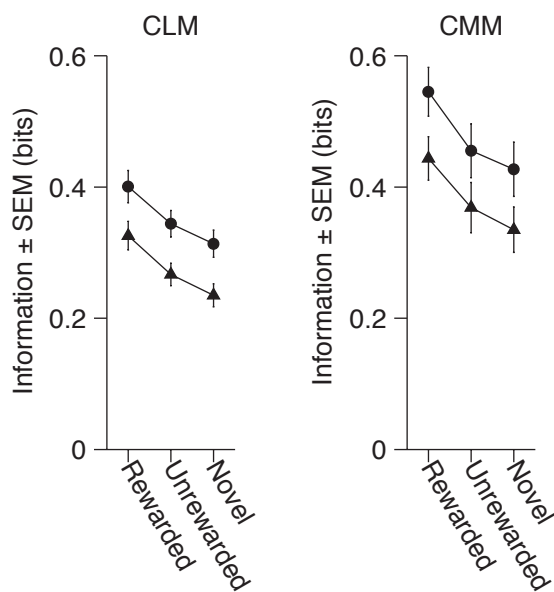


Figure 2.S5. Effects of treating multiple renditions of the same motif type as identical motifs for the purposes of information encoding in CLM (left) and CMM (right). Circles denote information when all renditions are considered independent (as in the main text). Triangles denote information when all renditions of a given motif type are considered to be identical. No differences are found between the two calculations (2-way repeated measures ANOVA interaction; CLM: $p = 0.87$; CMM: $p = 0.42$).

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References

- Amin N, Grace JA, Theunissen FE (2004) Neural response to bird's own song and tutor song in the zebra finch field L and caudal mesopallium. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 190:469-489.
- Asari H, Zador AM (2009) Long-lasting context dependence constrains neural encoding models in rodent auditory cortex. *J Neurophysiol* 102:2638-2656.
- Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R (2008) A synaptic basis for auditory-vocal integration in the songbird. *J Neurosci* 28:1509-1522.
- Binder JR, Frost JA, Hammeke TA, Bellgowan PS, Springer JA, Kaufman JN, Possing ET (2000) Human temporal lobe activation by speech and nonspeech sounds. *Cereb Cortex* 10:512-528.
- Blake DT, Heiser MA, Caywood M, Merzenich MM (2006) Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. *Neuron* 52:371-381.
- Bonke D, Scheich H, Langner G (1979) Responsiveness of units in the auditory neostriatum of the guinea fowl (*Numida meleagris*) to species-specific calls and synthetic stimuli *J Comp Physiol A* 132:1432-1351.
- Brenner N, Strong S, Koberle R, Bialek W (2000) Synergy in a neural code. *Neural Comput* 12:1531-1552.
- Chaiken M, Bohner J, Marler P (1993) Song Acquisition in European Starlings, *Sturnus vulgaris* - a comparison of the songs of live-tutored, tape-tutored, untutored, and wild-caught males. *Anim Behav* 46:1079-1090.
- Chechik G, Anderson MJ, Bar-Yosef O, Young ED, Tishby N, Nelken I (2006) Reduction of information redundancy in the ascending auditory pathway. *Neuron* 51:359-368.
- Cover T, Thomas J (2006) *Elements of Information Theory*, 2 Edition. Hoboken, New Jersey: John Wiley & Sons, Inc.
- Desimone R, Albright TD, Gross CG, Bruce C (1984) Stimulus-selective properties of inferior temporal neurons in the macaque. *J Neurosci* 4:2051-2062.
- Eens M (1997) Understanding the Complex Song of the European Starling: An Integrated Ethological Approach. *Adv Study Behav* 26:355-434.
- Felleman DJ, Van Essen DC (1991) Distributed hierarchical processing in the primate cerebral cortex. *Cereb Cortex* 1:1-47.
- Franco L, Rolls ET, Aggelopoulos NC, Jerez JM (2007) Neuronal selectivity, population sparseness, and ergodicity in the inferior temporal visual cortex. *Biol Cybern* 96:547-560.
- Freedman DJ, Assad JA (2006) Experience-dependent representation of visual categories in parietal cortex. *Nature* 443:85-88.

- Freedman DJ, Miller EK (2008) Neural mechanisms of visual categorization: insights from neurophysiology. *Neurosci Biobehav R* 32:311-329.
- Freedman DJ, Riesenhuber M, Poggio T, Miller EK (2001) Categorical representation of visual stimuli in the primate prefrontal cortex. *Science* 291:312-316.
- Freedman DJ, Riesenhuber M, Poggio T, Miller EK (2003) A comparison of primate prefrontal and inferior temporal cortices during visual categorization. *J Neurosci* 23:5235-5246.
- Gentner TQ (2008) Temporal scales of auditory objects underlying birdsong vocal recognition. *J Acoust Soc Am* 124:1350-1359.
- Gentner TQ, Hulse SH (2000) Perceptual classification based on the component structure of song in European starlings. *J Acoust Soc Am* 107:3369-3381.
- Gentner TQ, Margoliash D (2003) Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669-674.
- Gill P, Woolley SMN, Fremouw T, Theunissen FE (2008) What's that sound? Auditory area CLM encodes stimulus surprise, not intensity or intensity changes. *J Neurophysiol* 99:2809-2820.
- Hoffman KL, Logothetis NK (2009) Cortical mechanisms of sensory learning and object recognition. *Philos Trans R Soc Lond B Biol Sci* 364:321-329.
- Hsu A, Woolley SM, Fremouw TE, Theunissen FE (2004) Modulation power and phase spectrum of natural sounds enhance neural encoding performed by single auditory neurons. *J Neurosci* 24:9201-9211.
- Janata P, Margoliash D (1999) Gradual emergence of song selectivity in sensorimotor structures of the male zebra finch song system. *J Neurosci* 19:5108-5118.
- Kaas JH, Hackett TA (2000) Subdivisions of auditory cortex and processing streams in primates. *Proc Natl Acad Sci U S A* 97:11793-11799.
- Kikuchi Y, Horwitz B, Mishkin M (2010) Hierarchical auditory processing directed rostrally along the monkey's supratemporal plane. *J Neurosci* 30:13021-13030.
- Langner G, Bonke D, Scheich H (1981) Neuronal discrimination of natural and synthetic vowels in field L of trained mynah birds. *Exp Brain Res* 43:11-24.
- Lehky SR, Sejnowski TJ, Desimone R (2005) Selectivity and sparseness in the responses of striate complex cells. *Vision Res* 45:57-73.
- Macmillan NA, Creelman CD (2005) *Detection Theory, A User's Guide*. Mahwah, NJ: Lawrence Erlbaum Associates, Inc. .
- Margoliash D (1983) Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. *J Neurosci* 3:1039-1057.
- Maunsell JH, Newsome WT (1987) Visual processing in monkey extrastriate cortex. *Annu Rev Neurosci* 10:363-401.
- Meliza CD, Chi Z, Margoliash D (2010) Representations of conspecific song by starling secondary forebrain auditory neurons: toward a hierarchical framework. *J Neurophysiol* 103:1195-1208.
- Merzenich MM, deCharms RC (1996) Neural representations, experience, and change. In: *The Mind-Brain Continuum* (Llinas R, Churchland PS, eds), pp 61-81. Cambridge, MA: MIT Press.

- Messinger A, Squire LR, Zola SM, Albright TD (2001) Neuronal representations of stimulus associations develop in the temporal lobe during learning. *Proc Natl Acad Sci U S A* 98:12239-12244.
- Nahum M, Nelken I, Ahissar M (2008) Low-level information and high-level perception: the case of speech in noise. *Plos Biol* 6:e126.
- Nelken I (2004) Processing of complex stimuli and natural scenes in the auditory cortex. *Curr Opin Neurobiol* 14:474-480.
- Panzeri S, Senatore R, Montemurro MA, Petersen RS (2007) Correcting for the sampling bias problem in spike train information measures. *J Neurophysiol* 98:1064-1072.
- Pinaud R, Velho TAF, Jeong JK, Tremere LA, Leão RM, von Gersdorff H, Mello CV (2004) GABAergic neurons participate in the brain's response to birdsong auditory stimulation. *Eur J Neurosci* 20:1318-1330.
- Polley DB, Steinberg EE, Merzenich MM (2006) Perceptual learning directs auditory cortical map reorganization through top-down influences. *J Neurosci* 26:4970-4982.
- Prather JF, Nowicki S, Anderson RC, Peters S, Mooney R (2009) Neural correlates of categorical perception in learned vocal communication. *Nat Neurosci* 12:221-228.
- Rauschecker JP, Scott SK (2009) Maps and streams in the auditory cortex: nonhuman primates illuminate human speech processing. *Nat Neurosci* 12:718-724.
- Riesenhuber M, Poggio T (2000) Models of object recognition. *Nat Neurosci* 3 Suppl:1199-1204.
- Rolls ET, Tovee MJ (1995) Sparseness of the neuronal representation of stimuli in the primate temporal visual cortex. *J Neurophysiol* 73:713-726.
- Rolls ET, Baylis GC, Hasselmo ME, Nalwa V (1989) The effect of learning on the face selective responses of neurons in the cortex in the superior temporal sulcus of the monkey. *Exp Brain Res* 76:153-164.
- Russ BE, Ackelson AL, Baker AE, Cohen YE (2008) Coding of auditory-stimulus identity in the auditory non-spatial processing stream. *J Neurophysiol* 99:87-95.
- Rust NC, DiCarlo JJ (2010) Selectivity and tolerance ("invariance") both increase as visual information propagates from cortical area V4 to IT. *J Neurosci* 30:12978-12995.
- Seeba F, Klump GM (2009) Stimulus familiarity affects perceptual restoration in the European starling (*Sturnus vulgaris*). *PLoS One* 4:e5974.
- Seger CA, Miller EK (2010) Category learning in the brain. *Annu Rev Neurosci* 33:203-219.
- Sen K, Theunissen FE, Doupe AJ (2001) Feature analysis of natural sounds in the songbird auditory forebrain. *J Neurophysiol* 86:1445-1458.
- Shaevitz SS, Theunissen FE (2007) Functional connectivity between auditory areas field L and CLM and song system nucleus HVC in anesthetized zebra finches. *J Neurophysiol* 98:2747-2764.
- Sigala N, Logothetis NK (2002) Visual categorization shapes feature selectivity in the primate temporal cortex. *Nature* 415:318-320.
- Strong SP, Koberle R, van Steveninck RRD, Bialek W (1998) Entropy and information in neural spike trains. *Phys Rev Lett* 80:197-200.

- Thompson JV, Gentner TQ (2010) Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *J Neurophysiol* 103:1785-1797.
- Treves A, Panzeri S (1995) The Upward Bias in Measures of Information Derived from Limited Data Samples. *Neural Comput* 7:399-407.
- Van Essen DC, Anderson CH, Felleman DJ (1992) Information processing in the primate visual system: an integrated systems perspective. *Science* 255:419-423.
- Vates GE, Broome BM, Mello CV, Nottebohm F (1996) Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366:613-642.
- Vinje WE, Gallant JL (2000) Sparse coding and decorrelation in primary visual cortex during natural vision. *Science* 287:1273-1276.
- Willmore B, Tolhurst DJ (2001) Characterizing the sparseness of neural codes. *Network* 12:255-270.
- Winer JA, Miller LM, Lee CC, Schreiner CE (2005) Auditory thalamocortical transformation: structure and function. *Trends Neurosci* 28:255-263.
- Woolley SM, Fremouw TE, Hsu A, Theunissen FE (2005) Tuning for spectro-temporal modulations as a mechanism for auditory discrimination of natural sounds. *Nat Neurosci* 8:1371-1379.
- Woolley SM, Gill PR, Fremouw T, Theunissen FE (2009) Functional groups in the avian auditory system. *J Neurosci* 29:2780-2793.

III. Learning alters neural encoding of natural auditory signals in relation to their informativeness for behavior

Abstract

Learning plays an important role in the modification of neural encoding in the brain, by changing cortical maps of sensory space to increase the coverage of learned signal parameters and by forming single-neuron representations of complex learned signals. However, neural processing of natural sensory signals is a complex process that unfolds over time and involves coordinated activity across many regions of the brain. Thus, it is important to understand how learning modifies spatially and temporally distributed neural representations. Using European starlings trained to recognize short segments of natural birdsong (motifs), we show here that learning alters the neural encoding of motifs in a spatially and temporally dissociated manner within the caudal mesopallium (CM), a higher order auditory area in the songbird. Neurons in medial CM (CMM) primarily encode motif familiarity, whereas neurons in lateral CM (CLM) primarily encode the behavioral informativeness of motifs. Moreover, this encoding emerges over time, being most pronounced in CMM early in the motif and in CLM towards the end of the motif. Finally, the strength of encoding of familiarity and behavioral meaning decreased with duration of behavioral experience. Together, these findings implicate both CLM and CMM in a neural circuit that extracts behaviorally relevant information from complex natural stimuli.

Introduction

Plasticity alters the neural representations of sensory signals in the cortex as a function of familiarity and association with reward (Bakin and Weinberger, 1990; Recanzone et al., 1993; Gentner and Margoliash, 2003; Blake et al., 2006; Jeanne et al., 2011). Furthermore, subcortical signals of reward from the ventral tegmental area can elicit a similar reshaping of cortical encoding (Bao et al., 2001). However, in many cases, rewarded signals may be complex and high dimensional, such that multiple features can be informative while others uninformative for the task at hand. For example, one can distinguish a raven from

a crow by its size or its voice, but not by its color. Because the informativeness of natural sensory signals can vary substantially, it is important to understand how it influences the plasticity of neural encoding. By altering the importance of stimulus features, previous studies have demonstrated that categorical representations of non-natural signals in the brain can be manipulated by behavioral context (e.g. Sigala and Logothetis, 2002; Freedman and Assad, 2006; Mirabella et al., 2007; Zhang et al., 2010). However, the role of behavioral relevance on forming long-lasting representations of natural signals is currently unknown. The European starling (*Sturnus vulgaris*) is an excellent model for exploring the influence of learning on neural encoding of natural stimuli, because its behavior can be easily controlled in laboratory settings, quickly learns new songs, and has well-identified auditory processing circuitry (Gentner and Margoliash, 2003). Starlings sing spectrotemporally complex songs composed of discrete collections of notes, called motifs, which are thought to function in conspecific recognition in the wild (Gentner and Hulse, 2000; Gentner, 2008). This recognition is mediated, in part, by the activity of neurons in the caudomedial mesopallium (CM), a secondary auditory area, which is modulated by learned association with reward (Gentner and Margoliash, 2003), forms categorical representations of learned motifs (Jeanne et al., 2011), and may function in the processing of auditory feedback during juvenile song learning (Keller and Hahnloser, 2009).

Here, we explore how learned behavioral relevance alters neural encoding in lateral CM (CLM) and medial CM (CMM) by experimentally manipulating the information that motifs convey about the motor output required to obtain reward, while controlling for the temporal association with reward. We find that informative motifs elicit higher firing rates than uninformative or novel motifs and that the firing rate difference between informative and uninformative motifs are most pronounced in CLM neurons. Furthermore, the temporal profile of these responses is dynamic, with the biggest effects of informativeness emerging within CLM near the end of the motif. Finally, these effects diminish with increasing time spent training, consistent with the expansion-renormalization hypothesis of cortical plasticity (Reed et al., 2011). Collectively, our results show that the information that natural auditory signals convey about behavior plays an important role in its neural representation during learning.

Materials and Methods

All procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of California, San Diego.

Stimuli All stimuli were constructed from 12 motifs (stereotyped clusters of natural starling song) taken from recordings of songs from three adult European starlings. Motifs were divided into three sets: four motifs (A,B,C,D,) were labeled “informative,” four motifs (E,F,G,H) were labeled “uninformative,” and four motifs (I,J,K,L) were labeled “novel.” Pairs of motifs were aligned sequentially with a 20ms gap of silence between them to form the stimuli used for behavioral training. Each stimulus contained exactly one informative and one uninformative motif, and they could occur in either order. The resulting 32 stimuli were divided into two groups for behavioral training: the 16 stimuli containing motifs A or B were used as class 1 (i.e. go left) and the 16 stimuli containing motifs C or B were used for class 2 (i.e. go right). All uninformative motifs occurred with equal probability in both class 1 and class 2 stimuli. The complete set of 12 motifs was presented in isolation (i.e. not paired) during all neural recording sessions.

Behavioral Training Nine wild-caught adult European starlings (*Sturnus vulgaris*) were trained using a two-alternative choice operant conditioning paradigm with established techniques (Gentner and Margoliash, 2003) to distinguish between stimuli from class 1 and class 2. Prior to training, none of the subjects had any exposure to these stimuli. All training took place inside a sound attenuation chamber with an operant response panel (Fig. 3.1a). Starlings initiated trials by inserting their beak into the center port of the response panel, which initiated the playback of one of the 32 stimuli from the speaker inside the chamber. After the end of playback, starlings had two seconds to indicate their response by pecking in either the left or the right port. Incorrect responses were punished by extinguishing the lights for 10-90 seconds, during which time the starling could not initiate further trials. Correct responses were rewarded by two-second access to food on a fixed ratio schedule of reinforcement: starlings had to respond correctly to a fixed number of trials sequentially before receiving food. The number of correct trials required for reward was gradually increased over time from 1 to 6. A secondary reinforcer (flashing of LEDs on the response

panel) was used on correct trials when the food reward was not delivered. Incorrect responses reset the running count of correct trials. The fixed ratio reinforcement was necessary to ensure that all motifs were presented an equal number of times, and to ensure that starlings could not systematically ignore any of the stimuli. At the end of training, starlings were presented with randomly reinforced probe stimuli consisting of each of the 8 training motifs presented in isolation, to confirm that they learned the task appropriately.

Electrophysiology Approximately 24 hours prior to electrophysiological recording, a small pin was attached to the surface of the skull under isoflurane anesthesia (1.5-2% concentration), after which starlings were allowed to recover. On the recording day, starlings were anesthetized with urethane (20% by volume, 7-8ml/kg) and head-fixed via the attached pin to a stereotactic apparatus inside a sound-attenuating chamber. A small craniotomy was made dorsal to CLM and CMM, and multi-channel silicon electrode arrays (177 μm^2 electrode surface area, 50 μm spacing, 1x16 and 1x32 electrode layout; NeuroNexus). 1x32 electrode arrays were generally inserted at a 35° angle (relative to horizontal) and simultaneously measured neural activity across the medial-lateral axis of CM. 1x16 electrode arrays were generally inserted at a 90° angle (relative to horizontal) directly into CMM. For some subjects, only the 1x32 array was used. Motif stimuli were presented free field from a speaker 30cm from the bird. Electrode arrays were advanced while presenting the 12 motif stimuli until 2 or more auditory single units were isolated. Once single units were isolated, all 12 single motifs and the set of training motif pairs were presented pseudo-randomly in blocks while the extracellular electrical activity was amplified (5000 \times gain; AM Systems), filtered (high pass, 300Hz; low pass, 3-5kHz), sampled (20kHz), and saved digitally for offline analysis (Spike2; Cambridge Electronic Design). Electrodes were coated with Di-I to facilitate localization of penetration tracks in histological sections.

Histology At the end of the recording session, starlings were euthanized with an overdose of nembutal (150ml/kg), and perfused transcardially with 10% neutral buffered formalin. Brains were removed from the skull and placed in 30% solution for several days for cryoprotection. Brains were then cut into 50 μm coronal sections on a freezing microtome and mounted on glass slides. Electrode penetration

tracks were identified with the assistance of Di-I marking and epifluorescence microscopy. Tissue was then stained with cresyl violet to localize penetration tracks to neuroanatomical boundaries. The recording location of each neuron was aligned to the position of its corresponding electrode track and its lateral distance from the midline was measured. The boundary between CLM and CMM was taken to be 900 μm . Recording locations for CMM neurons ranged from 0-895 μm and recording locations for CLM neurons ranged from 900-2081 μm .

Data Analysis The data reported here are a superset of previously reported neural recordings (Jeanne, et. al, 2012, not yet published). Putative action potentials in the recorded voltage traces were identified by amplitude, and sorted into single units with principal components analysis on waveform shape using Spike2 software (Cambridge Electronic Design). Only spike waveforms that formed a clear cluster in principal component space and which had very few refractory period violations were considered to be single units. Because the recording sites on each multi-channel array were only 50 μm apart, stereotrode sorts were used to further improve sorting quality. Only sites that were driven by the auditory stimulus were used in subsequent analyses.

Behavioral criterion (the point at which birds learned the task) was taken to be the point when the bird first performed the task above chance for 5 consecutive blocks of 200 trials. Behavioral performance in each non-overlapping block of 200 trials was analyzed using d' ,

$$d' = zscore(\text{Hit rate}) - zscore(\text{False alarm rate}),$$

a measure of signal discriminability (Macmillan and Creelman, 2005). Chance performance was evaluated as the 95% confidence interval of the distribution of d' values for 5000 sets of randomly generated responses to each trial in a block.

For whole motif analysis, firing rates were averaged over the duration of the motif starting 30ms after onset, and ending 30ms after offset, to account for signal propagation delay. For the analysis of the temporal response profile, the response was partitioned into bins 56.5ms in duration. Because the motifs used in the experiment were not of identical duration (range: 565-957ms, median: 756ms), only the first 10 bins after the motif onset were within the duration of all 12 motifs. To compare firing rate profiles over the

course of the motif, we defined two 200ms time windows within each motif. The “early period” lasted from 50-250ms after the start of the motif; the “late period” lasted from 300 to 100ms prior to the start of the motif. Unless specified otherwise, firing rate responses were normalized by computing the z-score over all driven responses. For analyses that considered the temporal profile of neural responses, the z-scores were computed over the mean firing rates of all time windows for all motifs for a given neuron.

Statistical Analysis All data were tested for normality using the Lilliefors test evaluated at $p < 0.05$. Nonparametric tests were used when data were not normal. All reported p-values are for Wilcoxon Signed-Rank tests, unless otherwise noted. Central tendencies are reported as means \pm standard errors of the mean.

Results

We trained 9 starlings to distinguish pairs of motifs using a two-alternative choice operant conditioning paradigm (Gentner and Margoliash, 2003) (Fig. 3.1a). Stimuli were sequentially constructed pairs of motifs (stereotyped clusters of notes that comprise full songs; Fig. 3.1b) such that one of the two motifs (the “informative” motif) indicated the correct behavioral response (go left or go right) while the other motif had no relation to the correct response (the “uninformative” motif; Fig. 3.1c). All motif pairs were presented with equal probability such that exposure to the relevant and irrelevant motifs was identical. Starlings learned this task quickly, performing above criterion after 7422 ± 728 trials (Fig. 3.1d; Methods). Mean performance during the final 200 trial block was $84.5 \pm 1.6\%$. Following behavioral training, we recorded the electrical activity of multiple well-isolated single units within the caudomedial mesopallium (CMM) and caudolateral mesopallium (CLM; Fig. 3.1e), and compared the spiking activity in response to informative and uninformative motifs and a third set of novel motifs (Fig. 3.1f,g).

We first investigated whether learning modified the average firing rate responses to motifs within CM. Figure 3.2 illustrates the responses of one neuron from CLM (Fig. 3.2a) and one from CMM (Fig. 3.2b). These neurons responded with the most spikes during presentation of informative motifs. Some of the uninformative motifs elicited moderate responses (e.g. motif A in Fig. 3.2b), but the novel motifs

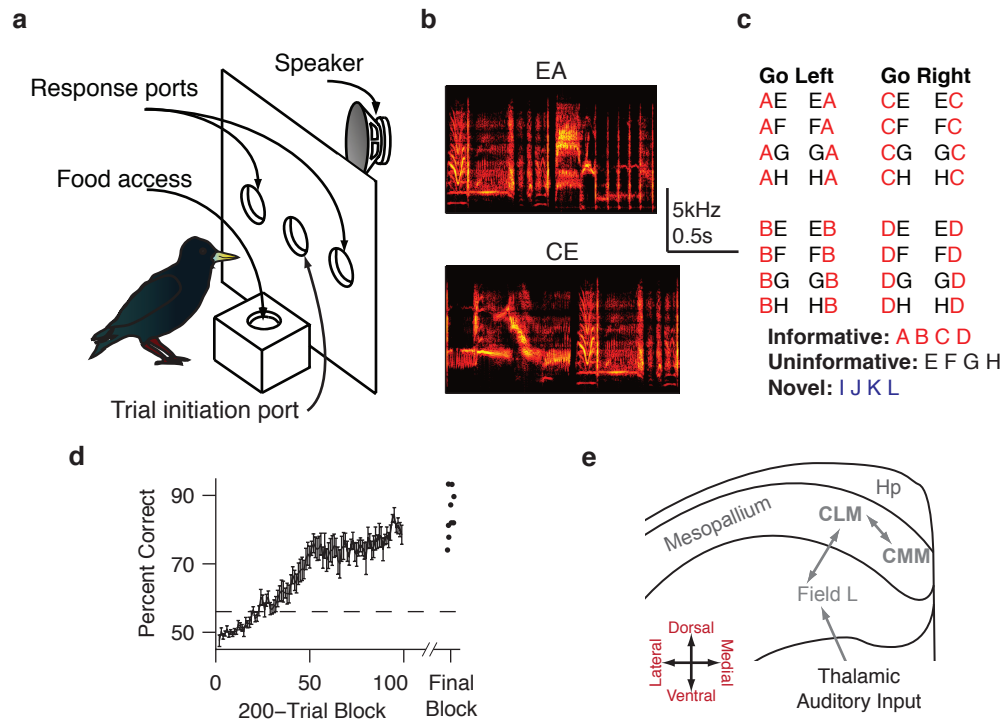


Figure 3.1. Behavioral training. **(a)** Setup of operant apparatus. **(b)** Example paired-motif stimulus used during training. Top: example go left stimulus composed from motifs E and A. Bottom: example go right stimulus composed from motifs C and E. **(c)** Complete set of stimuli used during training. Each letter denotes a single motif. Motifs in red are informative (i.e. indicate whether the correct response is go left or go right); motifs in black are uninformative (i.e. do not indicate whether the correct response is go left or go right). During electrophysiological recording, novel motifs (not used during training) are also presented. **(d)** Behavioral acquisition curve showing learning of the task. Each point is the mean percent correct within a non-overlapping 200 trial block. Dashed line indicates 95% confidence interval of above chance performance. **(e)** Schematic of starling avian forebrain anatomy showing CLM and CMM in relation to adjacent auditory areas.

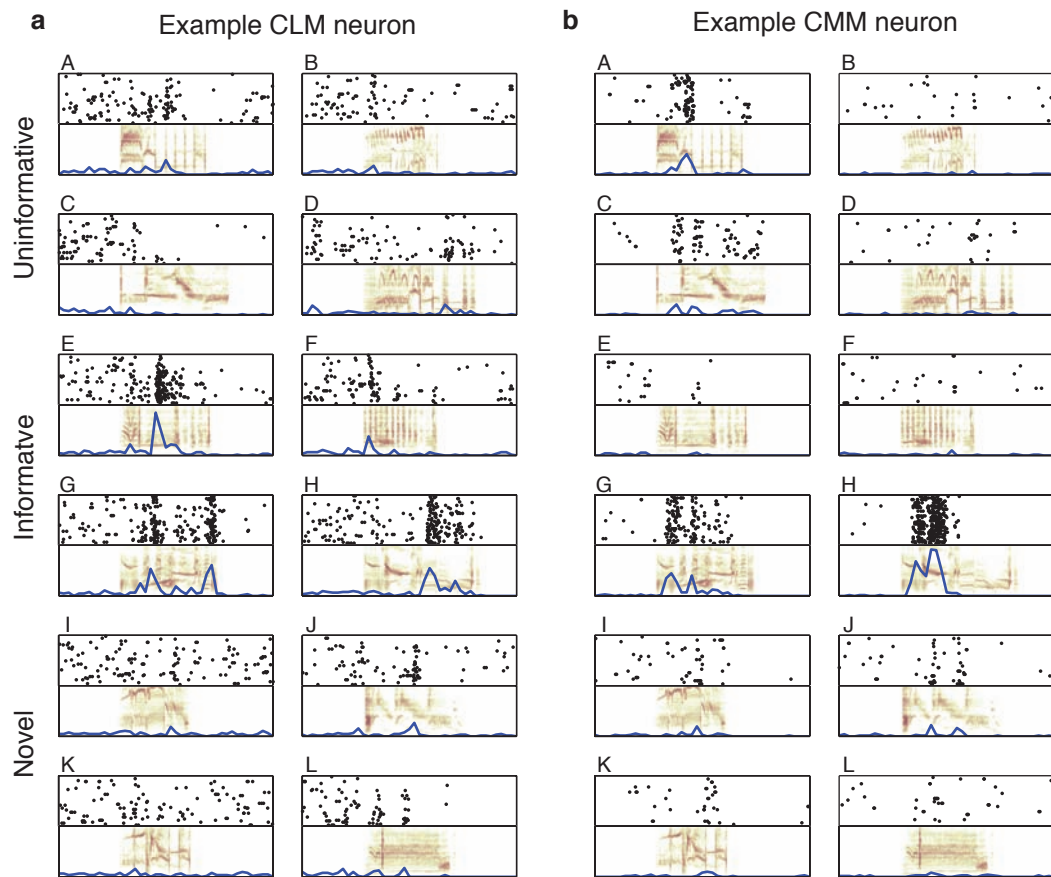


Figure 3.2. Raster plots and peri-stimulus time histograms of neural responses to two example cells from our dataset. **(a)** Example neuron from CLM. **(b)** Example neuron from CMM.

generally elicited very weak responses. On average, the behavioral category significantly modulated firing rates in neurons throughout CM (Friedman test, $p = 2.9 \times 10^{-6}$; Fig. 3.3a), with informative motifs eliciting higher rates ($4.03 \pm 0.025\text{Hz}$) than uninformative motifs ($3.84 \pm 0.24\text{Hz}$) and novel motifs ($3.66 \pm 0.25\text{Hz}$). Despite considerable variability from neuron to neuron, these data show that the information conveyed by motifs about behavior significantly alters their representation in the brain (Fig. 3.3b).

How does this representation change across the medial-lateral axis of CM? To address this question, we compared encoding of informative, uninformative, and novel motifs with the lateral position of the neuron. The difference in mean firing rate between familiar (informative and uninformative) motifs and novel motifs decreased significantly with distance from the midline (Pearson correlation coefficient: $r = -0.16$, $p = 0.0035$; Fig 3.4a). In contrast, the difference in mean firing rate between informative and uninformative motifs exhibited no such correlation with distance from the midline (Pearson correlation coefficient: $r = -0.0057$, $p = 0.92$; Fig. 3.4b). Consequently, CMM neurons primarily encoded familiarity, with firing rates in response to informative motifs ($3.67 \pm 0.33\text{Hz}$; Fig. 3.3c) and uninformative motifs ($3.49 \pm 0.30\text{Hz}$) significantly higher than firing rates in response to uninformative motifs ($3.10 \pm 0.30\text{Hz}$; Wilcoxon signed-rank tests, informative vs. novel: $p = 3.4 \times 10^{-5}$, uninformative vs. novel: $p = 0.0015$). However, no difference in firing rate was observed between informative and uninformative motifs (Wilcoxon signed-rank test: $p = 0.22$; Fig. 3.4d). CLM neurons, however, primarily encoded behavioral relevance, with firing rates in response to informative motifs ($4.03 \pm 0.25\text{Hz}$) significantly higher than firing rates in response to uninformative motifs ($3.84 \pm 0.24\text{Hz}$; Wilcoxon signed-rank test: $p = 0.024$) and novel motifs ($3.65 \pm 0.25\text{Hz}$; Wilcoxon signed-rank test: $p = 0.015$). No significant difference in firing rate was observed between uninformative and novel motifs (Wilcoxon signed-rank test: $p = 0.76$). Thus, while learning modifies neural encoding in both CLM and CMM, the nature of this encoding differs between the two regions (Two-way mixed model ANOVA interaction: $p = 0.026$).

We next investigated whether learning altered how neural responses in CLM and CMM unfold over time. The response profile of CMM neurons shows that, on average, responses to familiar motifs (informative and uninformative) are larger than responses to unfamiliar motifs throughout the duration of the motif (Fig. 3.5a). Consistently, both early in the motif (50-250ms after onset), and late in the motif

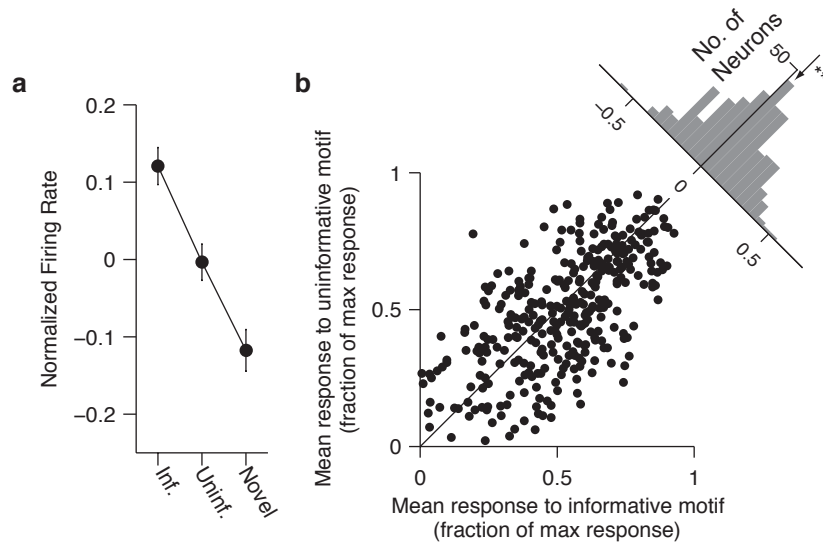


Figure 3.3. Motif Informativeness modulates firing rates in CM. **(a)** Mean normalized firing rates (z-score) for informative, uninformative, and novel motifs across all CM neurons. **(b)** Comparison of mean firing rate responses of all CM neurons to informative and uninformative motifs. The response to each motif is normalized by the highest response to any motif before taking the average firing rate within each class. Upper right inset: Histogram of differences in maximum-normalized firing rates between informative and uninformative motifs.

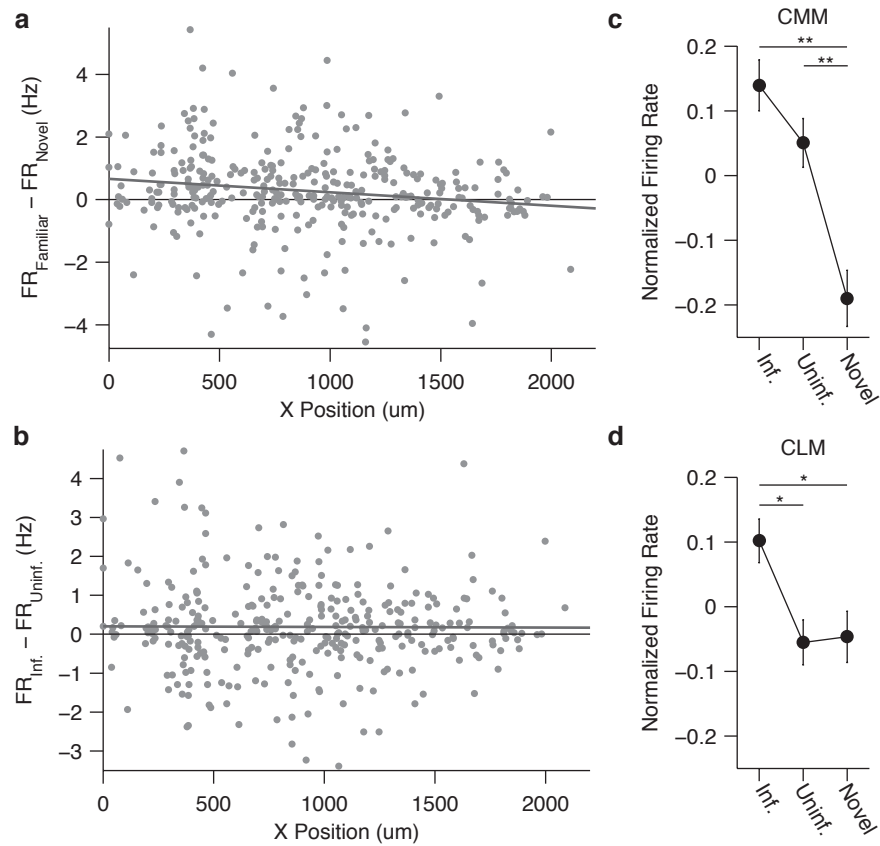


Figure 3.4. Neural coding varies spatially within CM. **(a)** Scatter plot of the difference in firing rate between familiar and novel motifs vs. position of neuron from the midline. This difference in firing rate decreases with distance from midline. **(b)** Scatter plot of the difference in firing rate between informative and uninformative motifs vs. position of neuron from the midline. This firing rate difference does not relate to the neuron's position from the midline. **(c)** Mean normalized (z-score) firing rates for informative, uninformative, and novel motifs within CMM. **(d)** Same as (c) for CLM neurons. Wilcoxon signed-rank test: * $p < 0.05$; ** $p < 0.01$.

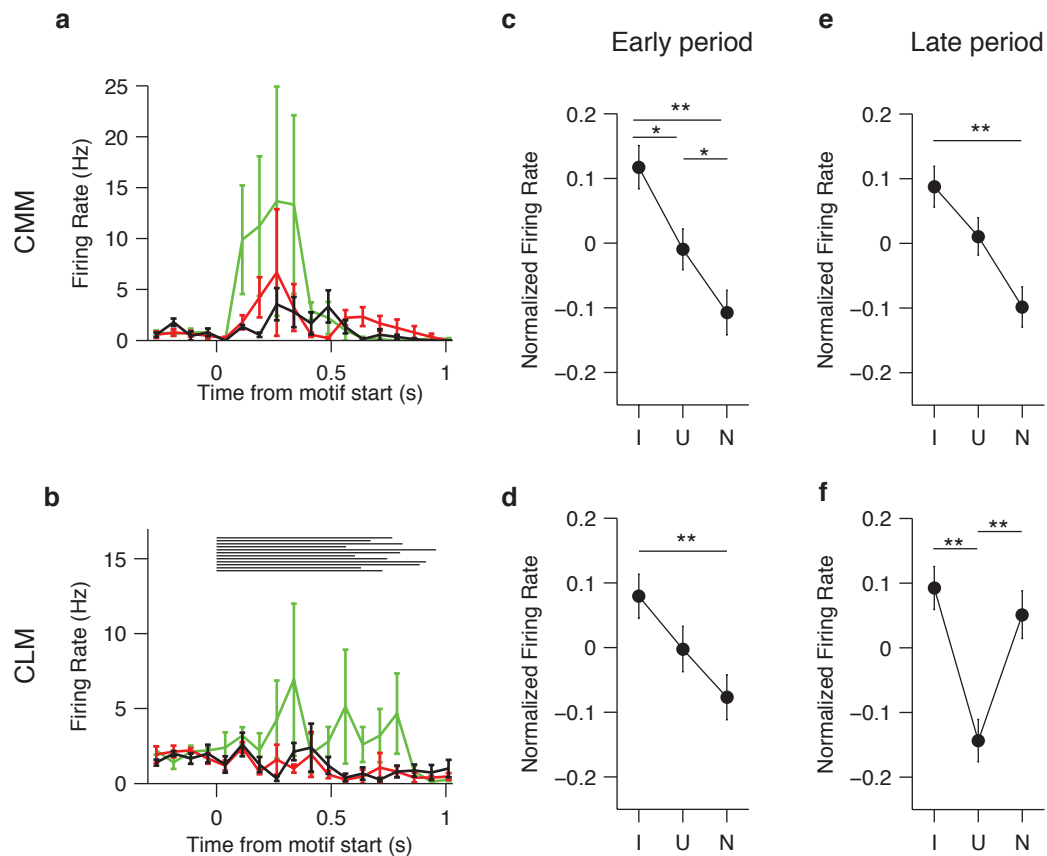


Figure 3.5. Encoding of informativeness varies over the timecourse of the motif. **(a)** Temporal profile of mean response to informative motifs (green), uninformative motifs (red), and novel motifs (black) for the CMM neuron shown in figure 3.2b. **(b)** Same as (a) but for the CLM neuron shown in figure 2a. **(c)** Mean normalized (z-score) responses of CMM neurons to motifs from each class during the early window (50-250ms after motif onset). **(d)** Same as (c) but for CLM neurons. **(e)** Mean normalized (z-score) responses of all CMM neurons to motifs from each class during the late window (300-100ms prior to end of motif). **(f)** Same as (e) but for all CLM neurons. Wilcoxon signed-rank test: * $p < 0.05$; ** $p < 0.01$.

(300-100ms prior to offset), CMM neurons responded with higher firing rates to familiar motifs than to unfamiliar motifs (early: Wilcoxon signed-rank test, $p = 3.1 \times 10^{-4}$; late: Wilcoxon signed-rank test: $p = 0.0074$; Fig. 3.4b,c). However, the response profile of CLM neurons shows that, on average, a subtle preference for familiar motifs early in the response gives way to a substantial suppression of uninformative motifs late in the response (Fig 3.5d). Consistently, early in the response, firing rates are higher for familiar motifs than for novel motifs (Wilcoxon signed-rank test: $p = 0.0013$; Fig. 3.5e). Yet late in the response, firing rates for familiar and novel motifs were indistinguishable (Wilcoxon signed-rank test: $p = 0.41$) but uninformative motifs were strongly suppressed (Wilcoxon signed-rank tests: uninformative vs. informative: $p = 2.8 \times 10^{-5}$; Uninformative vs. novel: $p = 0.0049$; Fig. 3.5f). Neurons in CLM therefore encode different aspects of behavioral information at different times during the motif (Two-way repeated measures ANOVA interaction: $p = 0.0031$), with the largest firing rate differences between informative and uninformative motifs not arising until nearly the end of the motif.

Because the cortical encoding of learned signals can change during the time after initial learning (Takehara-Nishiuchi and McNaughton, 2008), we wondered whether the effects of learning on neural coding relate to the extent of the bird's experience with the behavioral task. Although each bird reached behavioral criterion (Materials and Methods) after a similar number of trials, the number of trials performed after reaching this criterion was quite variable across birds (Fig. 3.6a). This variability in exposure was due to differences both in the number of days in which birds were trained and in the number of trials done per day (Fig. 3.6b). We therefore asked whether the number of trials performed after criterion influenced the neural encoding of differences between informative and uninformative motifs. Because CLM and CMM neurons encoded different features at different times during the motif, we analyzed responses during the early period (50-250ms after motif start) separately from the responses during the late period (300-100ms before motif end). In CMM, we found that additional experience decreased discriminability of informative motif from uninformative motifs (measured as d' -prime, Materials and Methods) during the early period ($r = -0.77$, $p = 0.015$; Fig. 3.6c), but had no effect during the late period ($p = 0.31$; Fig. 3.6d). In CLM, these effects were reversed: additional experience did not significantly alter discriminability during the early period ($p = 0.071$; Fig. 3.6e), but substantially decreased discriminability

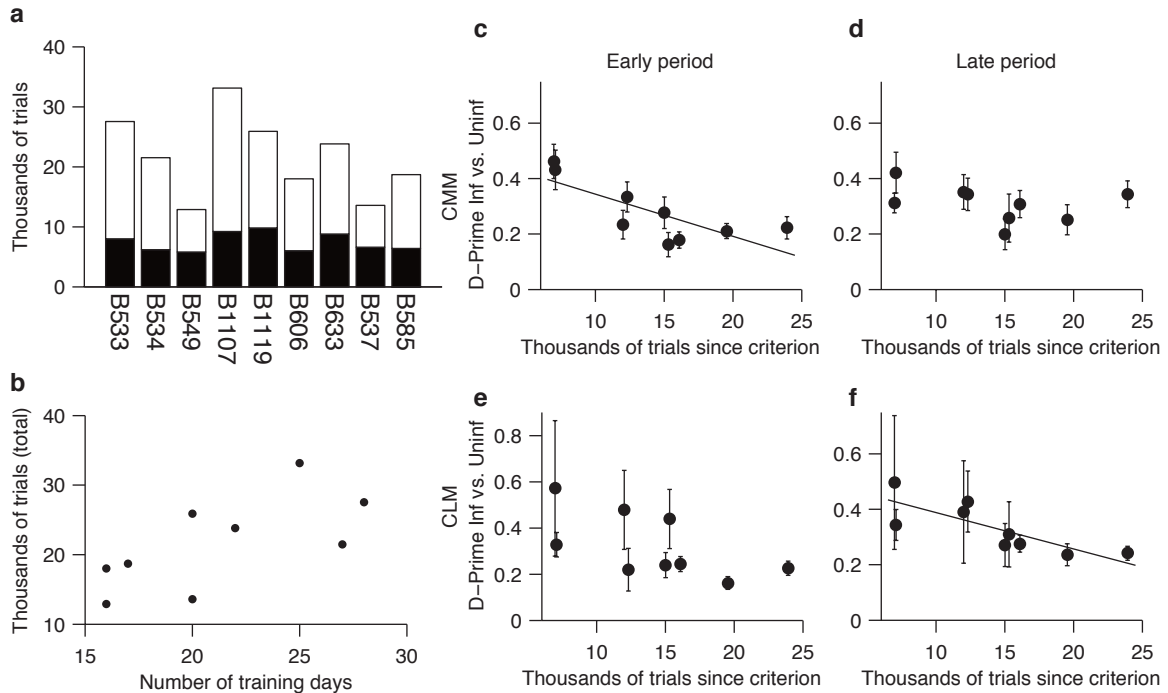


Figure 3.6. Discriminability of informative vs. uninformative motifs decreases with additional training. **(a)** Number of trials performed by each bird until reaching criterion (black bars; Materials and Methods) and number of trials performed by each bird after reaching criterion (white bars). Black and white bars are stacked. **(b)** Relationship between number of training days and number of trials performed. **(c-d)** Discriminability (d-prime; Materials and methods) of responses of CMM neurons during **(c)** the early period and **(d)** the late period to informative and uninformative motifs vs. number of trials performed. Each data point represents the mean d-prime value for all neurons recorded from that bird. **(e-f)** Same as **(c-e)** but for CLM neurons.

during the late period ($r = -0.80$, $p = 0.0094$; Fig. 3.6f). These results parallel our findings of population averaged firing rates where the largest differences between firing rates in response to informative and uninformative motifs occurred in CMM during the early period and in CLM during the late period. This suggests that cortical coding continues to change after learning, and does so in a spatially and temporally specific manner.

Discussion

The results described here demonstrate that learning alters the neural encoding of behaviorally relevant information in a spatially and temporally dissociated manner. In the natural world, any sensory signal has the potential to convey information to an organism. Through learning, an animal can discern which signals actually carry information about behavior and potential reward. We designed the experiments here to manipulate the information conveyed by motifs while controlling for amount of exposure and temporal association with reward (Fig. 3.1b,c). Our findings demonstrate that for neurons within CM, motifs that convey information about the proper motor task required to obtain reward (i.e. go left or go right) elicit stronger firing rate responses than motifs that do not carry this information (Fig. 3.3). These results are consistent with previous findings that reward and familiarity alter neural encoding in CM (Gentner and Margoliash, 2003; Jeanne et al., 2011), and in other vertebrate auditory areas (Blake et al., 2006; Polley et al., 2006; Thompson and Gentner, 2010). However, our study extends these results to show that learning can also modify neural encoding based on the information that signals provide about behavior.

Spatial dissociation of learning effects in CM

We also find that learning affects motif encoding in CMM differently from CLM. While CMM encodes the differences between familiar and novel motifs quite strongly, CLM predominantly encodes differences between informative and uninformative motifs. This occurs primarily because the distinction between uninformative and novel motifs is lost in CLM neurons. This may be a means for emphasizing behaviorally relevant motifs by CLM, which sends axonal projections to several regions of the song production circuitry, including HVC, the HVC shelf, and Nif (Vates et al., 1996; Bauer et al., 2008). This

function of CLM is also consistent with the hypothesis that CLM may play a primary role in providing the auditory feedback to the song system that is necessary for juvenile song learning and for adult song maintenance (Lei and Mooney, 2010).

In a previous study, we found that neural encoding of behavioral categories of motifs defined by reward was stronger in CMM than in CLM (Jeanne et al., 2011). This is not inconsistent with the present results. Because CMM neurons respond similarly to informative and uninformative motifs, one would expect CMM neurons to respond similarly to all rewarded motifs. In contrast, CLM neurons (when normalized for total response variance) have a comparably large difference in responses for informative and uninformative motifs so one would expect greater variability in responses to rewarded motifs. In the previous study, behavioral relevance of informative motifs was not controlled, and likely was highly variable both within and between subjects. Thus, CMM neurons encode rewarded motifs more categorically than do CLM neurons, which make a greater relative distinction based on behavioral relevance.

Temporal dissociation of learning effects in CM

The degree to which the behavioral relevance of motifs (i.e., informative, uninformative, or novel) is encoded in CLM and CMM varies over the duration of the motif. Studies of the neural encoding of dynamic signals often employ receptive field models to capture the relationship between stimulus and response dynamics (Adelson and Bergen, 1985; Nagel and Doupe, 2008; Geffen et al., 2009). Although our stimulus set was too small to make reliable estimates of receptive field models, we nonetheless find robust temporal structure in the responses to natural birdsong components that relates to the behavioral relevance acquired during training. Because the receptive fields of neurons in CM are high dimensional and strongly nonlinear (Gentner and Margoliash, 2003; Meliza et al., 2010), however, a subtle learning-induced change in the receptive field could have large effects on the neural responses. Such changes would be well poised to enable neurons to emphasize particular features within informative motifs. Because many studies of plasticity in high-level cortical areas use static or highly simplified stimuli (e.g. Sigala and Logothetis,

2002; Takehara-Nishiuchi and McNaughton, 2008), the temporal nature of the responses observed here may reflect mechanisms not previously observed.

Neurons in CLM, in particular, encoded information associated with behavior substantially differently early in the motif than late in the motif. Of particular interest was that the largest differences between neural responses of CLM neurons to informative and uninformative motifs occurred several hundred milliseconds prior to the end of the motif. This suggests that CLM integrates dynamic acoustic information over the course of the motif and, just prior to the end of the motif, reduces firing rates to uninformative motifs. In addition, such a response pattern may relate to the bird's decision-making process. The end of the informative motif contains the last acoustic material that can signal whether the bird needs to respond in the left port or the right port in order to obtain reward. When processing uninformative motifs, the response suppression could help to ensure that the bird does not accidentally base its behavioral decision on the uninformative motif. Further studies, ideally in awake, behaving starlings, will be necessary to confirming such a hypothesis.

Renormalization of plasticity over time

Our results also suggest that extended experience after training continues to alter neural encoding. Because behavioral performance remains high after initial learning, this finding may reflect a cortical reorganization that is independent of behavior, akin to mechanisms of memory consolidation from the medial temporal lobe to cortex (Squire and Alvarez, 1995). However, our observations are distinct from those attributed to memory consolidation in prefrontal cortex (Takehara-Nishiuchi and McNaughton, 2008) because the strength of neural encoding in CM decreases rather than increases with additional training. In contrast, our results are more consistent with the expansion-renormalization theory of cortical map plasticity, in which neural representations strengthen during initial learning, and then return to baseline levels over time (Yotsumoto et al., 2008; Takahashi et al., 2010; Reed et al., 2011). Our findings suggest that the same principles by which learning alters the tonotopic map in primary auditory cortex also alter representations of complex signals in higher-order cortical areas. In accordance with the expansion-renormalization theory, especially strong representations likely form during the early stages of learning that

assist the bird by highlighting multiple relevant features. As the bird settles onto a strategy for solving the task, the encoding of additional features becomes superfluous and subsides, leaving a sparser neural representation that is harder to detect in single unit recordings, but still sufficiently subserves behavior. Such a possibility could be tested with repeated recordings from the same bird to directly compare changes to neural representations over time, and behavioral experiments to probe the strategies adopted by individual birds.

Although our neural recordings were conducted under urethane anesthesia, it is likely that similar neural representations would be found under awake, behaving conditions. In the bird, urethane alters neuronal excitability, but does not affect tuning or discriminability during responses to natural birdsong (Capsius and Leppelsack, 1996; Schumacher et al., 2011). Furthermore, urethane anesthesia allows for strong control of novelty during neural recording: because it precludes cognitive involvement, novel stimuli remain novel even after many presentations. Of course, awake preparations will be important for understanding how neural encoding of motifs change as behavioral relevance changes.

Conclusion

Identifying the signals that convey information about behavior or reward is a critical function of learning. By prescribing which motifs convey information, we demonstrate that the informativeness of a signal modulates the firing rates of neurons in CM. Further, we show that this learning-dependent neural encoding is spatially and temporally organized within CM, and agrees with the expansion-renormalization theory of cortical plasticity. Collectively, our results show that the information a signal conveys about behavior plays a central role in the plasticity of cortical neural representations and warrants a careful re-examination of the role of informativeness (as compared to familiarity and association with reward) in shaping sensory neural coding in other areas of the brain.

Acknowledgements

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References

- Adelson EH, Bergen JR (1985) Spatiotemporal energy models for the perception of motion. *J Opt Soc Am A* 2:284-299.
- Bakin JS, Weinberger NM (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res* 536:271-286.
- Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412:79-83.
- Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R (2008) A synaptic basis for auditory-vocal integration in the songbird. *J Neurosci* 28:1509-1522.
- Blake DT, Heiser MA, Caywood M, Merzenich MM (2006) Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. *Neuron* 52:371-381.
- Capsius B, Leppelsack HJ (1996) Influence of urethane anesthesia on neural processing in the auditory cortex analogue of a songbird. *Hear Res* 96:59-70.
- Freedman DJ, Assad JA (2006) Experience-dependent representation of visual categories in parietal cortex. *Nature* 443:85-88.
- Geffen MN, Broome BM, Laurent G, Meister M (2009) Neural encoding of rapidly fluctuating odors. *Neuron* 61:570-586.
- Gentner TQ (2008) Temporal scales of auditory objects underlying birdsong vocal recognition. *J Acoust Soc Am* 124:1350-1359.
- Gentner TQ, Hulse SH (2000) Perceptual classification based on the component structure of song in European starlings. *J Acoust Soc Am* 107:3369-3381.
- Gentner TQ, Margoliash D (2003) Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669-674.
- Jeanne JM, Thompson JV, Sharpee TO, Gentner TQ (2011) Emergence of learned categorical representations within an auditory forebrain circuit. *J Neurosci* 31:2595-2606.
- Keller GB, Hahnloser RHR (2009) Neural processing of auditory feedback during vocal practice in a songbird. *Nature* 457:187-190.
- Lei H, Mooney R (2010) Manipulation of a central auditory representation shapes learned vocal output. *Neuron* 65:122-134.
- Macmillan NA, Creelman CD (2005) *Detection Theory, A User's Guide*. Mahwah, NJ: Lawrence Erlbaum Associates, Inc. .
- Meliza CD, Chi Z, Margoliash D (2010) Representations of conspecific song by starling secondary forebrain auditory neurons: toward a hierarchical framework. *J Neurophysiol* 103:1195-1208.

- Mirabella G, Bertini G, Samengo I, Kilavik BE, Frilli D, Della Libera C, Chelazzi L (2007) Neurons in area V4 of the macaque translate attended visual features into behaviorally relevant categories. *Neuron* 54:303-318.
- Nagel KI, Doupe AJ (2008) Organizing principles of spectro-temporal encoding in the avian primary auditory area field L. *Neuron* 58:938-955.
- Polley DB, Steinberg EE, Merzenich MM (2006) Perceptual learning directs auditory cortical map reorganization through top-down influences. *J Neurosci* 26:4970-4982.
- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13:87-103.
- Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron* 70:121-131.
- Schumacher JW, Schneider DM, Woolley SM (2011) Anesthetic state modulates excitability but not spectral tuning or neural discrimination in single auditory midbrain neurons. *J Neurophysiol* 106:500-514.
- Sigala N, Logothetis NK (2002) Visual categorization shapes feature selectivity in the primate temporal cortex. *Nature* 415:318-320.
- Squire LR, Alvarez P (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol* 5:169-177.
- Takahashi H, Funamizu A, Mitsumori Y, Kose H, Kanzaki R (2010) Progressive plasticity of auditory cortex during appetitive operant conditioning. *Biosystems* 101:37-41.
- Takehara-Nishiuchi K, McNaughton BL (2008) Spontaneous changes of neocortical code for associative memory during consolidation. *Science* 322:960-963.
- Thompson JV, Gentner TQ (2010) Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *J Neurophysiol* 103:1785-1797.
- Vates GE, Broome BM, Mello CV, Nottebohm F (1996) Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366:613-642.
- Yotsumoto Y, Watanabe T, Sasaki Y (2008) Different dynamics of performance and brain activation in the time course of perceptual learning. *Neuron* 57:827-833.
- Zhang J, Meeson A, Welchman AE, Kourtzi Z (2010) Learning alters the tuning of functional magnetic resonance imaging patterns for visual forms. *J Neurosci* 30:14127-14133.

IV. Selective, learning-dependent enhancement of a neural population code

Abstract

Plasticity in the encoding properties of single adult cortical neurons is well established (Bakin and Weinberger, 1990; Recanzone et al., 1993; Gentner and Margoliash, 2003; Jeanne et al., 2011) and required for learning (Reed et al., 2011). However, the cortical circuits that support learned behaviors comprise millions of neurons operating in coordination (Averbeck et al., 2006) and little is known about plasticity beyond the level of single neurons. The representational fidelity of a neuronal population depends on the relationship between shared neuronal variability (noise correlations) and similarity in average sensory tuning properties (signal correlations): positive relationships blur the responses to different stimuli while negative relationships sharpen them (Johnson, 1980; Oram et al., 1998; Abbott and Dayan, 1999; Averbeck et al., 2006; Gu et al., 2011). Most studies report a positive relationship between signal and noise correlations (Lee et al., 1998; Bair et al., 2001; Constantinidis and Goldman-Rakic, 2002; Kohn and Smith, 2005; Cohen and Maunsell, 2009; Gu et al., 2011). Here, using songbirds trained to recognize segments of natural birdsong (motifs), we show that the relationship between signal and noise correlations depends on the learned behavioral relevance of the sensory signal. Populations of putative projection neurons in the auditory cortex driven by behaviorally uninformative motifs show the typical positive relationship between signal and noise correlations. When the same populations are driven by behaviorally informative motifs, however, the signal and noise correlations are negatively related and this enhances discriminability in large neural populations. Thus, learning can selectively enhance the fidelity of behaviorally informative representations in a population code.

Introduction

Current understanding of the function of cortical circuits rests on two common intuitions: that cortical processing involves the orchestration of large populations of neurons and that cortical plasticity supports learning. Little is known, however, about how learning-dependent plasticity influences neural population representations. Neurons in sensory cortical areas exhibit a broad range of tuning properties and elicit variable responses to repeated presentations of the same stimulus. Response variability between

neurons is not independent, however, and these inter-neuronal correlations can have profound implications for encoding at the population level (Oram et al., 1998; Abbott and Dayan, 1999; Averbeck et al., 2006). In the cortex, trial-to-trial co-variability (noise correlation) typically increases with similarity of tuning (signal correlation), which is likely due to common inputs that provide correlated signal and noise to post-synaptic neurons (Bair et al., 2001; Kohn and Smith, 2005). Theoretical work demonstrates that this positive relationship between the signal and noise correlation can blur differences between encoded signals in the neural population response (Fig 4.1a) while a negative relationship between signal and noise correlations can sharpen response differences (Johnson, 1980; Oram et al., 1998; Abbott and Dayan, 1999; Averbeck et al., 2006) (c.f. Ecker et al., 2011) (Fig. 4.1b). Since the relationship between signal and noise correlations alters population encoding fidelity, we predicted that changes in this relationship may contribute to the selective representation of learned sensory signals. Because recognition learning can change the tuning properties of single neurons in the songbird auditory cortex (Gentner and Margoliash, 2003; Thompson and Gentner, 2010; Jeanne et al., 2011), we tested here whether this learning also alters the relationship between signal and noise correlations in this system.

Results

Using an established operant procedure (Gentner and Margoliash, 2003), we trained 9 European starlings (*Sturnus vulgaris*), a species of songbird, to recognize sets of stereotyped natural song segments called motifs (Fig 4.1b-d; Methods). Motif recognition underlies a range of natural behaviors (Gentner and Hulse, 2000), and can be tightly controlled in the laboratory (Gentner and Margoliash, 2003; Thompson and Gentner, 2010; Jeanne et al., 2011). On each trial we presented the bird with a pair of sequentially ordered motifs (e.g. Fig. 4.1c). One motif was always informative of the correct behavioral response for the trial (i.e. whether to poke at the left or right port to receive food; “informative” motifs) and the other motif was never informative for the correct response (“uninformative” motifs; Fig 4.1d). Informative and uninformative motifs occurred with equal frequency and in identical proximity (on average) to reward during training; all that differed between the two motif classes was their relevance for the animal’s response. This design permits the dissociation of the experience-dependent effects of reward from those

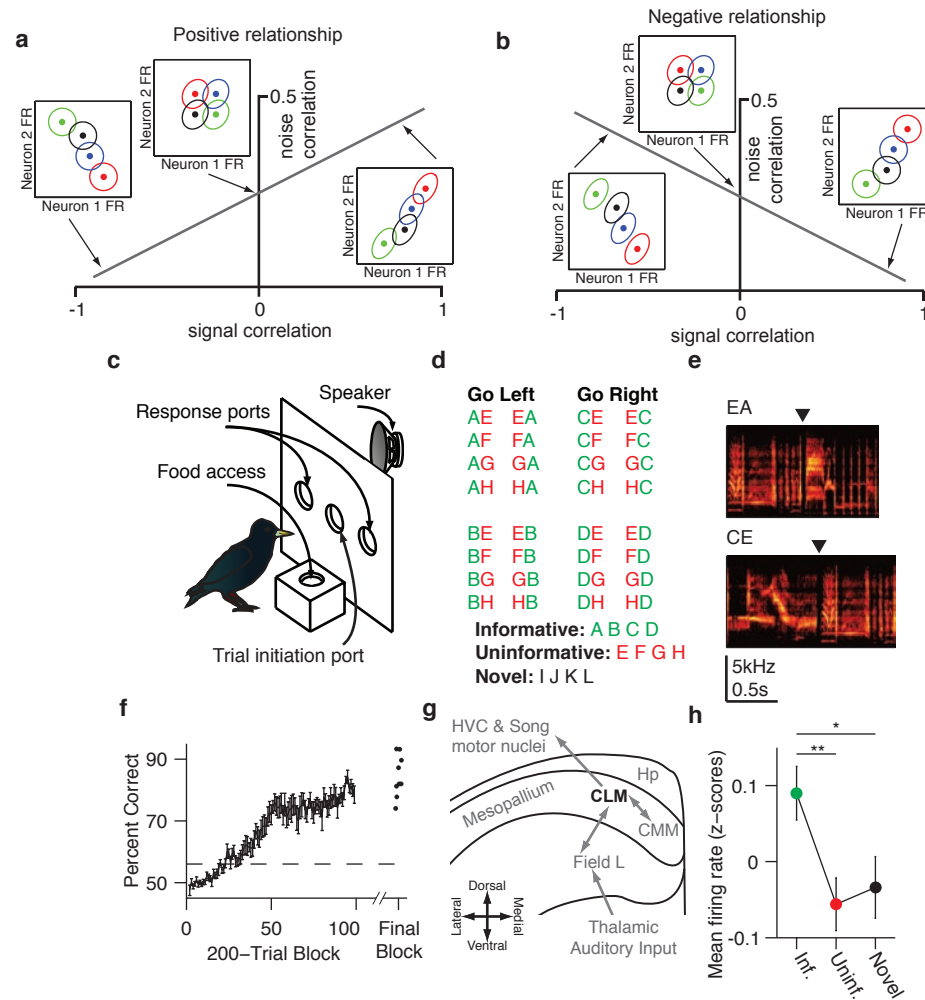


Figure 4.1 Experimental design. **(a,b)** Schematic of theoretical relationships between signal and noise correlations. Each colored dot denotes the mean response for two neurons to each of four stimuli. Each colored ellipse denotes the standard deviation of the two-dimensional response distribution for each stimulus. For the positive relationship in **(a)**, neuron pairs with positive signal correlation and large noise correlation have substantial overlap in their responses (inset right), while pairs with negative signal correlation and small noise correlation have less overlap (inset left). Center inset depicts pairs with zero signal correlation but moderate noise correlation. For the negative relationship in **(b)**, neuron pairs with positive signal correlation and small noise correlation have some overlap in their responses (inset right), while neuron pairs with negative signal correlation and large noise correlation have very little overlap (inset left). The negative relationship thus yields neural populations that discriminate between stimuli better than the positive relationship. **(c)** Schematic of behavioral training apparatus. **(d)** Experimental stimulus setup. Informative motifs (green) indicated whether to respond left or right. Uninformative motifs (red) were paired in sequence with relevant motifs and occurred with equal probability in left stimuli and right stimuli. Novel motifs (black) were never presented during behavioral training, but were presented during neural recording. **(e)** Example “go left” stimulus (top) and “go right” stimulus (bottom). Arrowheads denote 20ms silent periods between motifs. **(f)** Mean (\pm SEM) acquisition curve showing increase in performance with training. Blocks are non-overlapping. Dots at right denote behavioral performance for each bird during the 200 trials prior to neural recording. **(g)** Schematic of CLM within the avian auditory forebrain circuitry. Hp: hyperpallium. **(h)** Mean (\pm SEM) driven responses (z-score) for informative, uninformative, and novel motifs. Wilcoxon signed-rank test (applied to raw firing rates): ** $p < 0.005$; * $p < 0.05$.

associated with behavioral response. All birds learned to perform this task accurately (Fig. 4.1e). After training, we recorded the simultaneous activity of multiple well-isolated single neurons in the caudolateral mesopallium (CLM) in response to behaviorally informative and uninformative motifs and a third set of entirely novel motifs, under urethane anesthesia (Methods; Supplemental Figs. 4.1, 4.2). CLM is a higher-order auditory region in the songbird cortex that is specialized for processing learned songs (Jeanne et al., 2011), and projects auditory information into the vocal premotor region HVC (Fig. 4.1f) (Bauer et al., 2008). The behavioral training led to a modulation of firing rates in CLM neurons ($n = 134$ neurons from 9 birds; Friedman test, $p = 0.038$; Fig. 4.1g), with informative motifs eliciting significantly higher firing rates (4.92 ± 0.55 Hz) than both uninformative motifs (4.63 ± 0.53 Hz; Wilcoxon signed-rank test, $p = 0.0024$) and novel motifs (4.58 ± 0.52 Hz; Wilcoxon signed-rank test, $p = 0.042$). Thus, information that motifs provide about appropriate behavioral responses is important for shaping neural encoding in CLM.

Because connectivity and correlation within neural populations depends on cell type (Lee et al., 1998; Constantinidis and Goldman-Rakic, 2002; Hofer et al., 2011), we divided our dataset into wide spiking (WS) and narrow spiking (NS) neurons on the basis of action potential width (trough-to-peak duration; Fig 4.2a,b; Methods) (Bartho et al., 2004; Mitchell et al., 2007). The distribution of action potential widths is bimodal (Hartigan's dip test, $p = 0.041$; Fig 4.2c) (Hartigan and Hartigan, 1985; Mitchell et al., 2007). Based on network interactions and correlations between extracellular and intracellular features, previous studies have established that WS and NS neurons correspond to excitatory principal neurons and inhibitory interneurons, respectively (Harris et al., 2000; Bartho et al., 2004; Tamura et al., 2004). Consistent with these classifications, our sample of NS neurons ($n = 33$) elicited significantly higher spontaneous firing rates (4.76 ± 0.82 Hz) than our sample of WS neurons ($n = 101$; 1.83 ± 0.20 Hz; Wilcoxon rank-sum test, $p = 1.84 \times 10^{-6}$). Because our sample of simultaneously recorded pairs of NS neurons was relatively small ($n = 13$ pairs), we focus our population analysis on pairs of WS neurons ($n = 185$ pairs from 6 birds). We note, however, that results similar to those described below are also observed across all pairs of CLM neurons ($n = 252$ pairs from 8 birds; Supplemental Fig. 4.3).

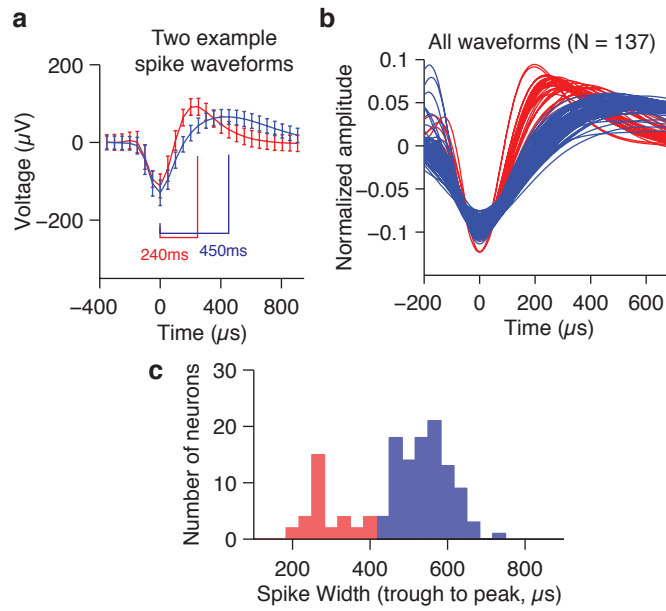


Figure 4.2 Spike shapes in CLM. **(a)** Mean (\pm S.D.) spike waveforms for wide (blue) and narrow (red) spiking neurons (recorded from the same electrode pad) depicting measurement of spike width from trough to peak. **(b)** Spike shapes of all neurons recorded in CLM. **(c)** Bimodal distribution of spike widths.

To determine whether learning altered the relationship between signal and noise correlations among neuron pairs, we compared signal and noise correlations during processing of informative, uninformative, and novel motifs. Figure 3 depicts the responses of two example pairs of WS neurons in CLM. The tuning of the neurons in the first pair was dissimilar for informative motifs, but similar for uninformative and novel motifs (Fig. 4.3a), leading to negative signal correlations for informative motifs and positive signal correlations for uninformative and novel motifs (Fig. 4.3b). Over repeated trials, however, the response variability was similar for the three sets of motifs (Fig. 4.3c). Thus, the noise correlations were uniformly strong and positive (Fig. 4.3d). In theory (Fig. 4.1b), the combination of a positive noise correlation and negative signal correlation in the responses to informative motifs should enhance discriminability in this neuron pair. The shared sign of the signal and noise correlations in the responses to uninformative and novel motifs, however, should impair the discriminability in this neuron pair. The second example pair shows dissimilar tuning (negative signal correlations) for all motifs (Fig. 4.3e,f), but a positive noise correlation for only the informative motifs (Fig. 4.3g,h). Again, this combination of signal and noise correlations should lead to an enhancement in the discriminability of this pair's responses to informative motifs relative to uninformative or novel motifs. These two example pairs show that stimulus-dependent differences in either signal or noise correlation can lead to selectively enhanced encoding of informative motifs (Fig. 4.1a,b).

Similar stimulus-dependent relationships between signal and noise correlations were present across our sample of CLM WS neuron pairs. For behaviorally informative motifs, this relationship was negative ($r = -0.15$, $p = 0.037$, Fig. 4.4a): larger signal correlations were accompanied by smaller noise correlations. For uninformative and novel motifs, in contrast, the relationship was positive (uninformative: $r = 0.16$, $p = 0.030$; novel: $r = 0.21$, $p = 0.0042$; Fig 4.4b,c): larger signal correlations were accompanied by larger noise correlations. The difference between these relationships was highly significant (ANCOVA motif class \times regression slope interaction, $p = 8.2 \times 10^{-4}$). These effects are not tied to differences in how the noise correlations are related to either firing rate or inter-neuronal distance (Supplemental Fig. 4.4). Likewise, no differences exist in the average signal or noise correlations evoked by the three classes of song motifs (Fig. 4d,e; repeated measures ANOVA, $p = 0.24$ and $p = 0.16$, noise and signal correlations,

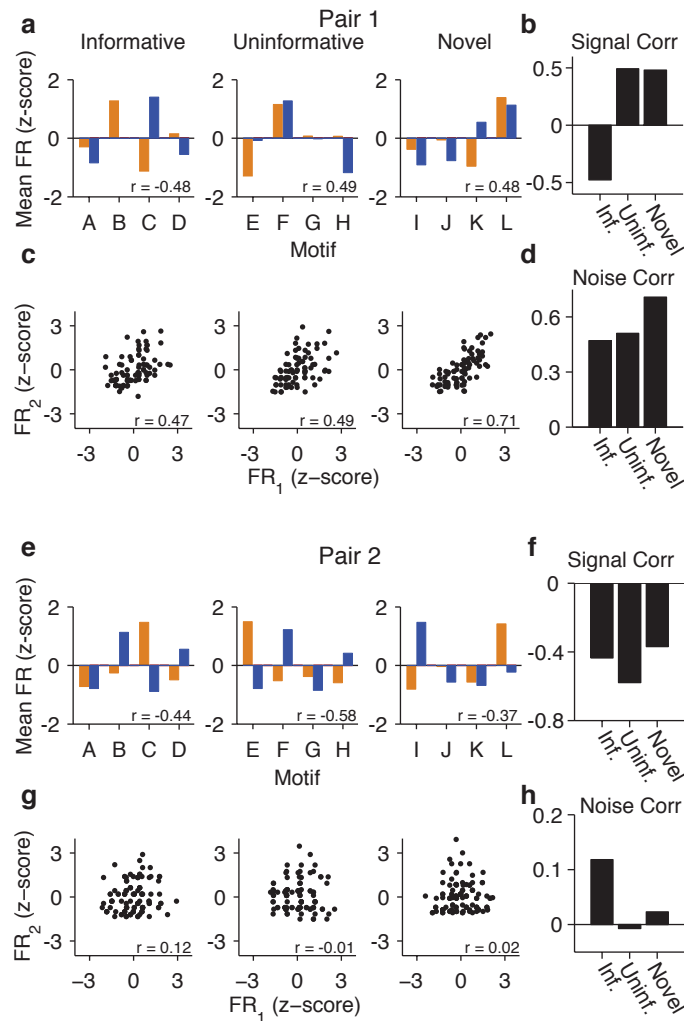


Figure 4.3 Signal and noise correlations in two sample pairs of WS neurons. **(a)** Normalized trial-averaged responses of informative (left), uninformative (center), and novel (right) motifs. Orange and blue bars depict neurons 1 and 2, respectively. r values in each plot denote the signal correlation for each set of motifs. **(b)** Signal correlations by motif class. **(c)** Trial-by-trial firing rates by motif class, ordered as in (a). Responses to each motif are converted to z-scores independently of the other motifs. r values in each plot denote the noise correlation (computed from raw firing rates for each motif separately and averaged). **(d)** Noise correlations by motif class. **(e-f)** Same as (a-d) but for a second sample pair.

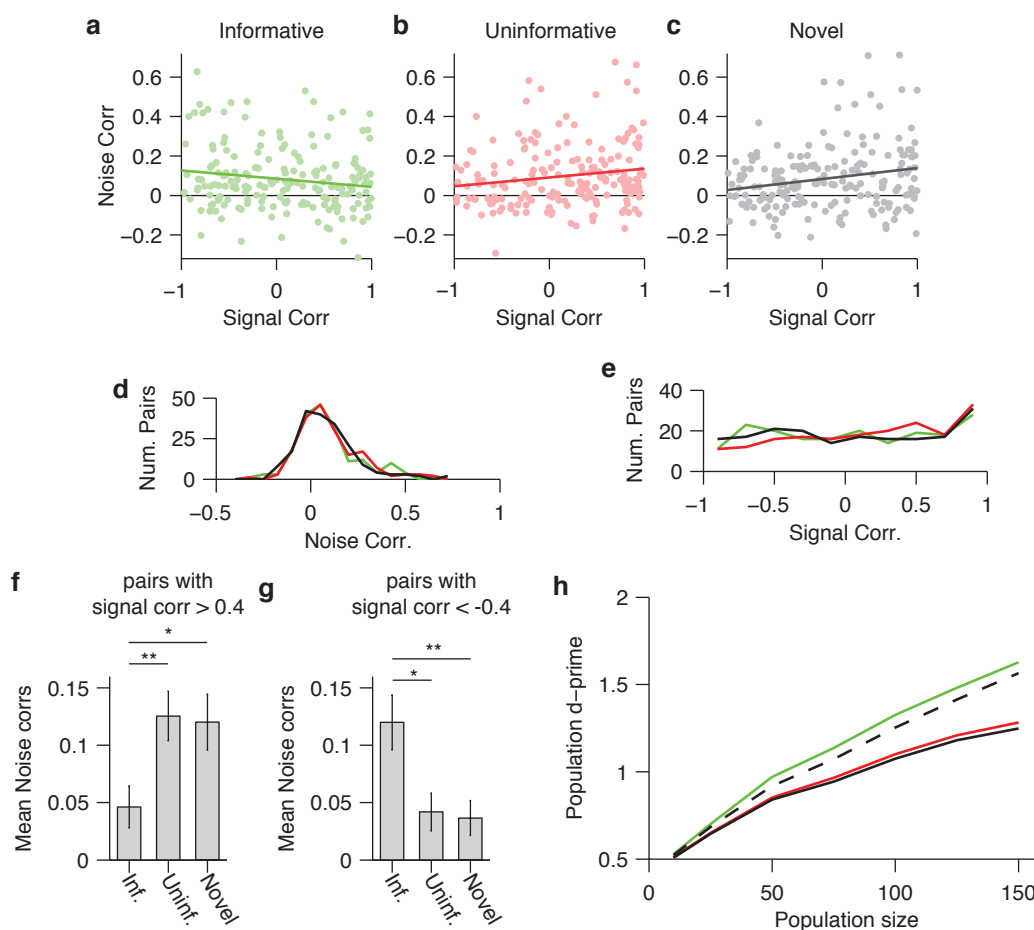


Figure 4.4 Learning alters signal and noise correlations to increase population coding fidelity. **(a-c)** Scatter plots of noise and signal correlations for all pairs for responses to each class of motifs. Black line depicts zero noise correlation; heavy colored lines are linear regression lines. **(d-e)** Distribution of noise (d) and signal (e) correlations from responses to informative (green), uninformative (red), and novel (black) motifs. No substantial differences are found. **(f)** Mean (\pm SEM) noise correlations for all pairs with signal correlation greater than 0.4 for informative ($n = 65$ pairs), uninformative ($n = 75$ pairs), and novel ($n = 64$ pairs) motifs. **(g)** Mean (\pm SEM) noise correlations for all pairs with signal correlation less than -0.4 for informative ($n = 54$ pairs), uninformative ($n = 39$ pairs), and novel ($n = 54$ pairs) motifs. **(h)** Model simulations of discriminability of left motifs from right motifs from populations of neurons with noise-signal correlation relationship defined by the linear regression from informative (green), uninformative (red), or novel (solid black) motifs and independent correlations (dashed black). Population d -prime is greater for correlations defined from informative motifs than for independent correlations or for correlations defined from uninformative or novel motifs for all population sizes (Wilcoxon signed-rank test, $p < 1 \times 10^{-4}$ for all comparisons). * $p < 0.05$, ** $p < 0.005$.

respectively). Rather, it is the relationship between the signal correlation and noise correlation that is stimulus-specific (see Fig. 4.1a,b). This specificity is particularly apparent in neuron pairs that have strong (either positive or negative) signal correlations (Fig. 4.4f,g). Among WS neuron pairs with strong positive signal correlations (greater than 0.4), the uninformative and novel motifs evoked significantly larger noise correlations than the informative motifs (ANOVA, $p = 0.015$; Fig. 4.4f). Similarly, in WS neuron pairs that had large negative signal correlations (less than -0.4) the uninformative and novel motifs evoked significantly weaker noise correlations than the informative motifs (ANOVA, $p = 0.0042$; Fig. 4.4g). We note that the magnitude of these differences is similar to previously reported behavior-dependent changes in noise correlation (Cohen & Maunsell 2009, Gu, et. al., 2011), except that these differences are not uniform with respect to signal correlation. Because the stimulus-specific effect is unique to the informative motifs, we conclude that learning selectively alters the relationship between the signal and noise correlations among WS neurons for those signals that inform behavior.

Does the observed negative relationship between signal and noise correlations for informative motifs improve population encoding? To address this, we constructed a model of population responses to motifs associated with left responses (“left motifs”) and motifs associated with right responses (“right motifs”) that preserves the empirically measured tuning functions and trial-to-trial variability of individual neurons. We then systematically varied the relationship between signal and noise correlations within the population (Methods). As expected, increasing the number of neurons increased the discriminability of left vs. right motifs. However, for large numbers of neurons, the populations simulated using the correlation structure from the informative motifs permitted significantly better discrimination of left vs. right motifs than the populations simulated using the correlation structure from the uninformative or novel motifs (Fig. 4.4h). In addition, the negative correlation relationship from the informative motifs even performed better than a model with independent noise (Fig. 4h). The negative relationship between signal and noise correlations for informative motifs thus improves population coding fidelity of behaviorally meaningful information.

Discussion

We demonstrate a long-lasting, stimulus-specific reversal of the population correlation structure that is more directly tied to learned behavioral responses than to reward. Noise correlations are typically thought to positively co-vary with signal correlations because common stimulus drive provides both signal and noise (Bair et al., 2001; Kohn and Smith, 2005), but our results demonstrate that the correlation structure is more flexible than previously appreciated. The negative relationship that we observe for informative motifs may result from the active modulation of neuronal correlation by local circuitry. One possibility is that common local circuit activity increases noise correlations among dissimilarly tuned neurons, while inhibitory mechanisms subtract out common noise from similarly tuned neurons (Renart et al., 2010). Other mechanisms are possible as well, and flexibility in the population correlation structure likely differs across neuronal subpopulations (Lee et al., 1998; Constantinidis and Goldman-Rakic, 2002; Hofer et al., 2011). In primate medial superior temporal cortex, for example, training can reduce noise correlations, but does so uniformly for all stimuli and without altering the slope of the relationship between signal and noise correlations (Gu et al., 2011). Changes in this slope have been observed, however, in motor cortex when monkeys make an overt arm movement (Lee et al., 1998). Our observation that learning evokes a stark reversal of the signal and noise correlation relationship for signals that provide information about the action required to receive reward (informative motifs) shows that, at least in some sensory regions, behavioral relevance plays an important role in structuring population correlations. Our observation that the shift in the correlation structure is strongest among the WS neurons is consistent with this. Because the WS neurons we identify are thought to be projection neurons, the plasticity in their population encoding may especially influence CLM's target regions, such as HVC (Bauer et al., 2008), a region known to control song production (Nottebohm et al., 1976; Long and Fee, 2008). CLM may thus bias the routing of auditory information into the song production system, and possibly other motor control systems, by emphasizing song components that are most instructive for behavior. Further experiments will be necessary to understand the mechanisms and functions of this plasticity, including in awake starlings actively engaged in similar recognition tasks. Nevertheless, we show that learning can selectively enhance the neuronal population code, confirming that a long-standing and theoretically well-grounded prediction

about the existence of negative signal-noise correlation relationships (Johnson, 1980; Oram et al., 1998) is realized for the representation of behaviorally meaningful sensory information.

Methods Summary

Starlings were trained to recognize sets of paired motifs (Fig. 4.1d-e; Supplemental Fig. 4.2) using established operant techniques (Gentner and Margoliash, 2003). Correlations were measured for pairs of simultaneously recorded neurons using the Pearson product-moment correlation coefficient of trial-averaged motif firing rates (signal correlations) and trial-to-trial response variability (noise correlations). Population modeling was based on previously reported techniques (Shadlen et al., 1996; Cohen and Newsome, 2008; Cohen and Maunsell, 2009), and designed such that only the noise correlation matrix varied between conditions.

Methods

All procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of California, San Diego.

Stimuli We constructed all stimuli from 12 motifs (stereotyped multi-note elements of natural starling song) recorded from the song repertoires of three adult European starlings. Motifs (565ms-957ms long) were grouped into three sets: four motifs (A,B,C,D) were labeled “informative,” four motifs (E,F,G,H) were labeled “uninformative,” and four motifs (I,J,K,L) were labeled “novel” (Supplemental Fig. 4.2). For behavioral training, we presented a sequential pair of motifs for each trial. Each pair contained exactly one informative and one uninformative motif, in either order, separated by a 20ms silent gap. This yielded 32 stimuli; the 16 containing motifs A or B were used as “left” stimuli and the 16 containing motifs C or D were used as “right” stimuli. All uninformative motifs occurred with equal probability in both left and right stimuli. Novel motifs were never presented during training. To ensure that learning effects were not due to intrinsic acoustic differences between motifs, motif assignment to informative, uninformative,

and novel categories was counter-balanced across birds. During neural recording sessions we presented each of the twelve motifs in isolation (i.e. not paired).

Behavioral Training Nine wild-caught adult European starlings (*Sturnus vulgaris*) were trained using a two-alternative choice operant conditioning paradigm (Gentner and Margoliash, 2003) to distinguish between the left stimuli and right stimuli described above. Prior to training, none of the subjects had any exposure to these stimuli. All training took place inside a sound attenuation chamber with an operant response panel (Fig. 4.1c). Birds initiated trials by inserting their beak into the center port of the response panel to start playback of one of the 32 stimuli from the speaker inside the chamber. Following playback, birds had two seconds to indicate their response by pecking in either the left or the right port. Incorrect responses were punished by extinguishing the lights and prohibiting trial initiation for 10-90 seconds. Correct responses were rewarded by 2-s access to food on a fixed ratio reinforcement schedule. The number of correct trials required for reward was gradually increased over time from 1 to 5. A secondary reinforcer (flashing of LEDs on the response panel) was used on correct trials when the food reward was not delivered. Incorrect responses reset the running count of correct trials. The fixed ratio schedule ensured that all stimuli were presented an equal number of times and that none were systematically ignored. At the end of training, starlings were presented with randomly reinforced (with secondary reinforcer only) probe stimuli consisting of each of the 8 training motifs in isolation (i.e. not paired) to obtain behavioral confirmation that all four informative motifs were recognized (data not shown). Probe trials were randomly interleaved with other trials, and accounted for 8-20% of all trials.

Electrophysiology Approximately 24 hours prior to electrophysiological recording, the animal was anesthetized (1.5-2% isoflurane), a small pin was attached to the surface of the skull just caudal to CLM, and the animal was allowed to recover. On the recording day, starlings were anesthetized with urethane (20% by volume, 7-8ml/kg) and head-fixed via the attached pin to a stereotactic apparatus inside a sound-attenuating chamber. A small craniotomy was made dorsal to CLM, and multi-channel silicon electrode arrays (177 μm^2 electrode surface area, 50 μm spacing, 1x16 and 1x32 electrode layout; NeuroNexus

technologies). 1x32 electrode arrays were generally inserted at a 35° angle (relative to horizontal) and simultaneously measured neural activity across the medial-lateral axis of CLM. 1x16 electrode arrays were generally inserted at a 90° angle (relative to horizontal). For some subjects, only the 1x32 array was used (Supplemental Fig. 4.1a). Motif stimuli were presented free field from a speaker 30cm from the bird. Electrode arrays were advanced while presenting the 12 motif stimuli until 2 or more auditory single units were isolated. Once single units were isolated, all 12 single motifs and the set of training motif pairs were presented pseudo-randomly in blocks while the extracellular electrical activity was amplified (5000× gain; AM Systems), filtered (high pass, 300Hz; low pass, 3-5kHz), sampled (20kHz), and saved digitally for offline analysis (Spike2; Cambridge Electronic Design). Electrodes were coated with Di-I to facilitate localization of penetration tracks in histological sections.

Histology At the end of the recording session, starlings were euthanized with an overdose of nembutal (150ml/kg), and perfused transcardially with 10% neutral buffered formalin. Brains were removed from the skull and placed in 30% sucrose solution for several days for cryoprotection. Brains were then cut into 50µm coronal sections on a freezing microtome and mounted on glass slides. Electrode penetration tracks were identified with the assistance of Di-I marking and epifluorescence microscopy (Supplemental Fig. 4.1b). Tissue was then stained with cresyl violet to localize penetration tracks to neuroanatomical boundaries. All electrode tracks were reconstructed from tissue sections, and the recording locations of all sites were measured. The medial boundary of CLM was taken to be 800µm from the midline. Recording locations for CLM neurons ranged from 810-2100µm from the midline, and from 507-2240µm from the dorsal surface (Supplemental Fig. 4.1c,d).

Data Analysis Putative action potentials in the recorded voltage traces were identified by amplitude, and sorted into single units with principal components analysis on waveform shape using Spike2 software (Cambridge Electronic Design). Only large amplitude spike waveforms that formed a clear cluster in principal component space and which had very few refractory period violations were considered to be single units. In our sample, 99.3% (133/134) of neurons had no refractory violations (inter-spike intervals

of less than 1ms) and one neuron had a single violation, which accounted for less than 0.005% of all measured ISIs for that neuron. Since presentation of informative, uninformative, and novel motifs were temporally interleaved, none of the effects reported here can be due to changes in neuron isolation or changes in anesthetic state. Because the recording sites on each multi-channel array were only 50 μ m apart, stereotrode sorts were used to further improve sorting quality. All but one of the WS neuron pairs analyzed here were recorded from different electrode channels on the multi-channel array. Omitting the one pair recorded from the same channel does not alter the main results. Only neurons that were driven by the auditory stimulus were used in subsequent analyses.

All further analysis was performed using custom written MATLAB (MathWorks) software. Spike shape classification was performed using spike width, the time from the initial trough to the subsequent peak (Fig. 4.2a). During recording, data from some birds were low pass filtered at 3kHz and others at 5kHz. Because differences in this cutoff frequency can alter the spike shape (Vigneswaran et al., 2011), we applied a first-order low-pass Butterworth filter with cutoff frequency at 3kHz to all spike shapes to equalize these differences. All mean spike waveforms were cubic spline interpolated to a 2.5 μ s sampling interval. The filtering slightly increased the spike widths of all neurons. Thus, our threshold of 425 μ s between WS and NS neurons is towards the upper end of the distribution of thresholds used in previous reports (Mitchell et al., 2007; Vigneswaran et al., 2011).

Signal correlations were computed for each pair of neurons as the Pearson product-moment correlation coefficient between the mean (averaged over trials) firing rates to the four motifs within the informative, uninformative, and novel classes. Noise correlations were computed for each individual motif for each pair across trials, then averaged for all motifs within a class. Because motifs were variable in duration (range: 565ms-957ms, mean: 756ms) and the size of the analysis window can affect measured correlation values (Cohen and Kohn, 2011), we use only the first 565ms (the minimum motif duration) of each response in the analyses reported here.

Population coding simulations were performed similarly to previous reports (Shadlen et al., 1996; Cohen and Newsome, 2008; Cohen and Maunsell, 2009). Briefly, neurons were selected from our sample randomly, with replacement, and the mean firing rates for each relevant motif were assembled into a 4 \times N

vector, where N is the number of neurons in the simulated population. To construct a correlation matrix, we computed the noise correlation as predicted by the linear regression fits for informative, uninformative, and novel motifs (Fig. 4.4a-c) for each pair of neurons. Because arbitrary symmetric matrices are not, in general, valid correlation matrices, we used the matrix square root to construct a valid matrix (Shadlen et al., 1996). Because this technique only approximates the desired noise correlation values, we manually adjusted the square root of the correlation matrix such that the simulated correlation values matched the empirical values. This technique also introduced variability in the noise correlation values, which was appropriate for our simulations, because the actual distributions of noise correlations have substantial variability (Fig. 4.4a-c). Single neuron variability was set by the empirically measured firing rate variance. We did not model correlations between three or more neurons, but note that their effects on population coding have been shown to be minimal in the cortex (Cohen and Maunsell, 2009).

Responses to 10000 trials were simulated 100 times for each population size. The simulated responses were four N -dimensional vectors (two for left motifs and two for right motifs) generated from a multivariate Gaussian distribution following the previously described mean and covariance values. Responses of each simulated neuron to the two left motifs were grouped and responses to the two right motifs were grouped. Population coding of left vs. right motifs was quantified by projecting the two resulting groups onto the line that connected their mean responses, and computing d -prime for these two distributions (difference in mean divided by root mean square of the standard deviations) (Cohen and Maunsell, 2009). Modeling of responses with independent noise correlations was performed by trial-shuffling the responses of populations simulated from the informative motif correlation structure.

Statistical Analysis All data were tested for normality using the Lilliefors test evaluated at $p < 0.05$. When available, nonparametric tests were used when data were not normal. Central tendencies are reported as means \pm standard errors of the mean, except where noted.

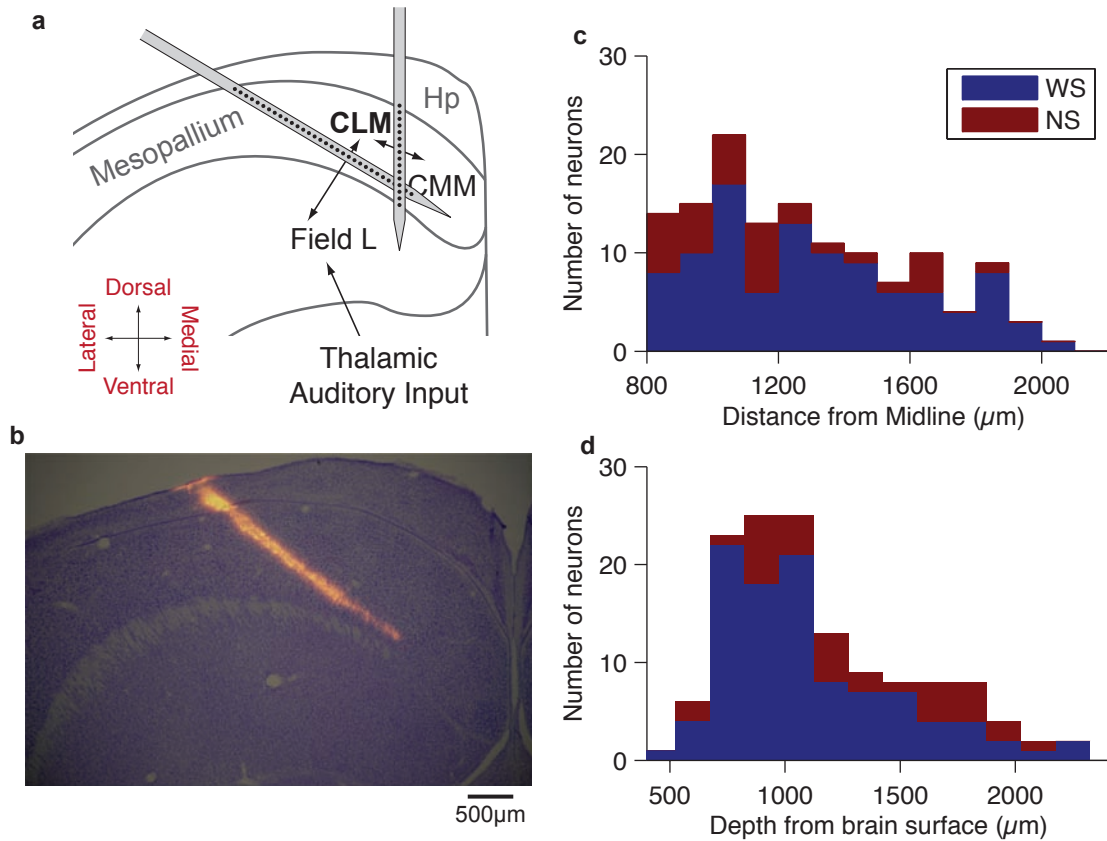


Figure 4.S1. Recording setup and histology. **(a)** Schematic showing approximate positioning of multi-channel electrode arrays within CLM. Most data was obtained from 1x32 linear probes inserted at a 35 degree angle. In some birds, a vertical penetration with a 1x16 linear probe was also used. **(b)** Nissl stained section showing fluorescent Di-I marking electrode penetration track into CLM. Orientation is same as in (a). Midline is to the right. **(c)** Distribution of distances from midline within CLM for wide spiking neurons (blue) and narrow spiking neurons (red). **(d)** Distribution of depths from the brain surface. Colors are as in (d).

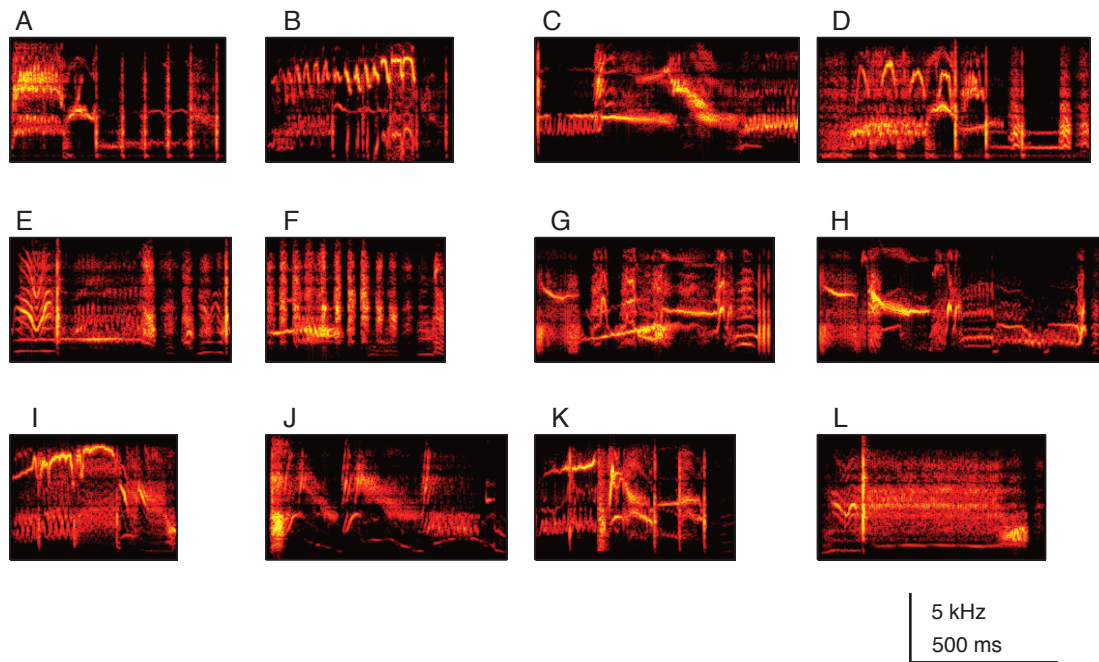


Figure. 4.S2. Spectrograms of motifs used in the study. Each spectrogram is a single motif and is identified by a letter A-L. During behavioral training, pairs of motifs were concatenated with 20ms of silence between them, as described by Fig. 1d,e. During neuronal recording, motifs were presented individually.

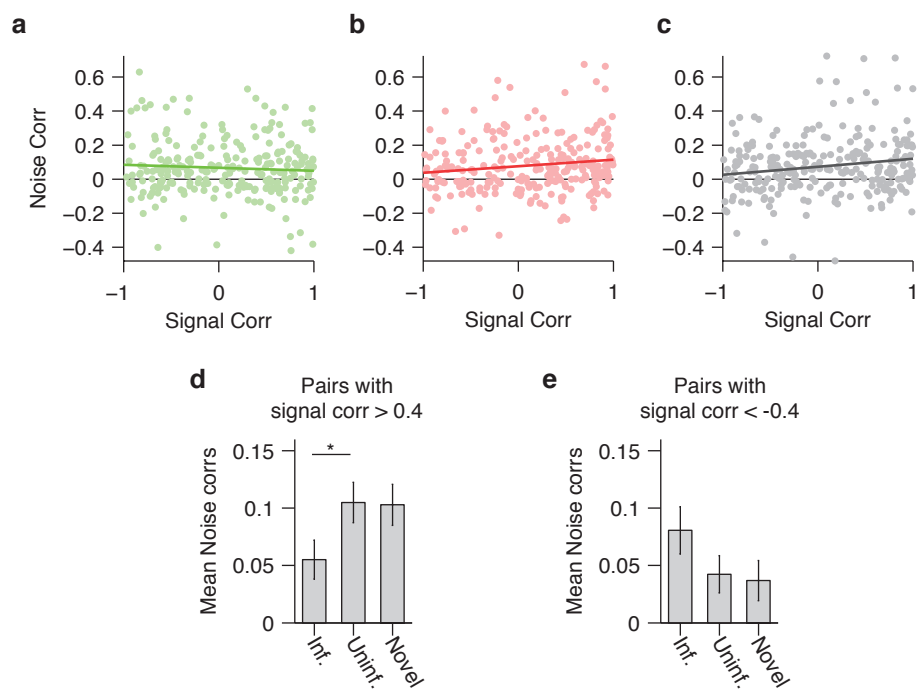


Figure 4.S3. Relationship between signal and noise correlations for all CLM neuron pairs. **(a-c)** Signal and noise correlations of pairs of all CLM neurons (including WS-WS, WS-NS, and NS-NS pairs) for informative (a), uninformative (b), and novel (c) motifs. The slopes of the linear regression fits for these three classes are significantly different (ANCOVA motif class \times regression slope interaction, $p = 0.024$). **(d)** Mean noise correlations for pairs of neurons with signal correlation greater than 0.4. **(e)** Mean noise correlations for pairs of neurons with signal correlation less than -0.4. * $p < 0.05$.

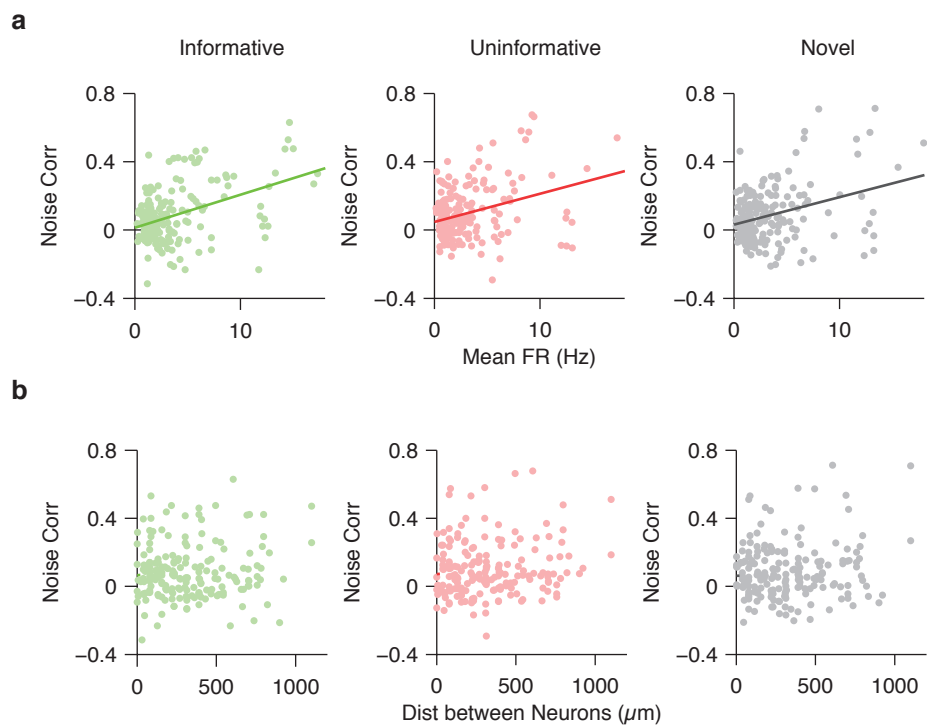


Figure 4.S4. Relationship between noise correlations and mean firing rate and inter-neuronal distance for pairs of WS neurons. **(a)** Scatter plots of mean firing rate and noise correlation. Noise correlation increases with mean firing rate of the pair, but no difference in this relationship exist between informative (left, green), uninformative (center, red), and novel (right, gray) motifs (ANCOVA motif class \times regression slope interaction, $p = 0.74$). **(b)** Scatter plots of distance between neurons and noise correlation. No difference in this relationship is observed between informative (left, green), uninformative (center, red), and novel (right, gray) motifs (ANCOVA motif class \times regression slope interaction, $p = 0.87$).

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References

- Abbott LF, Dayan P (1999) The effect of correlated variability on the accuracy of a population code. *Neural Computation*.
- Averbeck BB, Latham PE, Pouget A (2006) Neural correlations, population coding and computation. *Nature Reviews Neuroscience* 7:358-366.
- Bair W, Zohary E, Newsome WT (2001) Correlated firing in macaque visual area MT: time scales and relationship to behavior. *J Neurosci* 21:1676-1697.
- Bakin JS, Weinberger NM (1990) Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res* 536:271-286.
- Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G (2004) Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. *J Neurophysiol* 92:600-608.
- Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R (2008) A synaptic basis for auditory-vocal integration in the songbird. *J Neurosci* 28:1509-1522.
- Cohen M, Maunsell J (2009) Attention improves performance primarily by reducing interneuronal correlations. *Nat Neurosci*.
- Cohen MR, Newsome WT (2008) Context-dependent changes in functional circuitry in visual area MT. *Neuron* 60:162-173.
- Cohen MR, Kohn A (2011) Measuring and interpreting neuronal correlations. *Nat Neurosci* 14:811-819.
- Constantinidis C, Goldman-Rakic PS (2002) Correlated discharges among putative pyramidal neurons and interneurons in the primate prefrontal cortex. *J Neurophysiol* 88:3487-3497.
- Ecker AS, Berens P, Tolias AS, Bethge M (2011) The effect of noise correlations in populations of diversely tuned neurons. *J Neurosci* 31:14272-14283.
- Gentner TQ, Hulse SH (2000) Perceptual classification based on the component structure of song in European starlings. *J Acoust Soc Am* 107:3369-3381.
- Gentner TQ, Margoliash D (2003) Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669-674.
- Gu Y, Liu S, Fetsch CR, Yang Y, Fok S, Sunkara A, Deangelis GC, Angelaki DE (2011) Perceptual learning reduces interneuronal correlations in macaque visual cortex. *Neuron* 71:750-761.
- Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G (2000) Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J Neurophysiol* 84:401-414.
- Hartigan JA, Hartigan PM (1985) The Dip Test of Unimodality. *The Annals of Statistics* 13:70-84.
- Hofer SB, Ko H, Pichler B, Vogelstein J, Ros H, Zeng H, Lein E, Lesica NA, Mrsic-Flogel TD (2011) Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex. *Nat Neurosci* 14:1045-1052.

- Jeanne JM, Thompson JV, Sharpee TO, Gentner TQ (2011) Emergence of learned categorical representations within an auditory forebrain circuit. *J Neurosci* 31:2595-2606.
- Johnson KO (1980) Sensory discrimination: decision process. *J Neurophysiol* 43:1771-1792.
- Kohn A, Smith M (2005) Stimulus Dependence of Neuronal Correlation in Primary Visual Cortex of the Macaque. *Journal of Neuroscience* 25:3661.
- Lee D, Port NL, Kruse W, Georgopoulos AP (1998) Variability and correlated noise in the discharge of neurons in motor and parietal areas of the primate cortex. *J Neurosci* 18:1161-1170.
- Long MA, Fee MS (2008) Using temperature to analyse temporal dynamics in the songbird motor pathway. *Nature* 456:189-194.
- Mitchell JF, Sundberg KA, Reynolds JH (2007) Differential attention-dependent response modulation across cell classes in macaque visual area V4. *Neuron* 55:131-141.
- Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary, *Serinus canarius*. *J Comp Neurol* 165:457-486.
- Oram MW, Foldiak P, Perrett DI, Sengpiel F (1998) The 'Ideal Homunculus': decoding neural population signals. *Trends Neurosci* 21:259-265.
- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13:87-103.
- Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron* 70:121-131.
- Renart A, de la Rocha J, Bartho P, Hollender L, Parga N, Reyes A, Harris KD (2010) The asynchronous state in cortical circuits. *Science* 327:587-590.
- Shadlen MN, Britten KH, Newsome WT, Movshon JA (1996) A computational analysis of the relationship between neuronal and behavioral responses to visual motion. *J Neurosci* 16:1486-1510.
- Tamura H, Kaneko H, Kawasaki K, Fujita I (2004) Presumed inhibitory neurons in the macaque inferior temporal cortex: visual response properties and functional interactions with adjacent neurons. *J Neurophysiol* 91:2782-2796.
- Thompson JV, Gentner TQ (2010) Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *J Neurophysiol* 103:1785-1797.
- Vigneswaran G, Kraskov A, Lemon RN (2011) Large Identified Pyramidal Cells in Macaque Motor and Premotor Cortex Exhibit "Thin Spikes": Implications for Cell Type Classification. *J Neurosci* 31:14235-14242.

V. Conclusion

The preceding three chapters describe several important advances in how learning changes the function of neural circuits in the brain. Chapter 2 describes how neurons in CLM and CMM encode motifs from learned songs paired with reward with greater fidelity than motifs from unrewarded songs or novel songs. Chapter 3 describes how neurons in CLM respond with higher firing rates to motifs that are informative about the motor behavior required to achieve reward, while neurons in CMM respond with similarly high firing rates to both informative and uninformative motifs. Chapter 4 describes how populations of neurons in CLM respond to behaviorally informative motifs with a negative relationship between signal and noise correlations, which yields enhanced discrimination of informative motifs at a population level. Collectively, these results elucidate the learning-dependence of processing within the CLM-CMM circuit. Learning dependent processing within CLM had never been observed prior to this work, and the direct comparisons between CLM and CMM enabled by this work have highlighted important differences between these two regions, especially in their processing of behaviorally relevant song components. Because CLM projects to HVC (Bauer et al., 2008), an important brain region for the control of song production, the learning-dependent population coding we describe in CLM may be especially important for auditory feedback during juvenile song learning and adult song maintenance.

The studies described in Chapter 2 demonstrate that learning modifies the information encoding in both CLM and CMM in similar ways. To quantify this neural encoding, we measured the mutual information conveyed by neural firing rates about motif identity, a quantity that captures how variability in the stimulus correlates with variability in the response. In both CLM and CMM, mutual information about rewarded motifs was higher than that about unrewarded or novel motifs, indicating that a greater fraction of the response diversity of these neurons is allocated to rewarded motifs than other motifs. CLM may thus contribute to the particularly strong representations of learned songs observed in CMM (Gentner and Margoliash, 2003). Neurons in both CLM and CMM also encoded information about learned behavioral categories: responses to motifs or songs that were a part of the same training category (i.e. go, nogo, or novel songs) were more similar to each other than they were to motifs or songs of a different training

category. Although this categorization is not perfect, it is stronger for motifs than for songs and is stronger in CMM than in CLM, and is reminiscent of categorical encoding of objects observed in the visual system of monkeys (Freedman et al., 2001, 2003; Freedman and Assad, 2006). Thus, the abstraction of behaviorally defined categories of songs may emerge within the CLM-CMM circuitry and such representations may contribute to categorical encoding observed in the avian song production system (Prather et al., 2009).

Starling songs consist of many motifs, however, and thus there are many ways to distinguish between multiple songs. Are the motifs that are especially informative to this distinction encoded from other, more ambiguous motifs? The studies described in Chapter 3 demonstrate that the information a sensory signal carries about a behavior can influence neural encoding. By controlling for motif exposure and temporal association with reward during training, we could dissociate the effects of reward and exposure from the effects of information conveyed about behavior. We find that neurons in CLM elicit stronger responses for the informative motifs than for the uninformative or novel motifs. In contrast, neurons in CMM elicited similarly strong responses for both informative and uninformative motifs, but substantially weaker responses for novel motifs. In addition, the strength of these effects varied over the duration of the motifs, with the strongest difference in firing rates between informative and uninformative motifs occurring near the end of the motif among CLM neurons. Collectively, these findings highlight both a temporal and spatial dissociation of learning-dependent neural encoding within CM. Combined with the results from Chapter 2, these results suggest that different types of information may flow in different directions through the CLM-CMM circuit. While the formation of categorical representations may emerge from the medial flow of information from CLM to CMM, the preferential representation of behaviorally informative signals may emerge from the lateral flow of information from CMM to CLM. Further studies, however, will be necessary to fully understand the nature of this flow of information. Consistent with similar studies in monkey cortex (Sigala and Logothetis, 2002), these findings extend previous reports that reward and familiarity alter neural encoding in CM (Gentner and Margoliash, 2003; Jeanne et al., 2011) to show that the information that a signal conveys about a behavior also significantly alters neural encoding.

While the brain consists of billions of neurons, most studies of learning-related neural plasticity only investigate the effects on single neurons. It is widely accepted that neural processing in the cortex occurs in a distributed manner across large populations of neurons, and the nature of correlated activity between neurons can have a significant impact on the ability of the population to encode sensory signals (Johnson, 1980; Zohary et al., 1994; Abbott and Dayan, 1999; Averbeck et al., 2006). Nevertheless, learning has never been shown to enhance the encoding fidelity of a neural population code. The results shown in chapter 4, however, provide the first evidence that learning does enhance population-coding fidelity. Neural correlations can be divided into two classes: those derived from similarity in tuning functions (signal correlations) and those derived from trial-to-trial cofluctuations (noise correlations). While early experimental studies focused exclusively on noise correlations (Zohary et al., 1994), later studies showed that the relationship between signal and noise correlations is most important for neural coding in heterogeneous neuronal populations (Abbott and Dayan, 1999; Gu et al., 2011). In particular, a positive relationship between signal and noise correlations blurs the distinctions between the population representations of different stimuli whereas a negative relationship sharpens them (Figure 4.1a,b). To date, primarily negative relationships have been observed in cortical circuits (Bair et al., 2001; Kohn and Smith, 2005; Cohen and Maunsell, 2009; Gu et al., 2011). In CLM, we found the canonical positive relationship existed among putative excitatory neurons when processing uninformative and novel motifs, but observed a negative relationship when processing informative motifs. A simple neural population simulation showed that, for large neural populations, this shift in the correlation structure substantially enhanced the discriminability of informative motifs. Thus, these findings show for the first time that learning can alter the encoding ability of a neural population by altering the neural correlation structure. Such a result suggests that the neural correlation structure is more flexible than previously thought and implies that such neural population plasticity may be a fundamental principle of sensory cortical function.

These results suggest several directions for future research. First, it will be important to understand how sensory encoding in single neurons and neural populations changes during the course of learning. All the experiments described above were conducted in animals after learning had taken place. Historically, recording during learning has proven difficult in monkey models because of the large amounts

of time required to train them (Hoffman and Logothetis, 2009), although some studies have achieved some success (Messinger et al., 2001). In contrast, starlings can be trained very quickly, and the task can be optimized so that the learning of new sounds can occur on the timescale of minutes to hours (Daniel Knudsen, personal communication), which is fast enough to allow the monitoring of learning-driven modulations to single neuron activity with chronically implanted electrodes. Such techniques will also be important for addressing how interneuronal correlations change over the course of learning. Attention is known to reduce correlated activity in the cortex of monkeys (Cohen and Maunsell, 2009; Mitchell et al., 2009), so the related cognitive states that are involved during learning likely involve similar neuronal processes. Similarly, it will be important to understand how neural representations change after the initial learning. In the auditory cortex of rats learning a simple tone-discrimination task, the cortical area representing the learned tones expands during initial learning, and then renormalizes after extended training, following a “inverted U” shape (Reed et al., 2011). The data presented in this thesis are consistent with this (Figure 3.6), but tracking these changes in single neurons or populations after learning will be important to understand the precise time course of this neural plasticity.

A second direction for future research is to understand how different forebrain auditory regions interact. As shown in chapter 3, CLM and CMM encode different aspects of learned information, with CMM neurons responding most strongly to familiar songs and CLM neurons responding most strongly to behaviorally relevant songs. Neurons in NCM, however, respond qualitatively different: novel songs elicit stronger responses than learned songs (Thompson and Gentner, 2010). Somewhat similarly, preliminary evidence suggests that neurons in Field L1 respond more weakly to songs paired with reward than to unrewarded or novel songs, although evidence has not been found for the encoding of learning-related information in neurons in Field L2a and L3 (Emily Caporello, personal communication). In the forebrain, therefore, CM may be unique in its encoding of familiar and relevant information with higher firing rates. Because NCM is reciprocally connected with CMM and Field L1 is reciprocally connected with CLM (Vates et al., 1996), the inversely-related representation of learned songs suggests that CM may form an inhibitory relationship with both NCM and Field L. How does learning alter the flow of sensory information between these regions? Future experiments which simultaneously record neural activity in

CMM and NCM or in CLM and Field L could address this question. Unfortunately, given the vastness of the cortical network, the probability of finding synaptically coupled neurons is very small; coupled neuron pairs account for less than 1% of all simultaneously recorded neuron pairs in the medial prefrontal cortex (Fujisawa et al., 2008). Two approaches could prove helpful. First, a measure known as transfer entropy, the amount of the neural response not explained by its past response but that can be explained by the past response of another neuron, can be helpful in establishing the directionality in the flow of information even when neurons are not directly synaptically coupled (Schreiber, 2000; Gourévitch and Eggermont, 2007; Vicente et al., 2011). Second, comparing the local field potential (LFP), the spatially averaged neural activity over a region of several hundred microns in diameter (Mitzdorf, 1985; Katzner et al., 2009), between multiple regions can establish the broad-scale trends in information flow (Nauhaus et al., 2009) between forebrain regions. Both of these techniques would enable an assessment of whether the neural processing of learned songs flows through the forebrain network differently from novel songs, a question that has remained poorly explored (Salinas and Sejnowski, 2001). Furthermore, pharmacologically inactivating one region while measuring neural activity in another would complement these studies by providing causal evidence for the role of different brain regions on downstream neural representations (e.g. Bauer et al., 2008).

A third direction for future research is to further understand how processing differs between different cell types in CM. In chapter 4, we separate two classes of neuron on the basis of spike width, with the narrow spiking neurons generally thought to be inhibitory and the wide spiking neurons thought to be excitatory neurons (Harris et al., 2000; Bartho et al., 2004; Tamura et al., 2004). This correspondence, however, has recently been challenged (Vigneswaran et al., 2011). To more reliably classify cell type, two approaches could be taken. In one approach, recordings could be made using glass pipettes loaded with biocytin and, using the juxtacellular labeling technique, cells can be filled for later histological identification (Wilson and Sachdev, 2004). Physiological neural encoding properties could then be directly related to morphological properties, which have been shown to cluster into several neuron classes in the starling forebrain (Saini and Leppelsack, 1981). It is highly likely that neurons of different morphology serve different functions and elucidating this structure-function relationship is an important area of modern

neuroscience research (Bock et al., 2011; Briggman et al., 2011). In a second approach to classifying cell type, neurons could be identified based on their projection targets using antidromic stimulation. With this technique, a putative postsynaptic target region is stimulated electrically while recording a single neuron. If the stimulation creates a spike in the recorded neuron, this is taken as evidence that the neuron projects to the stimulated target region (Fuller and Schlag, 1976). Such a technique has proven highly successful in discriminating between projection targets of neurons other brain regions in songbirds, such as HVC (Dutar et al., 1998). Given the multiple projection targets of neurons in CLM and CMM (including Field L, NCM, and HVC), it will be important to understand how neurons with different projections encode learned sensory information. This will also provide important evidence for understanding how specific information flows through the auditory forebrain circuitry.

The experiments described in this thesis provide a new view into how learning changes the sensory processing capabilities of cortical circuits. I show that the processing of learned information changes between CLM and CMM and that this neural encoding depends both on the reward associated with a song and the information it provides with respect to behavior. Furthermore, I show that the interactions between neurons contribute to the formation of population-level representations that preferentially encode behaviorally relevant song components. It will be especially exciting to see how the learning-dependent representations in single neurons and neural populations in CLM and CMM interact with sensory processing within the larger auditory forebrain circuitry and ultimately, to understand how this circuitry causally influences perception, cognition, and behavior.

References

- Abbott LF, Dayan P (1999) The effect of correlated variability on the accuracy of a population code. *Neural Computation*.
- Averbeck BB, Latham PE, Pouget A (2006) Neural correlations, population coding and computation. *Nature Reviews Neuroscience* 7:358-366.
- Bair W, Zohary E, Newsome WT (2001) Correlated firing in macaque visual area MT: time scales and relationship to behavior. *J Neurosci* 21:1676-1697.
- Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G (2004) Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. *J Neurophysiol* 92:600-608.
- Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R (2008) A synaptic basis for auditory-vocal integration in the songbird. *J Neurosci* 28:1509-1522.
- Bock DD, Lee WC, Kerlin AM, Andermann ML, Hood G, Wetzel AW, Yurgenson S, Soucy ER, Kim HS, Reid RC (2011) Network anatomy and in vivo physiology of visual cortical neurons. *Nature* 471:177-182.
- Briggman KL, Helmstaedter M, Denk W (2011) Wiring specificity in the direction-selectivity circuit of the retina. *Nature* 471:183-188.
- Cohen M, Maunsell J (2009) Attention improves performance primarily by reducing interneuronal correlations. *Nat Neurosci*.
- Dutar P, Vu HM, Perkel DJ (1998) Multiple cell types distinguished by physiological, pharmacological, and anatomic properties in nucleus HVc of the adult zebra finch. *J Neurophysiol* 80:1828-1838.
- Freedman DJ, Assad JA (2006) Experience-dependent representation of visual categories in parietal cortex. *Nature* 443:85-88.
- Freedman DJ, Riesenhuber M, Poggio T, Miller EK (2001) Categorical representation of visual stimuli in the primate prefrontal cortex. *Science* 291:312-316.
- Freedman DJ, Riesenhuber M, Poggio T, Miller EK (2003) A comparison of primate prefrontal and inferior temporal cortices during visual categorization. *J Neurosci* 23:5235-5246.
- Fujisawa S, Amarasingham A, Harrison MT, Buzsaki G (2008) Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. *Nat Neurosci* 11:823-833.
- Fuller JH, Schlag JD (1976) Determination of antidromic excitation by the collision test: problems of interpretation. *Brain Res* 112:283-298.
- Gentner TQ, Margoliash D (2003) Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669-674.
- Gourévitch B, Eggermont JJ (2007) Evaluating information transfer between auditory cortical neurons. *J Neurophysiol* 97:2533-2543.
- Gu Y, Liu S, Fetsch CR, Yang Y, Fok S, Sunkara A, Deangelis GC, Angelaki DE (2011) Perceptual learning reduces interneuronal correlations in macaque visual cortex. *Neuron* 71:750-761.

- Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G (2000) Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J Neurophysiol* 84:401-414.
- Hoffman KL, Logothetis NK (2009) Cortical mechanisms of sensory learning and object recognition. *Philos Trans R Soc Lond B Biol Sci* 364:321-329.
- Jeanne JM, Thompson JV, Sharpee TO, Gentner TQ (2011) Emergence of learned categorical representations within an auditory forebrain circuit. *J Neurosci* 31:2595-2606.
- Johnson KO (1980) Sensory discrimination: decision process. *J Neurophysiol* 43:1771-1792.
- Katzner S, Nauhaus I, Benucci A, Bonin V, Ringach DL, Carandini M (2009) Local origin of field potentials in visual cortex. *Neuron* 61:35-41.
- Kohn A, Smith M (2005) Stimulus Dependence of Neuronal Correlation in Primary Visual Cortex of the Macaque. *Journal of Neuroscience* 25:3661.
- Messinger A, Squire LR, Zola SM, Albright TD (2001) Neuronal representations of stimulus associations develop in the temporal lobe during learning. *Proc Natl Acad Sci U S A* 98:12239-12244.
- Mitchell JF, Sundberg KA, Reynolds JH (2009) Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. *Neuron* 63:879-888.
- Mitzdorf U (1985) Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. *Physiol Rev* 65:37-100.
- Nauhaus I, Busse L, Carandini M, Ringach DL (2009) Stimulus contrast modulates functional connectivity in visual cortex. *Nat Neurosci* 12:70-76.
- Prather JF, Nowicki S, Anderson RC, Peters S, Mooney R (2009) Neural correlates of categorical perception in learned vocal communication. *Nat Neurosci* 12:221-228.
- Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron* 70:121-131.
- Saini KD, Leppelsack HJ (1981) Cell types of the auditory caudomedial neostriatum of the starling (*Sturnus vulgaris*). *J Comp Neurol* 198:209-229.
- Salinas E, Sejnowski TJ (2001) Correlated neuronal activity and the flow of neural information. *Nature Reviews Neuroscience* 2:539-550.
- Schreiber T (2000) Measuring information transfer. *Phys Rev Lett* 85:461-464.
- Sigala N, Logothetis NK (2002) Visual categorization shapes feature selectivity in the primate temporal cortex. *Nature* 415:318-320.
- Tamura H, Kaneko H, Kawasaki K, Fujita I (2004) Presumed inhibitory neurons in the macaque inferior temporal cortex: visual response properties and functional interactions with adjacent neurons. *J Neurophysiol* 91:2782-2796.
- Thompson JV, Gentner TQ (2010) Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *J Neurophysiol* 103:1785-1797.
- Vates GE, Broome BM, Mello CV, Nottebohm F (1996) Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366:613-642.

- Vicente R, Wibral M, Lindner M, Pipa G (2011) Transfer entropy--a model-free measure of effective connectivity for the neurosciences. *J Comput Neurosci* 30:45-67.
- Vigneswaran G, Kraskov A, Lemon RN (2011) Large identified pyramidal cells in macaque motor and premotor cortex exhibit "thin spikes": implications for cell type classification. *J Neurosci* 31:14235-14242.
- Wilson CJ, Sachdev RN (2004) Intracellular and juxtacellular staining with biocytin. *Curr Protoc Neurosci* Chapter 1:Unit 1 12.
- Zohary E, Shadlen MN, Newsome WT (1994) Correlated neuronal discharge rate and its implications for psychophysical performance. *Nature* 370:140-143.