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Neuronal representations underlying probabilistic sequence discrimination

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

in

Neurosciences with a Specialization in Computational Neurosciences

by

Justin Kiggins

Committee in charge:

Professor Timothy Gentner, Chair
Professor Thomas Albright
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2016

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Chair

University of California, San Diego

2016

DEDICATION

To my mother.

EPIGRAPH

“Ideas are tested by experiment.”
That is the core of science.
Everything else is bookkeeping.

Zombie Feynman
XKCD: Unscientific
xkcd.com/397/

TABLE OF CONTENTS

SIGNATURE PAGE	iii
DEDICATION	iv
EPIGRAPH	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
ACKNOWLEDGEMENTS	ix
VITA	xi
ABSTRACT OF THE DISSERTATION	xii
CHAPTER 1 Introduction to the Dissertation	1
1.1 Sequence perception in speech & language	2
1.2 Sequence specificity in motor learning	4
1.3 Sequence perception in vocal communication	5
1.4 Making decisions based upon sequences	6
1.5 Population coding of sequences	7
1.6 References	8
CHAPTER 2 Sequence-specific facilitation supports vocal sequence discrimination .	12
2.1 Abstract	13
2.2 Introduction	14
2.3 Methods	18
2.3.1 Subjects	19
2.3.2 Sequence elements	19
2.3.3 Behavior	19
2.3.4 Behavioral performance	21
2.4 Neurophysiology	22
2.4.1 Recording	22
2.4.2 Sequences for neurophysiological recording	23
2.4.3 Spike sorting	23
2.4.4 Putative cell types	24
2.4.5 Neurophysiology Data Analysis	24
2.4.6 Linear model of combination responses	24
2.4.7 Sequence selectivity	25
2.4.8 Discriminability	26
2.5 Results	26
2.5.1 Starlings discriminate between probabilistic auditory sequences	26
2.5.2 Behavioral discrimination depends on evidence in a sequence	27
2.5.3 Single neuron responses in NCM are sequence-specific	29
2.5.4 Sequence responses support the discrimination of diagnostic sequences	32
2.5.5 Sequences are better encoded in neurons which prefer familiar elements	34
2.5.6 NCM can discriminate between diagnostic sequences better than sequence elements	35

2.5.7	Sequences are facilitated by reward-association if it will improve sequence discrimination	38
2.5.8	Prediction of reward-associated sequences.....	39
2.6	Discussion.....	41
2.7	References.....	48
CHAPTER 3	State-dependent element encoding in neuronal populations during probabilistic sequence discrimination.....	51
3.1	Abstract.....	52
3.2	Introduction.....	52
3.3	Methods.....	55
3.3.1	Behavior.....	55
3.3.2	Sequence generation	56
3.3.3	Task acquisition & shaping procedures	58
3.3.4	Behavioral performance.....	58
3.3.5	Neural Probe Placement.....	59
3.3.6	Neural recording block design	59
3.3.7	Neural Recording Sessions	60
3.3.8	Identifying neurons	60
3.3.9	Putative cell types based on waveforms	61
3.3.10	Cluster Quality and Inclusion Criteria	62
3.3.11	Identifying Auditory Neurons.....	62
3.3.12	Mixed Selectivity.....	63
3.3.13	Estimating the Coding Capacity of Neuronal Populations with Linear Decoders.....	63
3.4	Results.....	64
3.4.1	Sequence Discrimination	64
3.4.2	Mixed Selectivity to Sequence Parameters.....	66
3.4.3	Population Encoding of Element Identity.....	69
3.4.4	Role of Coordinated Activity in Element Encoding	71
3.4.5	Inter-population Facilitation of Element Encoding	73
3.5	Discussion.....	76
3.6	References.....	83
CHAPTER 4	Conclusion to the Dissertation	87
4.1	Major Findings.....	88
4.2	Future Directions	89
4.3	References.....	94

LIST OF FIGURES

Figure 2.1. Training starlings to discriminate between probabilistically generated sequences of vocal elements.	27
Figure 2.2. Sequence discrimination is predicted by individual transitions.	29
Figure 2.3. Single units recorded from the avian forebrain.	30
Figure 2.4. Responses to sequences are not well predicted by the responses to isolated elements.	31
Figure 2.5. Familiar-tuned neurons discriminate between GO and NOGO sequences better than novel-tuned neurons.	33
Figure 2.6. Familiar-tuned neurons exhibit better discrimination between sequences than between elements.	37
Figure 2.7. Rewarded sequences of familiar-tuned neurons' best contrast are facilitated when the rewarded target is a higher firing rate than the non-rewarded target. ...	39
Figure 2.8. Offsets responses predict reward-associated sequences.	41
Figure 3.1. Starlings can discriminate between probabilistically generated sequences. ..	57
Figure 3.2. Net evidence in a sequence predicts starlings' decisions.	66
Figure 3.3. Awake behaving single unit population recordings during decision-making. ...	68
Figure 3.4. Mixed selectivity for element identity and position.	69
Figure 3.5. Encoding accuracy improves with larger populations.	71
Figure 3.6. Coordinated population activity improves element encoding.	73
Figure 3.7. Accurate decoding of element identity by FS and RS populations predicts accurate encoding across-, but not within-populations.	76

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ABSTRACT OF THE DISSERTATION

Neuronal representations underlying probabilistic sequence discrimination

by

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Doctor of Philosophy in Neurosciences with a Specialization in Computational
Neurosciences

University of California, San Diego, 2016

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How language and music are processed by the biological connection in our heads is one of the most significant challenges to neuroscience. A critical aspect of this challenge is that the signals are necessarily sequential and the brain must process events at multiple hierarchical levels simultaneously. Aspects of this are not unique to humans,

however, but shared with many animals who rely on vocal communication. In this work, we focus on characterizing the capacity of European starlings to discriminate between sequences and understanding how individual neurons and populations of neurons support sequence discrimination. We train starlings on novel behavioral protocols wherein subjects must discriminate between probabilistically generated sequences composed of vocal elements using only the sequential relationships between elements. When faced with uncertainty, subjects discriminate sequences in a way which is consistent with weighing the evidence afforded by the sequence. By recording the spiking activity of neurons in anesthetized subjects, we found that neurons in the caudomedial nidopallium (NCM) are sensitive to sequences and that their capacity to encode sequences is higher than their capacity to encode elements. Neuronal responses to sequences are shaped by a combination of reward-association and behavioral demands. Recordings of population activity in awake behaving subjects revealed that neurons in caudolateral mesopallium (CLM) exhibit mixed selectivity for elements and position, supporting robust population representations of element identity. Element encoding fidelity in CLM is state-dependent—accurate decoding of element identity from either fast-spiking or regular-spiking populations improves the reliability and encoding fidelity of the complementary population. These results emphasize the importance of behavioral goals in understanding how sequences are processed by neuronal populations.

CHAPTER 1

Introduction to the Dissertation

1.1 Sequence perception in speech & language

In many sensory modalities, information emerges over time and the sequence of sensory events is important for decision making. This is especially clear in the speech perception and language comprehension, where phoneme sequencing is critical to recognizing words and word order is critical to understand the meaning of a sentence. Importantly, speech streams are processed in parallel with the incoming speech stream. As more words are perceived, segmented, and identified, there is more evidence of the meaning of the sentence. A good example of this can be found by considering how evidence is accumulated in the sentence “The old man the boat.” First, we are tempted to assume that the evidence accumulated is simply the five words of the sentence. However, there are multiple steps of cognitive processing between the physical signal of air pressure on the eardrum to the percept of five words, let alone extracting the meaning of the sentence. One of the first needs of the auditory system is to parse the speech stream into its unitary elements: phonemes, in the case of human speech. Phonemes occupy learned categories in the the feature space of speech signals. Importantly, the boundaries between phonemes (if they occur at all) are learned. For example, Japanese does not distinguish between r and l ; accordingly, Japanese speakers do not perceive r and l to be distinguishable phonemes. The second challenge to the auditory system is that speech signals rarely have silence between words. Rather, each phoneme rolls into the next and one of the first challenges faced by the auditory system processing a speech signal is parsing boundaries between words. One key feature that assists in this process is the joint likelihoods of adjacent phonemes... phonemes that occur next to each other within words exhibit higher joint likelihoods than those which occur across word

boundaries. One should not assume that this processing is a simple feed-forward process. On the contrary, while the joint likelihoods of phonemes are used to segment words they are also used to resolve ambiguity in prior phoneme identities. As phonemes and word boundaries are established, words themselves can be resolved, which need to be assigned with lexical meaning. Lexical processing is complicated by homophonic relationships between words or words which can partake in multiple parts of speech. A major cue to the meaning of a word is the sequential context in which it is presented. This is all to say that evidence in a speech stream accumulates at each level of analysis (phonological, lexical, syntactical) simultaneously and does not simply flow from the bottom up, rather, each level provides evidence for the other levels throughout the speech stream.

Thus, rather than simply making a decision based on veridical features of the sensory environment, speech perception involves multiple cognitive processes which must transform and reshape the signal (acoustic power at different frequencies over time) into relevant sequential elements (the words in a sentence). Sequential evidence is ubiquitous in speech and language—properly perceiving a sequence of phonemes is important to identify whether a police officer yells “STOP!” or “POTS!” and the evidence of the appropriate response does not lie in the individual elements of the sequence but in their ordering. Similarly, garden path sentences, like “The old man the boat,” are a canonical example of the challenge of accumulating sequential evidence in language: the meaning of the sentence emerges sequentially and we typically don’t wait until the “end” of a sequence to try to make sense of it. Instead, as evidence of the meaning of the sentence is accumulated, we decide what it means piecemeal.

1.2 Sequence specificity in motor learning

One area where sequence-specific neuronal responses have been extensively studied is in the context of songbird motor learning. A defining characteristic of songbirds is that they learn their vocalizations. Many songbirds, like the commonly studied zebra finch, learn their songs as juveniles from an adult tutor (Brainard and Doupe, 2002). When finches learn, they learn both elements of the tutor song (called “notes”) as well as the sequence that they are arranged in (Menyhart et al., 2015; Takahashi et al., 2010). The premotor area HVC responds robustly to playback of the bird’s own song (Margoliash, 1986) and the sequence of these syllables matters: many neurons will respond to syllable B only when it is preceded by syllable A (Lewicki and Konishi, 1995; Margoliash, 1983; Margoliash and Fortune, 1992). In the auditory area Field L, this effect is less strong: many neurons show sensitivity to the sequence, but many others respond to each element alone (Lewicki and Arthur, 1996). This effect is consistent with a hierarchy of sequence representation, with HVC neurons integrating sequential information to drive sequence-specific responses. The songs of zebra finches, however, have a significant limitation when it comes to exploring the sequence statistics that drive this sensitivity: because their songs are largely static. Bengalese finches, however, sing songs with more variability in their sequences, which permits isolating the influences of elements and position identity by characterizing the statistical properties of the sequence (Honda and Okanoya, 1999). Sequence sensitivity in Bengalese finch HVC is diverse (Nishikawa et al., 2008) and reflects the statistical variability of the bird’s song (Bouchard and Brainard, 2013, 2016).

1.3 Sequence perception in vocal communication

In addition to learning motor sequences, the perceptual and cognitive challenges underlying speech and language perception are also faced by many animals which rely on vocal communication. Female Tungara frogs make decisions about whether to approach males as prospective mates according to the number of “chuck” elements in their characteristic “whine-chuck” vocalizations (Akre et al., 2011). Many animals make decisions based on representational alarm calls from conspecifics: prairie dogs (Kiriakis and Slobodchikoff, 2006), vervet monkeys (Seyfarth et al., 1980), etc. The Japanese great tit (*Parus minor*) further exhibits compositional syntax, where the meaning of a vocalization changes based on the syntax (Suzuki et al., 2016). Further, many animals can be trained to extract meaning from vocal signals. It is common knowledge that dogs can learn commands; at least one dog has learned extensive lexical assignment (Pilley and Reid, 2011) and that the sequential composition of a command affords evidence (Pilley, 2013). Among birds, European starlings show great faculty making decisions based on complex sequences (Comins and Gentner, 2010, 2014; Gentner et al., 2006). Chapters 1 and 2 extend these results to better understand the capacity of starling to discriminate between sequences based on their statistics.

To what extent then are sequences learned in auditory regions involved in perception? Following passive exposure to sequences of synthesized harmonic stacks, the secondary auditory areas NCM and CLM show suppression in response to more probable sequences, while Field L does not (Lu and Vicario, 2014). In mammalian auditory cortex, on the other hand, it has been shown that sequences which are associated with reward

tend to respond more robustly than those not associated with reward (Yin et al., 2008; Zhou et al., 2010). The rapid plasticity observed in NCM and CLM following passive exposure could lay the groundwork for facilitation of salient, behaviorally relevant sequences. Chapter 1 investigates further how sequence sensitivity in NCM is shaped by reward association and behavioral goals.

1.4 Making decisions based upon sequences

To build a complete picture of sequence perception, we have to consider how sequence information is used to make decisions. The dominant paradigm for studying the decision making at the level of individual neurons has been in the visual system, most often using random dot motion tasks (Gold and Shadlen, 2007). In these tasks, monkeys are presented with randomly moving dots in a subset of their field of view. The direction of motion of the dots are correlated with each other, however, and the subject is trained to make a decision about the direction in which they are correlated. From this paradigm, key steps in the decision making process have been elucidated and tied to anatomical regions. The visual area MT largely represents the direction of dot motion at any point in time, while activity in LIP slowly ramps. When the correlated activity is stronger, neurons in LIP ramp more quickly. From these studies an understanding of visual decision making has emerged where LIP neurons represent the cumulative evidence of the appropriate response and ramp their activity to a decision threshold. Evidence accumulation in LIP can be modelled as a drift-diffusion process, where evidence from MT pushes a decision variable to one of two outcomes. This is equivalent to a Bayesian model of evidence accumulation, where decisions are based the relative likelihood of the model given the

evidence (Bitzer et al., 2014). The Bayesian nature of the drift diffusion model is important, because, despite the differences between language comprehension and perceiving coherent dot motion, the sequential process of recognizing words in a sentence can also be explained as a Bayesian process (Norris, 2006). Because the drift diffusion model is agnostic to the representation of the unitary evidence, it is a viable candidate mechanism for the accumulation of evidence in speech and language as well. Indeed, recent work has shown that LIP integrates probabilistic evidence afforded by symbols as well (Kira et al., 2015; Yang and Shadlen, 2007). So while it is unclear how more abstract forms of evidence (like word identities) are accumulated in neuronal ensembles to drive decisions and what regions might accumulate evidence for other behavioral outcomes, sequence decision making is well situated to build upon to work that has been done in the visual system and extend it to auditory perception processes that are relevant to language comprehension. Chapters 1 and 2 develop probabilistic sequence discrimination behaviors that are amenable to neurophysiological studies of evidence accumulation and decision making in the context of vocal communication.

1.5 Population coding of sequences

Another feature of encoding parameters in population activity that is lost when only considering single-neuron tuning is the benefits of mixed selectivity. If individual neurons exhibit a mixture of tuning specificity to different parameters, then arbitrary combinations of parameters can be readily decoded from population activity with linear decoders (Rigotti et al., 2013). For example, if neurons in a population exhibit mixed selectivity for elements and position, then another region could readily decode sequences

or arbitrarily categorize sequences by learning the appropriate linear discrimination of the population response space. Mixed-selectivity can emerge in state-dependent networks, where the activity of the network is driven not only by incoming stimuli, but the interaction between a stimulus and the dynamic state of the network (Buonomano and Maass, 2009). Such networks naturally lend themselves to probabilistic sequence encoding, where the relevance of a sequence element is due not only to the element identity, but it's relationship to prior elements in the sequence. Population coding capacity is also sensitive to the ways in which the population activity covaries. Trial-to-trial correlations have typically been observed to have deleterious effects on stimulus encoding (Averbeck et al., 2006; Moreno-Bote et al., 2014), however these negative effects can be relieved or even become beneficial in certain behavioral contexts (Cohen and Newsome, 2008; Jeanne et al., 2013). Chapter 2 addresses how coordinated population activity in CLM improves element encoding fidelity.

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CHAPTER 2

Sequence-specific facilitation supports vocal sequence discrimination

2.1 Abstract

Understanding speech and language requires that we learn to perceive and make decisions based upon sequences of words and phonemes. Other organisms that rely on vocal communication are faced with similar demands to listen to sequences and decide how to react. Sequence-selective neuronal responses have been described in multiple species, sensory modalities and brain regions, but our understanding of how vocal communication sequences are learned to support decision making is lacking. In order to address this, we trained European starlings, a preeminent model for the neurobiological mechanisms involved in the processing of complex acoustic communication signals, to discriminate between sequences of vocal elements. The task is designed such that the relative order of elements is the only cue available to solve the task: element identity and position are irrelevant. We observe that subjects' decisions are predicted by a model where local sequence information is accumulated to provide evidence of the appropriate behavioral response. To understand how the neuronal representations of these sequences might underlie sequence discrimination, we recorded spiking activity from individual neurons in a vocalization-selective auditory region of the songbird forebrain in response to sequences that subjects had been trained to discriminate. The neurons in the caudomedial nidopallium (NCM) are highly sensitive to sequences—sequence responses are poorly predicted from the responses to sequence elements. When presented with sequences that subjects had been trained to discriminate, individual neurons discriminate these sequences better than they discriminate the composite elements in isolation. This improvement in neuronal discrimination of the learned sequences is caused in part by facilitation due to reward-association. However, we find that reward-associated

facilitation is specific to those sequences for which facilitation enhances neuronal discrimination—when facilitation would result in impaired discrimination, no facilitation is observed. Thus, behavioral demands constrain the mechanisms of sequence learning. Our results point to the importance of behavioral goals in understanding how sequences are learned and represented.

2.2 Introduction

Sequence processing is an integral aspect of speech and language perception. Yet our understanding of how single neurons represent learned sequences is poor. In particular, we know very little about how vocal sequences are learned and how their neuronal representations are shaped to support decision making. Sentence comprehension depends upon the perception of sequences of words. Word recognition depends upon the perception of the sequence of phonemes. Statistical regularities in the sequences of phonemes and words facilitate speech and language perception. Sensitivities to the statistical regularities of vocal sequences are established very early, well before infants learn to speak (Aslin et al., 1998), pointing to a fundamental role of learning sequence statistics in speech perception (Saffran et al., 1996). Into adulthood, the extent to which phonological and syntactical elements are consistent with expectations established from the statistics of a speech stream profoundly affects both recognition and comprehension (Levy, 2008).

To investigate the how vocal sequences are learned, we use the European starling, a preeminent model for a subset of the neurobiological mechanisms involved in the representation, categorization, rule-learning, and top-down cognitive processing of

complex acoustic communication signals. As vocal learners, songbirds are a neurobiological model for human speech production and perception (Doupe and Kuhl, 1999). While songbirds do not possess language, they do share with humans the necessity of solving the core cognitive challenges of vocal sequence recognition. Like human speech and language, the songs of oscine songbirds show temporal structure at multiple hierarchical levels, from low-level acoustic features to the sequencing of auditory objects (Kiggins et al., 2012). Sequence recognition requires at least two neurobiological mechanisms: a neuronal sensitivity to sequences and a means for such sensitivity to be modified through learning. Auditory sequence sensitivity has been demonstrated in bats (Suga et al., 1978), songbirds (Margoliash and Fortune, 1992; Lewicki and Arthur, 1996), monkeys (Brosch et al., 1999; Bartlett and Wang, 2005), cats (Brosch and Schreiner, 2000), and rodents (Kilgard and Merzenich, 2002; Asari and Zador, 2009), indicating that the mechanisms of sequence sensitivity are either broadly conserved or greatly varied. How these sensitivities are modified through learning is less clear. Among songbirds, learning to produce vocal sequences is associated with increased neuronal sensitivity to these vocal sequences. In the songbird forebrain, both auditory and associative regions are selective to the particular sequence of a bird's own vocalizations (Margoliash, 1983; Margoliash and Fortune, 1992; Lewicki and Konishi, 1995; Lewicki and Arthur, 1996). This song sequence sensitivity emerges in the motor-learning pathway in parallel with song learning (Doupe and Solis, 1997). Vocal learning, however, is a specific case of sequence learning. It's not clear how the auditory sequence sensitivity that emerges when a bird learns to produce a sequence is related to the perception of sequences for other behavioral goals. Sequence perception is broadly important in vocal communication

(Gentner and Hulse, 2000a, 2000b; Farris et al., 2002). Female starlings listen to the statistical regularities of male song in order to assess reproductive fitness (Gentner and Hulse, 2000b). More generally, starlings use sequence statistics as a cue for individual recognition (Gentner and Hulse, 2000a). Importantly, starlings must make decisions about how to respond appropriately to a vocal sequence that they learned to recognize. Yet, we know relatively little about the neural mechanisms that shape and modify vocal sequence representations to support decision-making.

Due to their exquisite ability to recognize and make decisions based on vocal communication signals they hear and our knowledge of the brain regions that support auditory perception, the European starling is an ideal model for addressing these questions. Starlings can be readily trained to make decisions about things they hear and they are very good at recognizing sequences with complex syntax (Gentner et al., 2006; Comins and Gentner, 2010). The avian auditory forebrain contains regions which are hierarchically connected—the thalamorecipient region Field L2 projects to Field L1 and Field L3, which in turn project to the vocalization-sensitive regions of the Caudal Nidopallium (NCM) and the Caudal Mesopallium (CM). Neurons in the CM and NCM reflect this hierarchical structure in their tuning, responding preferentially and selectively to conspecific vocalizations over synthetic stimuli such as tones or white noise. Their response characteristics are more complex than a simple feed-forward model of increasingly selective receptive fields would predict—in fact, individual neurons in NCM can be driven by multiple orthogonal features (Kozlov and Gentner, 2016) and the computational role of individual neurons are highly flexible (Kozlov and Gentner, 2014).

Spiking responses in CM and NCM are also shaped through experience—associations with both reward and behavioral goals modify the response properties of individual neurons and population activity (Gentner and Margoliash, 2003; Jeanne et al., 2013). Associative learning drives long-lasting changes in tuning of NCM neurons, principally through response suppression (Thompson and Gentner, 2010), mediated by stimulus-specific inhibition (Thompson et al., 2013). These characteristics point to NCM and CM and candidate regions for encoding sequences used for behavioral goals.

It is well established that reward plays an important role in associative learning, enhancing the representations of associated stimuli. Reward plays a role in sequence representation as well—sequences which are associated with reward tend to respond more robustly than those not associated with reward (Yin et al., 2008; Zhou et al., 2010). However, if subjects are using neuronal representations of sequences or other stimuli in order to make decisions about appropriate behavioral responses, the indiscriminate facilitation of reward-associated stimuli risks hurting some decisions as much as it facilitates others. Take, for example, a neuron which responds strongly to stimulus A and weakly to stimulus B. Naively, this neuron has some capacity to discriminate between the two stimuli. If stimulus B is then associated with reward and facilitated, this facilitation will actually impede the capacity of our hypothetical neuron to discriminate between these two stimuli. Therefore, it is important to understand how reward-associated facilitation is constrained behavioral demands to discriminate between stimuli.

In order to understand how sequences are represented, we trained European starlings to discriminate among sequences according to their statistical regularities then

recorded spiking responses to sequences in NCM. A primary goal in the design of our sequence discrimination task was to isolate the influence of sequencing on representations from position cues or the identity of sequence elements. In a simple task design wherein subjects learn to discriminate between two static sequences (say, ABCD versus DACB), subjects can solve the task by listening for a particular element in an absolute position in the sequence rather than learning the full sequence per se (for example, listening D versus B in the final position). Indeed, prior investigations into the strategies starlings use to discriminate sequences point to the primacy of element-position cues in their decision making (Comins and Gentner, 2010). In order to focus on the information afforded by the sequence exclusively, we developed a sequence discrimination task where subjects were required discriminate among sequences based solely upon the order of elements within a sequence—neither the identities of individual elements nor their absolute positioning afforded any diagnostic value. After behavioral training, we presented sequences to anesthetized birds while recording the spiking responses of single units from NCM. We find that neurons in NCM are more selective for sequences than would be expected from the responses to isolated elements. This sequence sensitivity provides a rich substrate for sequence encoding—neurons in NCM discriminate between sequences that birds had been trained to discriminate better than their constituent elements. We find that the improved discrimination of learned sequences can be explained in part by the facilitation of reward-associated sequences contingent upon an improvement in sequence discrimination.

2.3 Methods

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

2.3.1 *Subjects*

Subjects for the experiment were eight adult European starlings (*Sturnus vulgaris*), wild-caught in Southern California. We did not record sex. Prior to behavioral experiments, subjects were housed in aviaries with full access to food and water. Light schedules were matched to local San Diego sunrise and sunset times.

2.3.2 *Sequence elements*

Stimuli used in this study for both behavioral training and neurophysiology were all constructed from a set of 12 conspecific song elements sampled from the previously recorded songs of 2 male European starlings (Fig 1A). The elements were chosen to be spectrotemporally diverse and of similar duration (range: 797-845ms). Elements were scaled to 68db average power during the duration of the element and 5ms linear ramps were added to the beginning and end of each wav file.

2.3.3 *Behavior*

Starlings were trained on a standard GO/NOGO operant conditioning task (Gentner and Margoliash, 2003) using a variable ratio (VR) reinforcement schedule (Ferster and Skinner, 1957). During behavioral training, subjects were housed in a sound attenuating chamber (Acoustic Systems) with access to an operant panel as described previously Fig 1A; (Thompson and Gentner, 2010). Subjects were provided free access to water and received food by engaging in the operant task.

For each subject, 8 of the 12 elements were used for the behavioral training, while the other 4 elements were reserved for later use in the neural recording experiment. Stimuli for trials consisted of 8-element long sequences generated according to one of two first-order Markov models associated with the stimulus class (GO or NOGO) of the trial (Fig 1C). Each model contains a unique set of 8 transitions that occur with high probability, α . Thus, there are 8 ordered pairs that are more likely to occur in sequences generated by that model. That is, for each model, any given element (say, B) is most likely to be followed by the “next” element (say, C) with probability α , whereas it might jump to in an ambiguous direction (say, E) with probability $(1-\alpha)/7$. Notably, it might transition in the opposing model’s dominant path (say, to A) with probability $(1-\alpha)/7$, yielding a misinformative ordered pair. Thus, there is some overlap between the two models in the sequences that they might produce. Any given value of α determines both the relative odds that a single ordered pair (say BC) was generated by the each model, as well as the odds that the entire sequence (say BCDEFGHA) was generated by each model. Thus, as α is decreased, the overlap between the two models increases, yielding more ambiguous sequences and increasing the overall difficulty of the task.

For each trial, a sequence was generated with a silent period in between sequence elements (mean 100ms). For two subjects, the silent period was fixed at 100ms (std. dev. = 0ms). For the remaining 6 subjects, the ISI was sampled randomly from a normal distribution for each interval (mean=100ms, st. dev. = 15ms). Playback of the sequence began immediately when a trial-initiation peck was detected, then pecks were queried during a 2-second response window following sequence playback. If the subject pecked

within the response window, the trial outcome was recorded as a response, otherwise, the trial outcome was recorded as a non-response. Correctly responding to GO sequences were rewarded with a brief flash of a green LED on every trial followed by 2 seconds of food access according to the VR schedule. Incorrectly responding to a NOGO sequence was punished by turning off the lights for 5–90 seconds. Failure to peck during the response window resulted in neither a food reward nor a timeout. The light schedule followed local sunrise and sunset times and during daylight hours subjects were free to initiate trials at will during this time.

For task acquisition, subjects began with the parameter α set to the highest (and therefore, least difficult) level used in the study, 0.93. As necessary, we extended response windows and/or timeout durations, or forced correction trials temporarily to support acquisition of the task. Once a subject's performance increased above chance performance, we terminated the use of correction trials and/or extended response windows and began to incrementally increase the VR to 2 (range: 1 through 3) or 3 (range: 1 through 5), ensuring that subjects received sufficient access to food. Once performance was very strong and stable, we began increasing the difficulty of the task by incrementally decreasing the value of the parameter α .

2.3.4 Behavioral performance

To summarize the final performance for each subject, we analyzed the last 10,000 trials performed by each subject. These were the most difficult trials for each subject (α range, 0.67–0.44), performed well after the subject had learned the task. To evaluate how the subject's response decision depends on the evidence, we performed a logistic

regression on the response outcome for trials conditioned on the number of reward-associated (GO) and non-reward-associated (NOGO) ordered pairs in presented strings. To evaluate how the subject's response depends on the comparison of evidence for each model, we performed a logistic regression conditioned using the difference between the number of GO ordered pairs and NOGO ordered pairs as the terms. These regressions were implemented in R as a generalized linear model with a logit link function.

2.4 Neurophysiology

Following behavioral training, six birds were anesthetized and extracellular recordings were obtained from the secondary auditory forebrain in response to isolated ordered pairs.

2.4.1 *Recording*

Prior to performing neural recordings, subjects were anesthetized with isoflourane (1.0–2.0% concentration in oxygen) and head-fixed. The upper layer of skull & trabecular were removed above NCM. A headpin was affixed to the skull caudal to the craniotomy with dental acrylic.

For the neural recordings, the subject was anesthetized with urethane (20% by volume, 7ml/kg) and head fixed in a stereotactic apparatus inside of a sound-attenuating chamber. A craniotomy was performed above NCM. A linear 32-channel silicon probe with 177um pads (Neuronexus) was coated with Di-I, then advanced through the dura until spiking activity was visible on one or more channels in response to samples of starling song. The extracellular waveform was amplified (5000 gain), filtered (high pass: 0.1 Hz, low pass: 5 kHz), sampled (25 kHz), and stored for offline analysis (Spike2, v 7,

Cambridge Electronic Design). We recorded multiple blocks at a given recording site, subject to maintenance of good isolation. The probe was then advanced to yield new units and the recording procedure was repeated until we estimated the probe to be located in the ventral portion of NCM based on the total depth that the probe had been advanced. We confirmed position of the probe and inferred the location of cellular recording sites in NCM though post-mortem histology (Fig 3B).

2.4.2 Sequences for neurophysiological recording

For each subject, we presented sequences composed of the 8 familiar sequence elements and 4 novel elements. Elements were presented in isolation and in two-element long sequences (ordered pairs) with a silent inter-element interval of 100ms while recording extracellular activity in the secondary auditory nucleus NCM. For most subjects, the first blocks recorded at each site consisted of all elements in isolation, all reward-associated sequences, and all non-reward-associated pairs, interleaved. If isolation of putative single units was maintained, further blocks would include all 144 sequences of novel and familiar elements as well. All trials were separated by at least two seconds of silence.

2.4.3 Spike sorting

Recording traces were digitally high-pass filtered (cutoff at 300Hz) and putative single units were identified from spike sorting in principal component space, with timepoints in the excised spike waveform as the input dimensions to the PCA. Only large amplitude waveforms that formed clear clusters in principal component space were considered to be single units and subjected to analyses. 96% of neurons had no refractory

period violations (interspike interval of less than 1ms). The remaining six neurons had between 1 and 3 refractory period violations, accounting for between 0.0025% and 0.29% of all interspike intervals.

2.4.4 Putative cell types

Excised spike shapes were resampled at 200kHz with a spline interpolation ($k=3$). Spike widths were calculated as the distance from the spike trough to the post-spike peak. A clear cluster of putative fast-spiking cells (FS) stood out (Figure 3C) with high spontaneous firing rates and narrow spike widths, contrasting with the putative regular spiking (RS) cells with low spontaneous firing rates and wide spike widths.

2.4.5 Neurophysiology Data Analysis

Unless otherwise noted, all analyses were performed using custom scripts in Python using the NumPy and SciPy packages. Data were stored in a custom Postgres database and queried using the Django ORM. All statistical analyses were computed using the `scipy.stats` package.

2.4.6 Linear model of combination responses

If cells in NCM do not exhibit any sequence sensitivity, then we would expect responses to sequences to be well predicted as a linear combination of the responses to each sequence element in isolation. Thus, to determine the overall nonlinearity of each cell, we modeled the response of each cell during the second element of ordered pair sequences as a linear summation between the expected offset response to the first element

(the context) and the expected driven response to the second element (the target) for each of the GO and NOGO sequences.

$$R_{\text{COMB}} = \max(\alpha R_{\text{TAR}} + \beta R_{\text{CON}}, 0)$$

R_{COMB} is the number of spikes the cell elicits in response to the presentation of the target and context together, R_{CON} is the offset response expected from the context presented alone and R_{TAR} is the strength of the driven response to the target presented alone. For each cell, α and β were fit to the GO and NOGO sequence trials using a least-squares approach (`scipy.optimize.curve_fit`). We then characterized the deviation from this model in two ways. First, if a neuron's observed response to pairs of stimuli is sufficiently explained by the linear model, then we expect the residuals of the fit to be normally distributed. Using D'Agostino and Pearson's test for normality (`scipy.stats.mstats.normaltest`, $p < 0.05$), we considered neurons whose residuals were non-normal as nonlinear. Secondly, we defined a sequence sensitivity score: the Mean Squared Error of the prediction normalized by the observed response. This yields a sequence sensitivity metric which varies from 0 to 1, where 0 indicates that all spikes in the observed responses are accounted for by the linear model (that is, not sequence sensitive) and 1 indicates that none of the spikes in the combination response are accounted for by the linear model (highly sequence sensitive).

2.4.7 Sequence selectivity

The selectivity of each unit's response among sequences and among their linear predictions were measured by calculating the sparseness index (Vinje & Gallant, 2000).

This measure yields a value of 0 for a unit that shows the same response to all sequences and a value of 1 for a unit which only responds to a single sequence.

2.4.8 Discriminability

In order to characterize the capacity of a neuron to discriminate between sequences, we used a logistic regression (scikit-learn; `sklearn.linear_model.LogisticRegression`) to perform a binary classification based on the spiking activity of the cell. For any given pair of sequences (or pair of elements), we trained the logistic regression and determined the accuracy of the classifier. We calculated chance performance of the classifier by shuffling the relationship between sequence identity and spiking activity 1000 times and recalculating the performance on each shuffle to determine a null distribution. The neuron's discrimination was considered to be significant if performance exceeded the 95-percent confidence interval of chance performance.

2.5 Results

2.5.1 Starlings discriminate between probabilistic auditory sequences

Subjects reached criterion of performing consistently above chance (10 blocks of 200 trials) after many thousands of trials (median 54.5 blocks; range: 14-91). One subject was unable to learn the task and was removed from the study. The remaining 6 subjects continued to perform well as the task difficulty was slowly increased, with all subjects performing well above chance (mean accuracy 74.94% $\pm$ 3.52%) during the final 10,000 trials (mean difficulty: 0.54; range: 0.44-0.72).

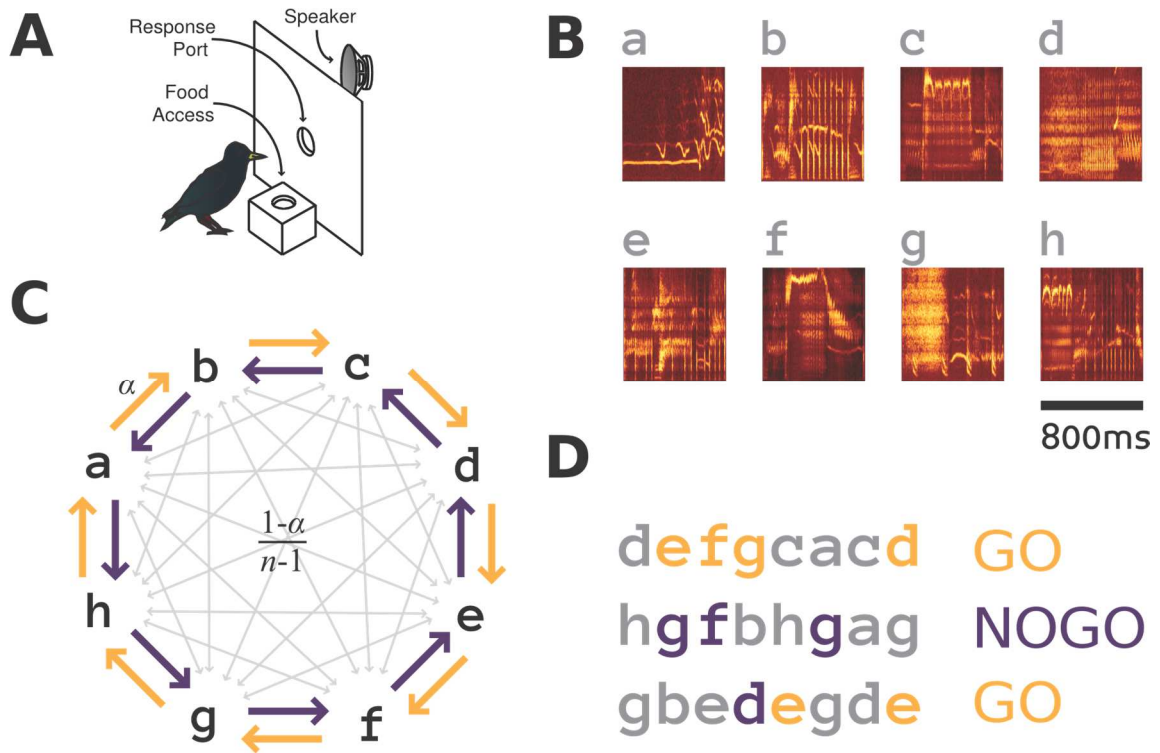


Figure 2.1. Training starlings to discriminate between probabilistically generated sequences of vocal elements.

A: Schematic of operant training panel. *B*: Spectrograms of 8 example elements, labeled 'a' through 'h', which composed sequences presented during behavioral training. Elements were excised from the songs of conspecifics. *C*: Illustration of the Markov models used to generate sequences, with GO (orange) and NOGO (violet), and non-diagnostic (grey) transitions. α is the likelihood that a GO transition will occur on a GO trial or a NOGO transition on a NOGO trial, and thus governs the difficulty of the task. *D*: Example GO and NOGO sequences. Each letter represents a motif shown in (A), colored according to the evidence of the generative model provided by the preceding transition. (Orange=GO, Violet=NOGO, Grey=ambiguous)

2.5.2 Behavioral discrimination depends on evidence in a sequence

Sequence elements alone provide subjects with no diagnostic information regarding the appropriate behavioral response. Instead, the diagnostic value of a given element is contingent upon the prior element in the sequence. Thus, each ordered pair

within a sequence provides a discrete value of evidence for the appropriate behavioral response. If a subject is accumulating evidence and making a response decision based on this evidence, we would expect not only that the presence of certain ordered pairs would predict the subject's response, but that response probabilities would change depending on the number of evidence-affording pairs in a sequence. Analyzing the subject's response likelihood as a function of the number of GO and NOGO ordered pairs present in each sequence indicates that they are weighing both types of evidence in their response decision, increasing their response likelihood with increasing numbers of GO pairs (data not shown: all beta coefficients for the GO term were positive and significant) and decreasing the response likelihood with increasing numbers of NOGO pairs (Fig 2A; data not shown: all beta coefficients for the NOGO term were negative and significant).

Given a subject's task of predicting the appropriate response given noisy evidence, the simplest strategy of an ideal observer with full knowledge of the two models would be to count the number of GO ordered pairs and NOGO ordered pairs and to respond when number of GO pairs exceeds the number of NOGO pairs. The net evidence (difference between the number of GO and NOGO pairs) present in a string is proportional to the log-likelihood of that the string was generated by the GO versus the NOGO model. We can thus simplify our behavioral model to see that response likelihoods change as a function of the net evidence (Figure 2B).

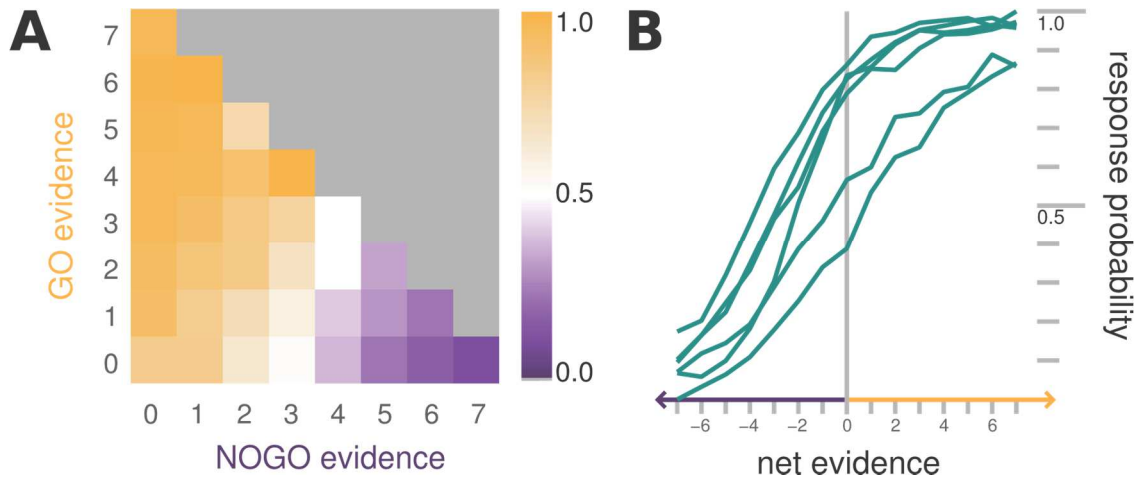


Figure 2.2. Sequence discrimination is predicted by individual transitions.

A: Example behavioral data from one bird after criterion performance. For each quantity of GO and NOGO evidence in a sequence, the color indicates the likelihood of a response. *B:* For six birds, the likelihood of responses are plotted as a function of the net evidence.

2.5.3 *Single neuron responses in NCM are sequence-specific*

We define sequence specificity as the tendency for a neuron to respond to a sequence of elements in a way that is not predicted by the responses to those elements in isolation. To characterize the sequence specificity of each neuron, we compared its responses the second element (the target) of GO and NOGO ordered pairs with the responses predicted by a linear summation of the first and second element (see Methods). Any deviations from the linear model are therefore evidence of sequence-specific tuning (Fig 4A). Overall, responses to sequences were poorly predicted by the linear summation model, with FS neurons exhibiting greater sequence specificity than FS neurons: 103 of 138 (74.6%) RS neurons were poorly fit by the linear summation model, whereas only 2 of 18 (11.1%) FS neurons were poorly fit (Fig 4D; see Methods). Similarly, the linear summation model accounts for a smaller fraction of the activity of regular-spiking cells than fast-spiking cells (Fig 4C; $p < 0.001$, Wilcoxon rank-sum). Sequence specificity also

gives rise to an increased selectivity among sequences. That is, the responses to specific sequences were more likely to be unique than would be expected from the linear model. The selectivity gain due to the sequence specific component of the response was greater for RS than FS neurons (Wilcoxon rank sums: $p = 0.036$), though both classes showed significant increase in selectivity among sequences (Wilcoxon signed rank; RS: $p < 0.0001$, FS: $p < 0.0001$).

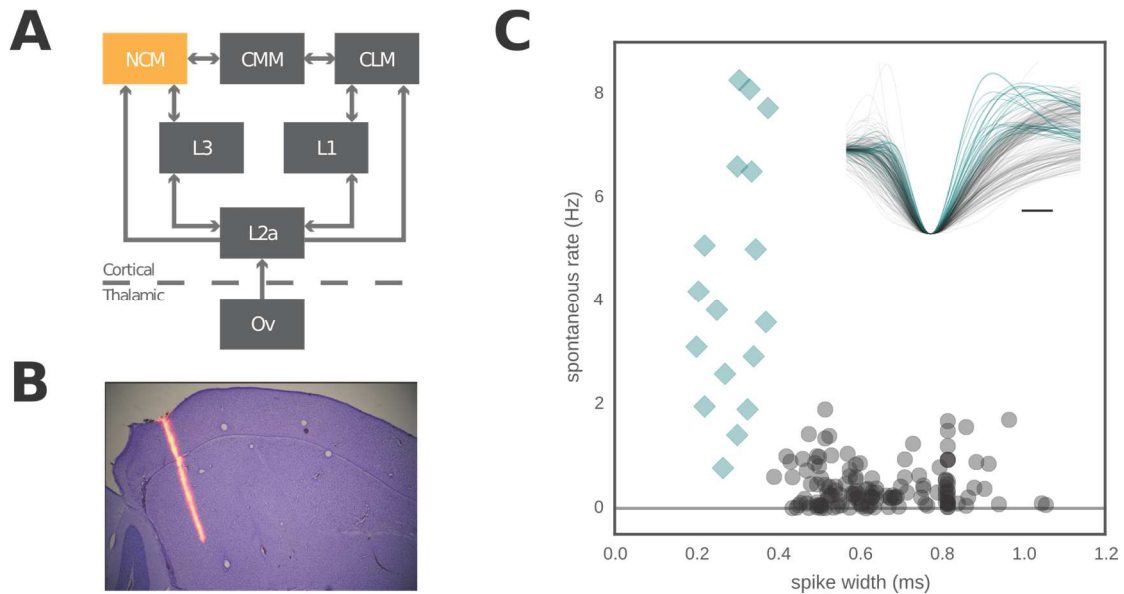


Figure 2.3. Single units recorded from the avian forebrain.

A: Schematic of the avian auditory forebrain. NCM=Caudomedial Nidopallium, CMM=Caudomedial Mesopallium, CLM=Caudolateral Mesopallium, L3=Field L3, L1 = Field L1, L2a = Field L2a, Ov = Nucleus Ovoidalis. B: Example electrode track from one bird. Superimposed images of Di-I and Nissl-stained tissue. C: Clustering of regular-spiking (RS) and fast-spiking (FS) neurons. Spontaneous rate is plotted against spike width. Teal diamonds = FS. Grey circles = RS. C, *Inset*: Average spike shape for all RS (grey) and FS (teal) units, aligned and normalized to trough depths.

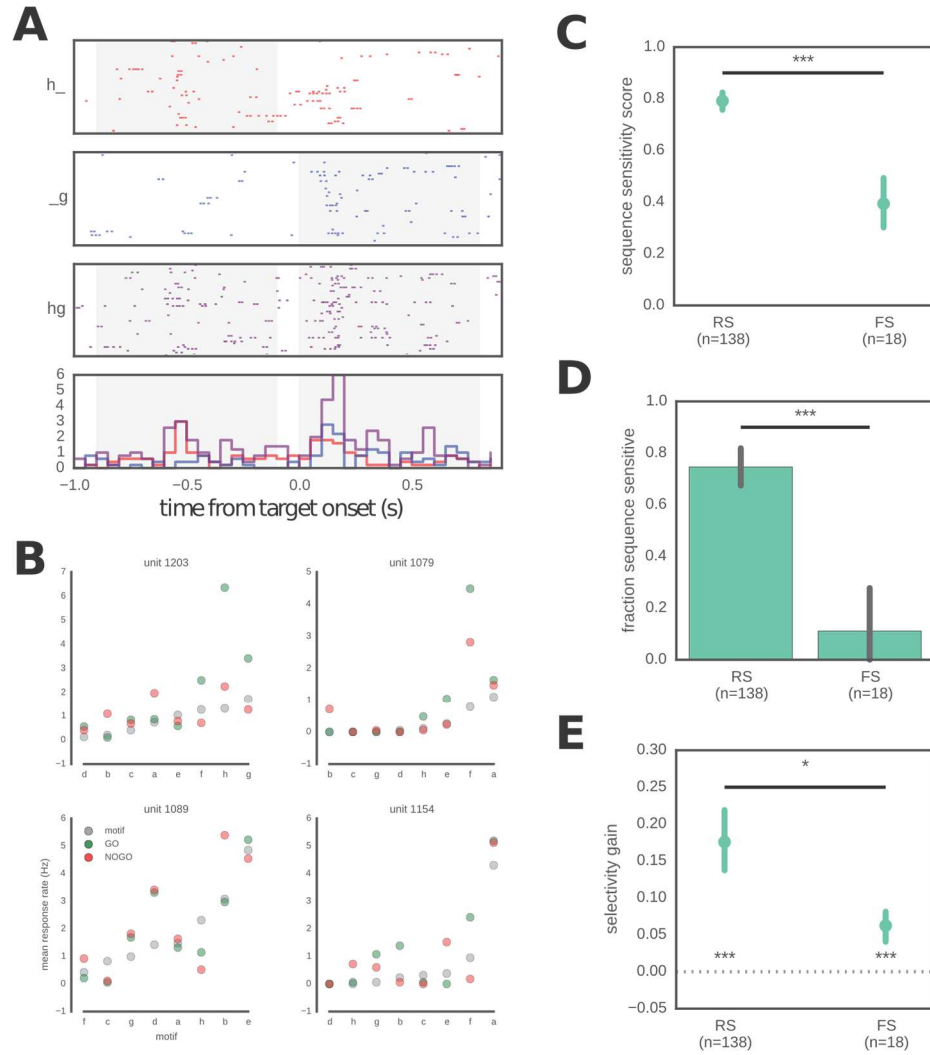


Figure 2.4. Responses to sequences are not well predicted by the responses to isolated elements.

A: Example raster plots and PSTH of one neuron showing its responses to sequence elements in isolation (red, blue) and in sequence (violet). Auditory stimulus epochs are indicated in grey. PSTH is binned in 100ms epochs, aligned to the onset of the second element. B: Example responses to elements alone (grey), in a GO sequence (green), and in a NOGO sequence (red) for 4 example units. Responses are mean firing rates during the first 790ms of the element. C: Sequence sensitivity (see *Methods*) of fast-spiking (FS) and regular-spiking (RS) units. Wilcoxon rank-sum: *** $P < 0.001$. Error bars indicate bootstrapped 95% confidence intervals, unless otherwise noted. D: Fraction of FS and RS units which are sequence sensitive (see *Methods*). Fisher's exact test: *** $P < 0.001$. E: Increase in selectivity among GO and NOGO sequences as compared with expected selectivity from the linear model. Values greater than zero indicate increased selectivity than expected from the linear model. Wilcoxon rank-sum: * $P < 0.05$, Wilcoxon signed-rank: *** $P < 0.001$.

2.5.4 Sequence responses support the discrimination of diagnostic sequences

In order to perform the task, subjects must be able to discriminate between reward-associated and non-reward associated sequences. We hypothesize that sequence specific responses we observed enable sequence discrimination. One prediction of this hypothesis is that potentially diagnostic elements (such as A and C) will be better discriminated when embedded within behaviorally-relevant sequences (BA vs BC) than when presented in isolation. For each subject, there are 8 such contrasts of interest where preceding a target element (A or C) with a common context element (B) creates a contrast between a reward-associated (GO) sequence (BC) and non-reward-associated (NOGO) sequence (BA). (Fig 5A)

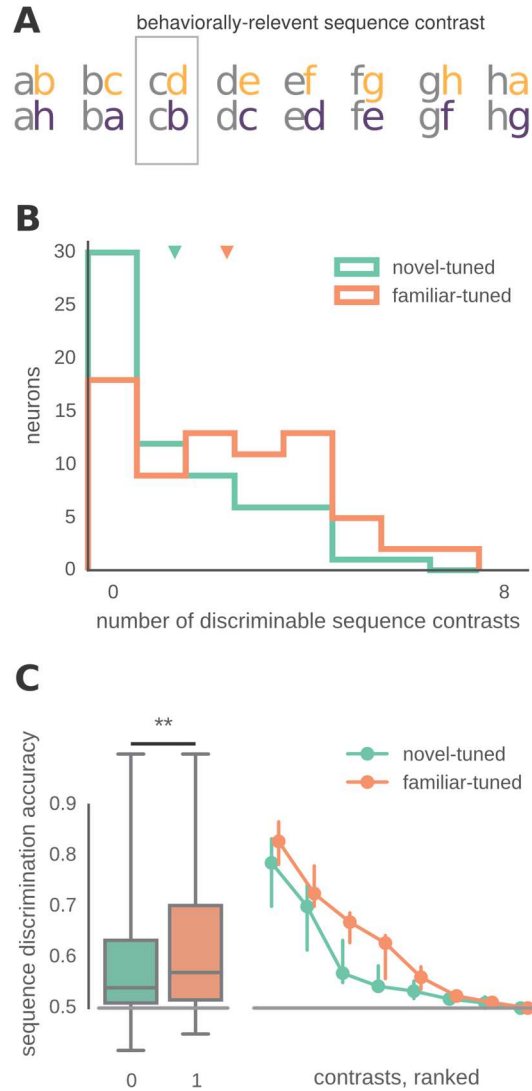


Figure 2.5. Familiar-tuned neurons discriminate between GO and NOGO sequences better than novel-tuned neurons.

A: For each bird, there are 8 pairs of sequences that form behaviorally-relevant contrasts, where GO or NOGO evidence emerges with the second. An example pair is outlined in grey. B: Familiar-tuned neurons encode more sequence contrasts than novel-tuned neurons. Histogram of the number of contrasts which can be discriminated firing rates for novel-tuned (green) and familiar-tuned (orange) neurons. Familiar-tuned neurons can discriminate an average of 2.34 contrasts, while novel-tuned neurons discriminate an average of 1.28 contrasts (Wilcoxon rank-sum, $P < 0.001$). C: Familiar-tuned neurons are more accurate at discriminating sequence contrasts than novel-tuned neurons. Left: box plot of average sequence discrimination accuracy of familiar-tuned and novel-tuned neurons. Familiar-tuned neurons tend to be more accurate than novel-tuned neurons (Wilcoxon rank-sum: * $P < 0.05$). Right: Each neuron's sequence discrimination accuracy is ranked and median accuracy of familiar-tuned and novel-tuned neurons is shown at each rank.

2.5.5 Sequences are better encoded in neurons which prefer familiar elements

Neurons in NCM exhibit diverse tuning, with some neurons responding more strongly to novel elements and some responding more strongly to familiar elements. Sequence sensitivity requires that a neuron receives inputs containing information about all elements of a sequence. In order to explore how sequence coding supports sequence perception and putative learning mechanisms to learn to discriminate sequences, we partitioned neurons into a familiar-tuned population, which showed preferences for the elements used to create sequences and a novel-tuned population, which showed preferences for novel elements. We expect that neurons which are preferentially tuned to familiar elements are more likely to be recruited by mechanisms of sequence learning.

For each neuron, we determined its capacity to discriminate between reward and non-reward associated sequences in each of 8 behaviorally-relevant sequence contrasts (BA vs. BC, CB vs. CD, DC vs. DE, etc, Fig 5A) by training a logistic regression to classify responses to each of the two sequences. High selectivity among sequences (Fig 4B, 4E) implies that individual neurons should have relatively little capacity to discriminate arbitrary pairs of sequences, as most stimuli elicit little or no response, but for some contrasts (e.g. where one sequence drives a strong response and the other does not), discrimination will be very good. We observe that familiar-tuned neurons discriminate among more behaviorally-relevant sequences than novel-tuned neurons (Fig 5B; FT mean: 1.27, NT mean: 2.34, Wilcoxon rank-sum: $P < 0.001$). We then restrict our analysis to those neurons which could discriminate between at least one pair of

behaviorally relevant sequences (55 of 73 familiar-tuned neurons and 35 of 65 novel tuned) and observe that, consistent with familiar-tuned neurons discriminating between more sequences, they also discriminate between behaviorally relevant sequences with greater accuracy on average than novel-tuned neurons (Fig 5C, Wilcoxon rank-sum, $p=0.033$).

2.5.6 NCM can discriminate between diagnostic sequences better than sequence elements

Familiar-tuned neurons are better at discriminating among behaviorally relevant sequences than novel-tuned neurons, however, to what extent is this specific to sequence discrimination and not simply a result of better discrimination between the target elements? For each behaviorally relevant sequence contrast (e.g. BA vs BC) there is a corollary element contrast (e.g. A vs C) which we can use to directly compare the accuracy of neurons discriminating behaviorally relevant sequences with the eight corollary element contrasts. For each neuron, we identify its “preferred sequence contrast” (that is, the sequence contrast which can be most accurately classified) and compare it to its “preferred element contrast” (the element contrast which can be most accurately classified). We exclude from our analysis 44 neurons which showed no significant discriminative capacity for either sequences or their corollary elements.

We observe that among the 57 familiar-tuned neurons, the preferred sequence contrast tends to show higher discriminability than the preferred element contrast (mean preferred sequence discrimination accuracy=0.820, mean preferred element discrimination accuracy=0.785; Fig 6C; Wilcoxon signed-rank test, $p=0.006$), whereas for the novel-

tuned neurons, sequence discrimination does not outperform element discrimination (n=37; mean preferred sequence discrimination score=0.776, mean preferred element discrimination score=0.760; Fig 6D; Wilcoxon signed-rank test, $p=0.217$).

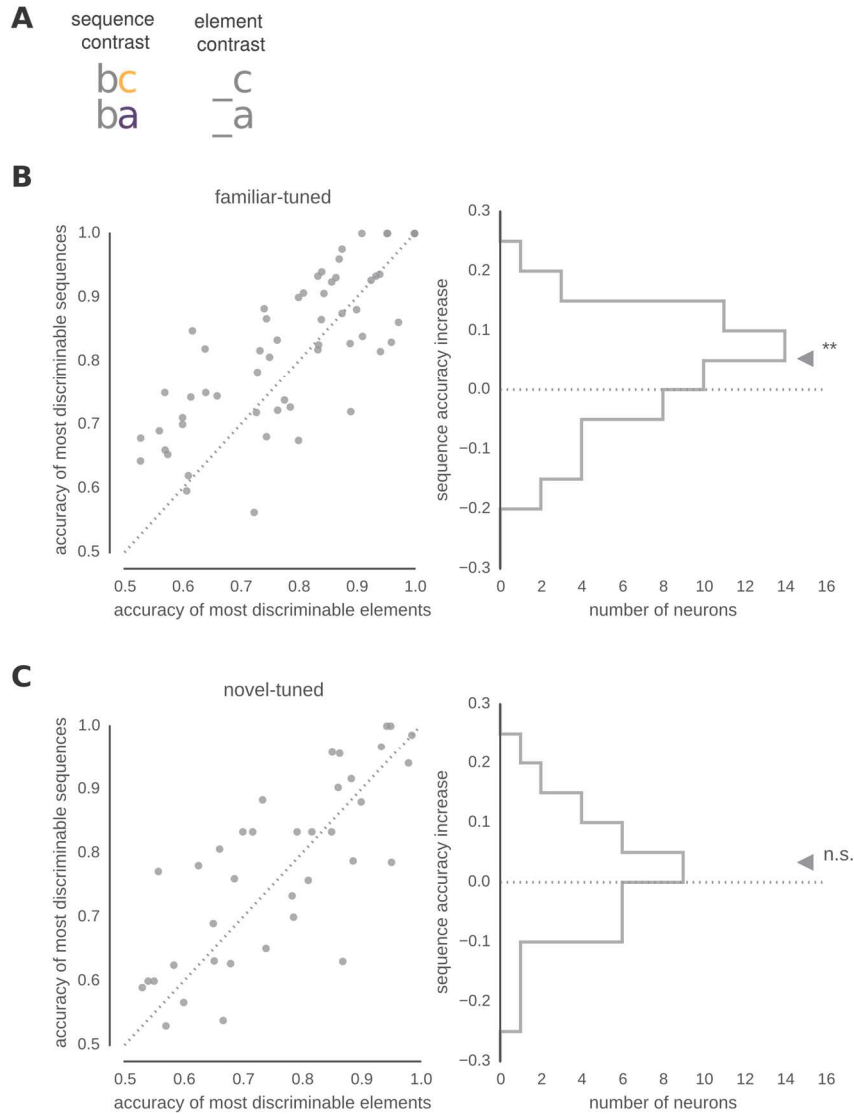


Figure 2.6. Familiar-tuned neurons exhibit better discrimination between sequences than between elements.

A: Schematic of sequences and motifs used for discrimination analysis. We compare sequences where the first element is shared and the second element creates a “contrast” between GO and NOGO sequences. In this example, *bc* and *ba* are GO and NOGO sequences differing only by the second element. For each sequence contrast, we also create a element contrast between the second elements presented in isolation. In this example, *c* and *a* are contrasted. B: Sequence discrimination of familiar-tuned population for each neuron’s best discrimination. (Left) The accuracy of each unit’s best-performing sequence contrast is plotted against the accuracy of its best-performing element contrast. (Right) Histogram of the differences between the accuracy of the preferred sequence contrast and the preferred motif contrast. Mean difference denoted by grey triangle. Wilcoxon signed-rank: ** $P < 0.01$ C: As in (B), for the novel-tuned population. (Wilcoxon signed-rank: n.s. $p > 0.05$)

2.5.7 Sequences are facilitated by reward-association if it will improve sequence discrimination

It follows then that the preferred sequence discrimination also tends to outperform the discrimination of its corollary element contrast. Importantly, the sequence discrimination is between sequences which the subject has learned to discriminate and thus reflects the behavioral demands of the sequence discrimination training. What then, are the changes in response properties of these neurons which underlie the improvement in sequence discrimination? As the logistic regression performs a linear discrimination among firing rates, any performance above chance necessitates that one of the two sequences drives a higher mean firing rate than the other. Therefore, any improvement in a single neuron's discriminability between two sequences over the corollary elements must be due to either facilitation of the higher firing rate element, suppression of the lower firing rate element, a decrease in the trial-to-trial variance in the firing rate of either target, or some combination of these factors. Further, these mechanisms may be differentially recruited for GO sequences, which have been extensively associated with reward, and NOGO sequences. Therefore, we split our population into neurons for which the element which fulfills the GO sequence drives a stronger firing rate (Fig 5, blue) or the element which fulfills the NOGO sequence (Fig 5, green). We observe facilitation of GO sequences only when the GO sequence drives stronger responses than the NOGO sequence (Wilcoxon signed rank: $p < 0.001$); when the NOGO target is preferred, we do not see facilitation of the reward-associated GO sequence (Wilcoxon signed rank: $p = 0.65$). For non-reward-associated NOGO sequences, we do not observe facilitation or

suppression, regardless of whether the NOGO sequence is the preferred (Wilcoxon signed rank: $p=0.06$) or not (Wilcoxon signed rank: $p=0.95$). Reward-associated facilitation is not observed for novel-tuned neurons (data not shown).

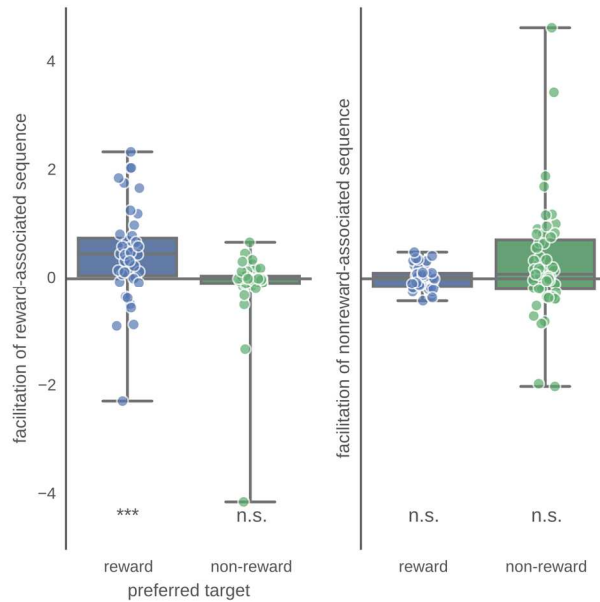


Figure 2.7. Rewarded sequences of familiar-tuned neurons' best contrast are facilitated when the rewarded target is a higher firing rate than the non-rewarded target.

Facilitation of a target is plotted for sequences where the target completes a reward (blue) or non-reward (green) sequence, and whether the rewarded sequence is preferred (Left) or the non-reward sequence is preferred. Deviations above 0 indicate facilitation, while deviations below 0 indicate suppression. Wilcoxon signed-rank: n.s. $p > 0.05$, *** $p < 0.001$

2.5.8 Prediction of reward-associated sequences

Sequence elements establish a context wherein subsequent elements provide evidence. For example, whereas element A and C alone afford no information regarding the appropriate response, if they are preceded by element B then they provide evidence.

Our results indicate that this context improves the neuronal discrimination of sequences. This temporal integration requires that information about the identity of familiar elements must be maintained until the subsequent elements are presented. On the other hand, novel elements do not establish any predictions of upcoming elements or context for decision making. We predicted that familiar elements will exhibit sustained responses following the elements.

As expected, we observed that familiar-tuned neurons show stronger offsets following familiar elements than following novel elements (add stats). On the other hand, novel-tuned neurons exhibit relatively weak offsets to both familiar and novel elements (data not shown). One possibility is that the offset of familiar-tuned neurons to familiar elements predict subsequent elements in the learned sequences. To what extent do the offset responses predict either the GO or NOGO sequence? For each neuron, we calculated the correlation between the eight offsets and both the GO and NOGO sequences that they predict (Fig 8B). We find that offsets tend to be more correlated with GO sequences than with NOGO sequences (Fig 8C; Wilcoxon signed rank: $p=0.028$).

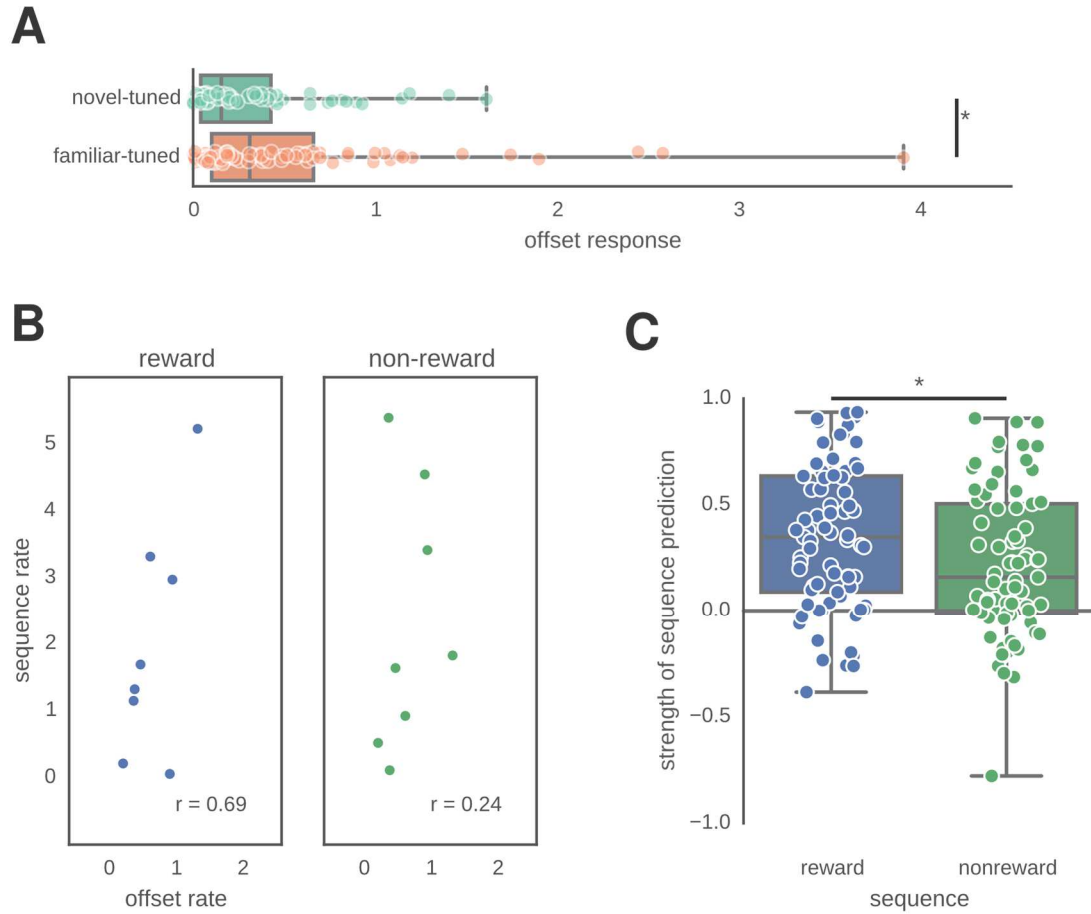


Figure 2.8. Offsets responses predict reward-associated sequences.

A: Familiar-tuned neurons exhibit larger offset responses to sequence elements. Box plots of offset responses for novel-tuned (green) and familiar-tuned (orange) neurons. Points indicate individual neurons' mean offset response. B: Mean offset responses for each of the 8 sequence elements is plotted against the mean responses to the reward (left, blue) and non-reward (right, green) sequences. "r" indicates the Pearson correlation between the offset rates and sequence rates. C: Boxplots showing the distribution of correlations between offset rates and reward sequence rates (left, blue) and non-reward sequence rates (right, green). Overlaid points indicate each neuron's offset-sequence correlation. Wilcoxon signed-rank: * $P < 0.05$

2.6 Discussion

A wide range of sensory neurons are sensitive to sequentially presented stimuli but their utility in decision making and how they emerge from goal-directed learning processes is unclear. Here, we show that auditory sequence sensitivity is shaped to

support behavioral demands. In the secondary auditory area NCM, we observe sequence-specific facilitation which is reward-mediated and supports behaviorally-relevant sequence discrimination.

As has been demonstrated before, we show that starlings can exclusively utilize the relative positioning of acoustic elements within a sequence to guide behavioral responses (Comins and Gentner, 2010). In the current study, however, we've built upon the prior work in three important ways. First, we show that starlings can discriminate between two classes of sequences which are defined according to their first-order transition statistics and matched with respect to the entropy of generated sequences. Second, they can perform this discrimination even with ambiguity regarding the class of the sequence. Sequences are generated by probabilistic Markov models, therefore every presented sequence has some chance of being generated under each of the two models. Third, given a sequence of complex elements, there is a chance that diagnostic local features will spontaneously emerge at the boundary between two successive elements. To prevent this, we inserted a silent gap with a variable length between elements. The performance of starlings on this task in light of these complicating factors indicate that starlings are very good at perceiving statistics of sequences of complex vocal elements and excel at using these statistics to make decisions about sequences they hear. One way of interpreting this task is that the subject must infer which of two first-order Markov models generated a sequence. There are, however, alternative strategies which could be employed to solve this task sufficiently to perform above chance. For example, even though both GO and NOGO ordered pairs afford evidence, subjects could ignore all

ordered pairs in one class or the other. While it is impossible to exclude all alternative strategies, our analyses indicate that the number of both GO and NOGO ordered pairs contributed to subjects' decisions and that other heuristic approaches don't appear to be utilized. In all, their performance is consistent with an inference model wherein they differentially weigh the evidence of the appropriate behavioral response based upon the ordered pairs which occur in the presented sequence and the extent to which a sequence is consistent with the learned statistics of the two classes.

Our results confirm that NCM shows robust sequence sensitivity and extends prior work to elucidate how such sensitivity emerges from behavioral demands. Sequence responses are not well predicted by the responses to sequence elements. We also show that sequence sensitivity depends upon cell type, as RS putative projection neurons are more sequence-sensitive than their FS putative inhibitory counterparts. For both FS and RS neurons, the nonlinear component of the sequence response affords an improvement in coding fidelity for sequences, but this improvement is greater for RS units. These results indicate that RS and FS neurons may play very different roles in representing sequences.

Throughout much of our analysis, neurons which were tuned for familiar elements exhibited learning-dependent changes which were not observed among novel-tuned neurons. Familiar-tuned neurons sustained their familiarity-preference for hundreds of milliseconds after the stimulus was gone, while novelty-tuned units did not. Familiar-tuned neurons exhibited greater selectivity among reward-associated sequences. And finally, familiar-tuned neurons were better able to discriminate among familiar sequences.

Taken together, these unique features of familiar-tuned units presents the intriguing possibility that these neurons have been recruited to represent behaviorally-relevant sequences due to their naïve tuning to sequence elements. Indeed, we would not expect a cell which receives little or no synaptic input in response to familiar elements to be of much use in coding sequences such elements. On the other hand, those neurons which are tuned to familiar elements are well positioned to sequence-sensitive responses through facilitative or suppressive mechanisms. The effects of associative learning on neuronal tuning depends on the naïve tuning relative to the learned stimulus {Weinberger, 2004}. Neurons for which their receptive field does not encompass the learned target exhibit little or no changes in their tuning due to associative learning, whereas units whose naïve best-frequency is near to a target may shift their tuning {Weinberger, 1997}. However, to our knowledge it has not been shown how learning-dependent tuning to higher order stimulus features (such as a sequence) might be shaped by lower-order tuning properties (such as the sequence elements). Nonetheless, we suggest that a similar principle might be at play wherein neurons are targets of learning mechanisms dependent upon their existing tuning and capacity to support behavioral goals.

The facilitation of sequences observed here contrasts with prior work in NCM which has shown suppression of responses to highly probable sequences (Schneider and Woolley, 2013; Lu and Vicario, 2014). Such suppression can be explained as an effect of exposure through sequence-specific adaptation (Lu and Vicario, 2014). Our observation of reward-associated facilitation cannot be explained by sequence-specific adaptation. This difference may reflect different effects of cumulative exposure and goal-directed

perception. First, the prior study observed relatively short term effects of exposure statistics, while our subjects experienced many thousands of presentations over weeks and months. It is not clear how the mechanisms that drive suppression over the course of a few hours are related to the mechanisms that shape sequence representations over many days and weeks and months of exposure. Second, our task places an important behavioral demand upon our subjects, as they must decide how to respond appropriately to sequences they hear. This goal-directed learning appears to shape sequence representations in order to facilitate discrimination—a important ethological constraint on sequence representation that is absent in prior work.

Our results do bear some similarity to other associative sequence learning studies which observed enhancement of responses for reward-associated sequences (Yin et al., 2008; Zhou et al., 2010). However, our results are different from these prior studies in two important ways. First, our behavioral training uses sequences composed of vocal communication signals rather than tones. By using spectrotemporally complex vocal communication stimuli, we were able to elicit responses in higher-order areas than primary auditory cortex. More importantly, however, using vocal communication elements affords insight into the mechanisms of these ecologically-guided decision-making. Secondly, unlike prior studies where only one or two discrete sequences are reward-associated, our study trained animals on probabilistic sequences governed by first-order statistics. The probabilistic nature of our task is important in isolating sequence-specific effects from position and element identity. For example, a behavior where a subject is only asked to discriminate between the sequence ABCD and the

sequence DCBA allows for solution strategies where the sequencing of elements can be ignored in favor of identifying any number of position-element combinations (e.g. ---D vs ---A). Our task design requires decisions based on the sequencing of elements and therefore provides specific insight into the mechanisms of sequence encoding.

There have been a number of proposals for the source of sequence sensitivity, including recurrent excitation (Drew and Abbott, 2002), delayed inhibition (Schneider and Woolley, 2013), and short term synaptic plasticity (Goudar and Buonomano, 2015). A key requirement of sequence sensitivity is a mechanism for working memory that ensures the responsiveness of neurons to select elements in the sequence contingent upon the identity of the prior elements. The sustained offset responses we observe following familiar elements might reflect this mechanism. This could arise from stimulus-specific differences in recurrent excitatory or inhibitory activity enabling stimulus information to persist in the network dynamics. Our results also point to sequence elements carrying a predictive role. Based on the subject's experience, the presence of a given element gives the subject information about the likely subsequent element. This offers the intriguing possibility is that the sustained offset responses we observe are not simply maintaining a memory of past events, but are establishing a prediction of future events. Indeed, we observe that these offsets correlate more strongly with reward-associated sequences than non-reward sequences. While it is possible that this correlation with reward-associated sequences reflects an "optimistic prediction," it seems more likely that they reflect changes in the responsivity of the neurons, enabling the sequence-specific reward facilitation what we observe.

Importantly, our results indicate that the mechanisms of sequence sensitivity are themselves targets of goal-directed learning and that sequence sensitivity can be shaped to support new behavioral demands. Our results also constrain the conditions under which sequence-specific responses may be modulated through learning. First, the learning-associated effects we observed were largely constrained to neurons which were preferentially tuned to sequence elements. This is analogous to other cognitive tasks, where subjects must make decisions not only on the first order features of the environment, but on a higher-order relationship between features. The modulation of responses by higher order relationships depends upon a neuron receiving lower-order features as inputs. For example, spatial attention drives modulations of a neuron's responsiveness contingent on the spatial receptive field of the neuron. Neurons which are tuned for a region of space which is far from either a target of attention or a distractor exhibit very little attentional modulation (Reynolds and Heeger, 2009). Similarly, we observe that neurons which are preferentially tuned for elements which do not comprise the sequences we presented show very little effect of sequence learning. These neurons are preferentially tuned to features which are not present in the set of familiar elements and therefore have relatively little capacity to support the task demands. Second, we find that reward-associated facilitation is contingent upon the improved sequence discrimination. This contingency is an important constraint for sequence learning. Just as non-specific reward facilitation would improve the ability to discriminate between some sequences, it risks impairing the discrimination of other sequences. Our data indicate that this risk is mitigated by selective facilitation of rewarded sequences only when such facilitation will improve a neuron's capacity to discriminate among. These results echo

prior work showing that both reward-association and the utility of a stimulus for meeting behavioral goals are important in stimulus tuning (Jeanne et al., 2013). In future work, it will be important to separate the roles of reward association and action selection in understanding sequence discrimination. Further, awake behaving experiments will be important to assess whether improved neuronal discrimination of sequences results in a concomitant improvement in behavioral discrimination.

Chapter 2, in full, is currently being prepared for submission for publication of the material. Kiggins, Justin T.; Gentner, Timothy Q. The dissertation author was the primary investigator and author of this manuscript.

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CHAPTER 3

State-dependent element encoding in neuronal populations during probabilistic sequence discrimination

3.1 Abstract

Sequence perception is a critical component of speech and language, but the neuronal mechanisms of sequence perception are unknown. Element identities must be accurately decoded in order to perceive sequences. At the same time, sequence statistics establish expectations that can facilitate element identification. To better understand how vocal sequences are encoded, we trained European starlings on a probabilistic sequence discrimination task and recorded populations of neurons while starlings categorized sequences. We find that neurons in the caudolateral mesopallium (CLM) exhibit mixed selectivity to the position and identity of elements and that element identities are more readily decoded from population activity when neuronal activity is coordinated—an encoding benefit that could be attributable to either mixed selectivity or beneficial correlations among neurons. We also found that accurate encoding of elements in population activity was state-dependent—accurate decoding of element identity from populations of either fast-spiking (FS) putative interneurons or regular spiking (RS) neurons predicted accurate discrimination in the complementary population, but not in the same population. This improvement in the fidelity of element representations is due to improved reliability of the target population representation.

3.2 Introduction

The challenge of extracting meaning from a sequential signal is critical to language comprehension and faced by many animals which rely on vocal communication, but the neuronal basis of this process is unclear. Many animals make decisions based on

representational alarm calls from conspecifics (Kiriakis and Slobodchikoff, 2006; Seyfarth et al., 1980). The Japanese great tit (*Parus minor*) further exhibits compositional syntax—the sequencing of elements changes the way that birds respond to the certain alarm calls (Suzuki et al., 2016). European starlings make decisions in the wild based upon the vocal signals of other starlings, and they rely on sequential information to do so (Gentner and Hulse, 2000a, 2000b). In all of these cases sequence perception involves multiple cognitive processes which must transform and reshape the raw acoustic signal into relevant sequential elements whose order can be analyzed (Kiggins et al., 2012). This transformation is not simply feed-forward process, however, where sequence elements are discretized then the sequence is analyzed. Instead, the multiple levels of processing interact and influence one another. For example, the sequence itself establishes expectations about upcoming elements that can affect perception of those elements (Warren, 1970). However, the utility of leveraging predictive statistics to decode new sequence elements is dependent upon the accurate encoding of sequence history. Misidentified elements could yield a different prediction, impairing the ability to resolve new elements.

We sought to understand how elements are encoded by neuronal populations during a sequence categorization task and how accurately encoding past elements affects encoding current elements. To do this, we trained European starlings on a probabilistic sequence discrimination task, where the only information available to perform the task was the order of elements in sequences, then recorded populations of neurons from the secondary auditory area CLM while birds made decisions. Starlings are an ideal

candidate for exploring the neural mechanisms of vocal perception. They can be readily trained to make decisions about sequences in the laboratory (Comins and Gentner, 2010, 2014; Gentner et al., 2006). Second, recent work has shown how the avian auditory system preferentially encode vocal signals and how these representations are shaped by associative learning (Gentner and Margoliash, 2003; Jeanne et al., 2011; Thompson et al., 2013). Finally, starlings are amenable to awake-behaving neural recording paradigms (Knudsen and Gentner, 2013) which is critical for understanding how neuronal representations of sequences contribute to decision making. For this study, we recorded from the caudolateral mesopallium (CLM) while starlings categorized sequences. CLM is a secondary auditory area which preferentially encodes conspecific song (Grace et al., 2003) and where the coordinated activity of populations of neurons in CLM improve the coding fidelity of meaningful vocal signals (Jeanne et al., 2013).

Sequences were generated according to first-order Markov statistics, so that the task could be solved by learning the transition statistics between elements and making a decision based upon the evidence afforded by the sequence the subject heard. We find that the subject performance is consistent with making decisions based upon the available evidence in a sequence. We then used silicon microprobes on a microdrive to record populations of fast-spiking and regular-spiking neurons in CLM while subjects discriminated between sequences. We found that neurons were sensitive to both elements and sequences and populations. We explored the encoding of sequence elements by training linear classifiers to decode element identity from the spiking responses of simultaneously recorded populations of neurons while birds were listening to sequences

before making decisions. We found that the coordinated activity of neuronal populations was important for encoding element identity. Interestingly, we found that FS and RS populations interacted during sequence presentation. Accurate decoding of elements by either population at each position predicts accurate decoding of elements across populations at the same and future positions.

3.3 Methods

3.3.1 Behavior

Starlings were trained on a standard two alternative choice (2AC) operant conditioning task using a variable ratio (VR) reinforcement schedule (Ferster & Skinner, 1957). During behavioral training, subjects were housed in a sound attenuating chamber (Acoustic Systems) with an operant panel as described previously {Jeanne, Sharpee, & Gentner, 2013; Jeanne, Thompson, Sharpee, & Gentner, 2011; Thompson & Gentner, 2010}. Subjects were provided free access to water and received food through engaging in the operant task. The light schedule followed a 14:8 hour light/dark cycle and subjects were free to initiate trials at any time during light cycles.

For each subject, strings were assigned either to the L or R sequence class. Playback of the sequence began immediately when a trial-initiation peck was detected. After the end of the playback, the program queried for pecks in the left and right ports during a 2-second response window. If the subject pecked within the response window, the trial outcome was recorded as a response, otherwise, the trial outcome was recorded as a non-response. Correct responses (a left peck in response to a L string or a right peck in response to a R string) were rewarded with a brief flash of a green LED on every trial

followed by 2 seconds of food access according to the VR schedule. Incorrect responses were punished by turning off the lights and preventing food access or trial initiation for 30 seconds. Incorrect trials were followed by a “correction” trial, where the same sequence was presented to the subject, but no food reward was available. Non-responses were neither rewarded nor punished.

3.3.2 Sequence generation

Sequences were generated from 5 vocal elements excised from conspecific starling song. Elements were 400ms in duration and spectrotemporally diverse. L and R sequences contained the same elements but differed according to the statistics of the transitions between elements. L sequences were generated according to a first-order Markov model where each element was more likely to transition to one of two other elements (that is, ‘a’ is most likely to be followed by ‘b’ or ‘d’) and less likely to transition to the other two (‘a’ is less likely to be followed by ‘c’ or ‘e’). Elements did not repeat. R sequences were generated by a complementary Markov model which inverted the transition statistics of the L model: that is, ‘a’ is less likely to be followed by ‘b’ or ‘d’ and more likely to be followed by ‘c’ or ‘e’, etc. (Figure 1A). The odds of each model generating a “likely” transition versus an “unlikely” transition is equal to the odds that a single transition was observed under one model rather than the other. We define this parameter as the Odds Ratio (OR). The OR defines the amount of overlap between the distributions of transition statistics under each of the two Markov models, and therefore is a difficulty parameters that governs the uncertainty in the task. Sequences were created

by concatenating the elements together with a brief silent interval between elements (mean duration=100ms, st. dev. = 15ms).

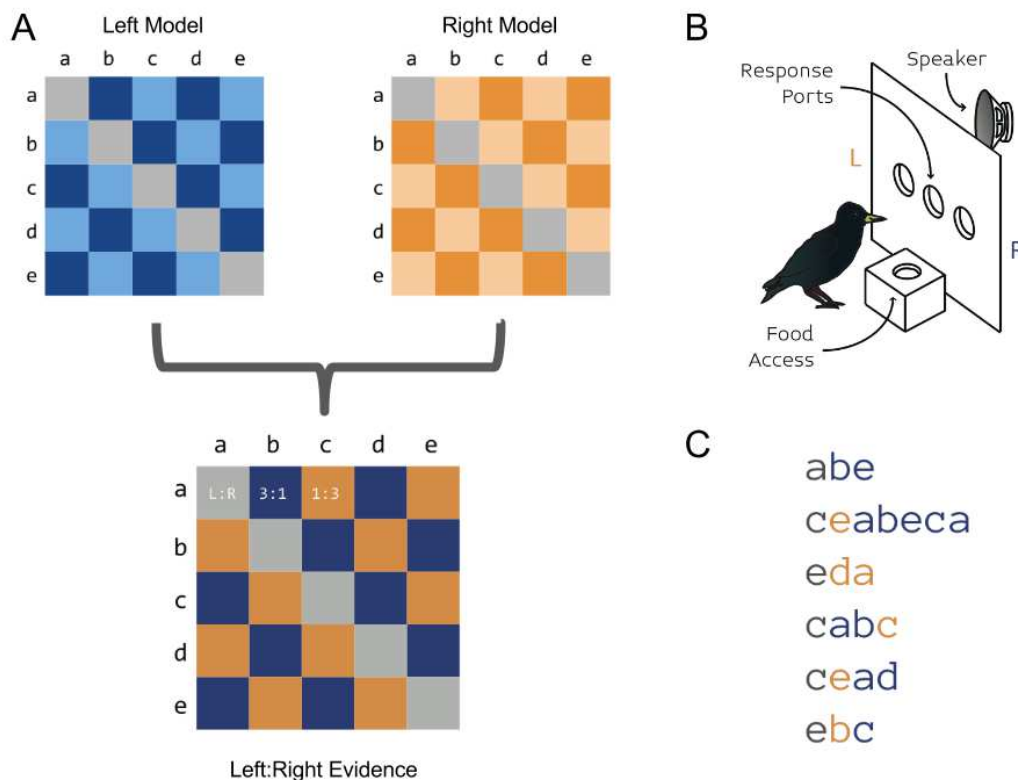


Figure 3.1. Starlings can discriminate between probabilistically generated sequences.

A: Sequences are generated from one of two Markov models. Each model (Left and Right, in shades of blue and orange, respectively) comprises a set of highly probable transitions (dark) and less probable transitions (light). Repetitions (in grey) do not occur. When sequences are generated from these two models, this results in a set of transitions that are more likely to be generated by one model than the other and therefore provides a unit of evidence that the sequence was generated by the model. The odds that a given transition will be generated by a given is equal to the ratio between the likelihood of a high-likelihood transition and a low-likelihood transition. B: Schematic diagram of the operant conditioning panel. Subjects initiate a trial by pecking the center port, then a sequence of acoustical elements generated by either the Left or Right Markov Model is played through the speaker. The subject then decides whether to peck in the Left or Right port. Correctly inferring the model that generated the sequence results in food presentation. An incorrect response results in a short time-out. C: Example sequences generated by the task. Each element is color coded according to the evidence it affords in combination with the prior element.

3.3.3 Task acquisition & shaping procedures

For task acquisition, subjects began with the OR greater or equal to 20 and all sequences of length $n=10$. Once a subject's performance increased above chance, we incrementally increased the VR to 2 or 3 while ensuring that subjects received sufficient access to food. Once performance was very strong and stable, we began shaping performance to prepare subjects for neurophysiological recording by incrementally decreasing the OR to 3, incrementally reducing the minimum sequence length to $n_{\min} 3$, and imposing a hazard function to determine sequence length: for each element after the second in a sequence, there was a constant likelihood that the element would terminate the sequence. Subjects were trained until they reached criterion performance: OR=3, $n_{\min}=3$, hazard rate = 0.5.

3.3.4 Behavioral performance

In order to summarize the final performance for each subject, we analyzed 10,000 trials once subjects achieved criterion performance on the task. To evaluate how each subject's decisions depend on the magnitude of left and right evidence presented in sequences, we performed a logistic regression, predicting subject's responses (L or R) based upon the number of L-evidence and R-evidence transitions in the sequences. To evaluate how each subject's decisions depend on the net magnitude of evidence presented in sequences, we performed a logistic regression, predicting subject's responses (L or R) based upon the difference between the magnitudes of L-evidence and R-evidence transitions in the sequences.

3.3.5 *Neural Probe Placement*

We recorded extracellular activity in CLM from two subjects. Subjects were transferred from behavioral training boxes to a neural recording box, where they resumed performing sequence discrimination trials. Once we confirmed that their behavior was stable at criterion performance in the new environment, we implanted a 32 channel silicon microprobe into the starling forebrain, as described previously (Knudsen and Gentner, 2013). Briefly, a silicon microprobe (Neuronexus) was mounted to a screw microdrive and placed over CLM, then lowered until auditory-responsive units were observed. Signals were bandpass filtered, amplified, and digitally sampled. Experimental control was governed by custom scripts written in Spike2 (ChronicScript.s2s, <https://github.com/gentnerlab/probe-the-broab/>).

3.3.6 *Neural recording block design*

Neural recording blocks were designed to compactly present a subset of possible sequences whose statistics were closely matched to key statistics of the training protocol. For example, at the criterion odds ratio of 3, the sequence `abc` should be presented 10 times. For 9 of those trials, left responses should be rewarded and right responses should be punished, and for one trial, a left response should be punished and a right response should be rewarded. There are 5 blocks of 3 elements for which we define sequences which maintain the OR of the full probabilistic task: `abc`, `bcd`, `cde`, `dea`, and `eab`. We can then create 318-trial blocks of OR-conforming sequences which allow us to ask specific questions about how elements are represented based on their local role in sequences.

3.3.7 *Neural Recording Sessions*

On a typical recording day, the headstage was connected to the amplifier and ADC through a commutator. We turned off the lights in the recording chamber and ran an initial passive search block wherein we played back 2-element sequences while the subject was passively listening to identify putative auditory-responsive spiking neurons. Once we identified putative units that responded to at least one 2-element sequence, we turned on the lights and queued a block (described above) that included the 2-element sequence we identified. Following culmination of the block, we either presented another sequence discrimination block, or ran another passive block to identify a new 2-element sequence or moved the probe to a new depth to search for new auditory units.

3.3.8 *Identifying neurons*

We used the Klustakwik Masked EM algorithm (Rossant et al., 2016) in order to identify spiking neurons. First, we used a custom Spike2 script to export recorded data to mat files (MatlabExport.s2s, <https://github.com/gentnerlab/probe-the-broab/>). We then used a custom Python script to reduce common noise across channels and migrate the data to the Kwik format used by Klusta. Movement artifacts tended to be common across electrodes, although with different amplitudes, presumably due to variance in the impedances of individual electrode sites and other unknown factors. We found that using a Common Average Reference (Ludwig et al., 2009) across all channels tended to exacerbate movement artifacts when there were some channels with larger artifacts than others, so we implemented a Weighted Average Reference as follows. After removing channels which were clearly unusable (line noise or extremely large movement artifacts),

we declared WAR on the artifacts of the remaining channels: for each channel we subtracted off the component of the signal which could be predicted from a linear combination of the other channels. Following spike detection and automatic clustering using Klusta, clusters were manually merged and labelled as Noise (for non-neuronal signals like artifacts and noise floor), MUA (for multi-unit activity with high response rates and numerous refractory period violations) or Good (putative single units) by an expert physiologist.

3.3.9 Putative cell types based on waveforms

In order to distinguish between Fast-Spiking (FS) putative interneurons and Regular-Spiking (RS) cells, we clustered neurons based on their mean waveforms. Although we did not observe a clear bimodal distribution of spike widths, inspection of the spike shapes indicated that this may be due to a failure of the post-spike “peak” of the waveform to rise above the noise floor of the recordings. Visual observation canonical FS and RS spike shapes indicate that the time between the trough and the peak is but one dimension that distinguishes these putative cell types—for example, FS neurons often have a small peak before the trough onset, a narrower spike trough, and a more rapid decay from the peak than RS. In order to take advantage of these additional features which might still be present in our data, we trained a linear classifier to distinguish between FS and RS cells based on the shape of the spikes using a dataset of spike shapes from an anesthetized acute recording with a clear bimodal distribution in spike widths. The linear classifier successfully matched the labels based on peak to trough spike widths in a held out portion of the dataset with 98% consistency with labels defined by the spike

width. We then used this classifier to assign each of the neurons recorded from awake behaving data to either the FS or RS cell types based upon the full waveform.

3.3.10 Cluster Quality and Inclusion Criteria

Putative single units for which greater than 0.5% of spikes exhibited refractory period violations (interspike intervals < 1ms) were considered to be multi-unit and were not considered in the analysis.

3.3.11 Identifying Auditory Neurons

We included neurons whose responses to any presented sequence differed from baseline rates. We characterized sequence responses according to the number of spikes elicited during the final element in a sequence. A “sequence” is therefore defined as a specific combination of the preceding elements and the current element. For example, if the elements ‘a’, ‘b’, and ‘c’ were presented in a trial, the trial comprises 3 sequences: the single-element sequence ‘a’, the 2-element sequence ‘ab’, and the 3-element sequence ‘abc’. The responses to each of these sequences are then number of spikes elicited by the final element in each (‘a’, ‘b’, and ‘c’). We sampled spikes in the 500ms window beginning at the start of the element and continuing for 100ms after the end of the element. A neuron was considered to be auditory-responsive if a least one presented sequence elicited responses which deviated significantly (paired T-test, $p < 0.01$) from the neuron’s baseline firing rate during the inter-trial period.

3.3.12 Mixed Selectivity

We characterized the extent to which neurons exhibit “mixed selectivity” by comparing the ability to decode the nine unique element-position combinations (a1,a2,a3,b1,b2,...) using a multinomial logistic regression with simulated element-selective and simulated position-selective. Pseudo selective neurons were simulated by shuffling within the selective. For example, to simulate a neuron with only element selectivity, we randomly shuffled element presentations across positions. Larger accuracy values decoding the intact responses than the shuffled responses are evidence of mixed selectivity. We summarized the population’s tendency for mixed selectivity by dividing each neuron’s performance decoding unique element-positions by the neuron’s performance on the same decoder with simulated element- and position-selective responses.

3.3.13 Estimating the Coding Capacity of Neuronal Populations with Linear Decoders

To measure the capacity of populations of simultaneously recorded neurons to encode element identity, we consider the decoding accuracy of a linear classifier be a proxy for encoding capacity (Jeanne et al., 2013; Seriès et al., 2004). We used logistic regression, as implemented in the scikit-learn package for Python, to discriminate between either pairs of elements or between all three of the elements presented during a neural recording block. Within each neural recording block, sequences were presented composed of three elements, creating 3 contrasts which are relevant. For example, for a block comprising elements a, b, and c, a/b, b/c and c/a are relevant contrasts. For each block, we inspected the capacity of the simultaneously recorded population(s) of RS and

FS neurons to encode each of the 3 possible contrasting elements as follows. For each simultaneously recorded population, we trained classifiers for different sized populations to discriminate between two of the three elements. In order to inspect the effect of population size on coding capacity, we sampled populations ranging in size from 1 to n , where n is the number of simultaneously recorded neurons for the block.

Discrimination accuracy was generally observed to increase with increasing population size (Figure X), so to characterize the coding capacity of each population, we estimated the upper bound on the population coding capacity by fitting a 4-parameter generalized logistic function to the population size vs accuracy curve for each recording block presented to the population.

$$accuracy(s) = A + \frac{K - A}{Qe^{-Bt}}$$

Where s is the size of the sampled population. The parameter K corresponds to the right asymptote of the logistic function and we interpret this parameter as the capacity of the population to encode elements.

3.4 Results

3.4.1 Sequence Discrimination

Six starlings were trained to criterion (consistent performance above chance with an odds ratio of 3 and a sequence duration hazard rate of 0.5). If subjects accumulate evidence toward their decision, we would predict that additional evidence would result in greater accuracy in classification. For all six subjects, we observed that their classification accuracy increased with increasing evidence (2D). An ideal strategy for

solving this task is to respond based on the the log-odds of the evidence (Figure 2B). If the likelihood that a given sequence was generated by the L model is greater than the likelihood the R model generated the sequence, then an ideal observer would respond left. For any given OR, the log odds that a sequence was generated by the left model is proportional to the net left evidence in a sequence. For all subjects, responses were well predicted by the log-likelihood of the model given the evidence available in sequences (Figure 2C; logistic regression sensitivity coefficient: mean=0.63, range=0.36-0.937).

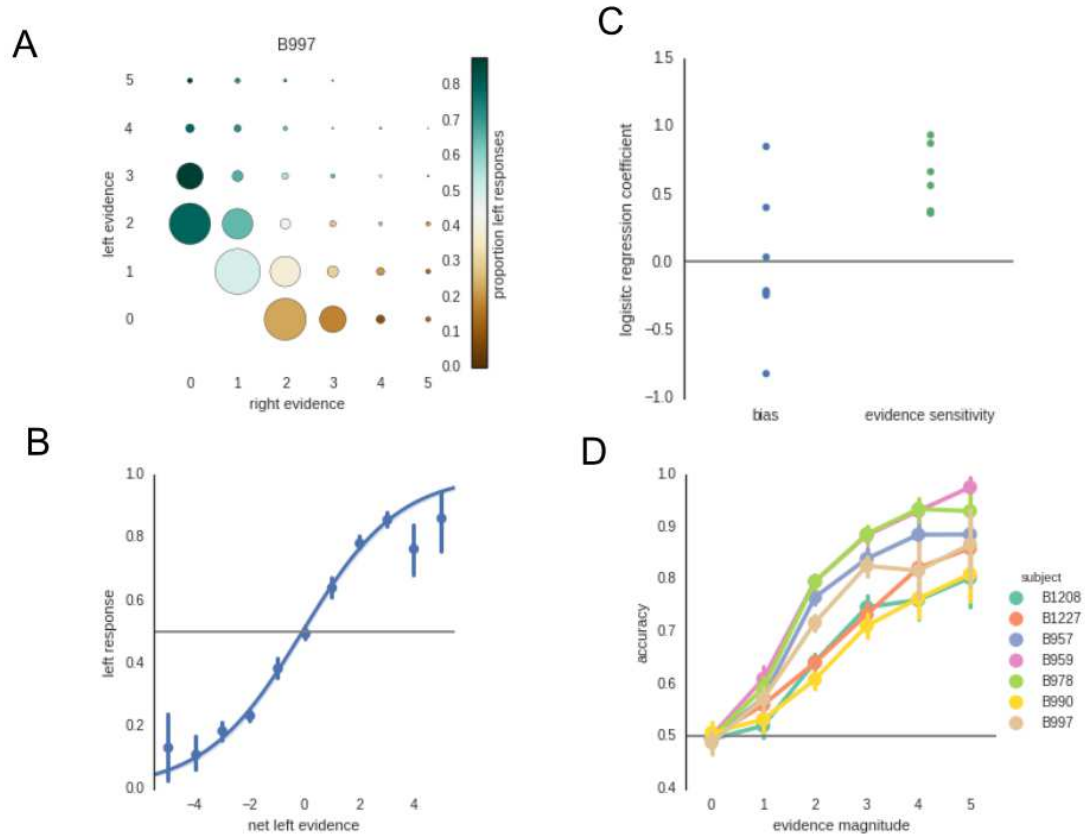


Figure 3.2. Net evidence in a sequence predicts starlings' decisions.

A: Responses are predicted by the combination of left and right evidence. Left response likelihood for an example bird (B997) is shown for different levels of left and right evidence. The area of each circle is proportional to the number of trials generated with a given combination of left and right evidence. Circles are colored according to the likelihood of a left response (green: left, brown: right). B: Responses are predicted by the difference between left and right evidence. For a B997, the likelihood of a left response is plotted with bootstrapped 95% confidence intervals for each level of net evidence (left evidence - right evidence). More left evidence than right evidence predicts left responses. Left response likelihood can be estimated by a logistic regression (solid blue line). C: Trained subjects ($n=7$) all show sensitivity to the net evidence and no systematic bias. D: Accurately inferring the model which generated the sequence increases with increasing evidence for all subjects.

3.4.2 Mixed Selectivity to Sequence Parameters

Neurons in CLM showed diverse responses to sequences, with some neurons primarily exhibiting sensitivity to the identity of elements in sequences (Fig 3B), the position of elements (Fig 3C), and mixed selectivity to both position and element identity

(Fig 3D). To characterize the the tendency for mixed selectivity, we first calculated each neuron's capacity to encode the nine element-position pairs (3 elements in 3 positions). Then, for each neuron, we synthesized its response to element-position pairs as a pseudo-element-selective neuron by shuffling presentations within each element and across position (thus breaking any position-selectivity). We followed the same procedure to synthesize pseudo-position-selective responses, then compared the full decoding of element-positions with the pseudo-element-selective and pseudo-position-selective responses (Figure 3E,F). We found that neurons showed greater selectivity for element-position combinations than would be expected by their element- or position- selectivity (ANOVA, main effect of cell type $p < 0.001$, main effect of shuffling $p < 0.001$, cell type shuffling interaction $p > 0.05$. Paired T-test between intact decoding and position-shuffled: $p < 0.0001$. Paired T-test between intact decoding and element-shuffled: $p < 0.0001$).

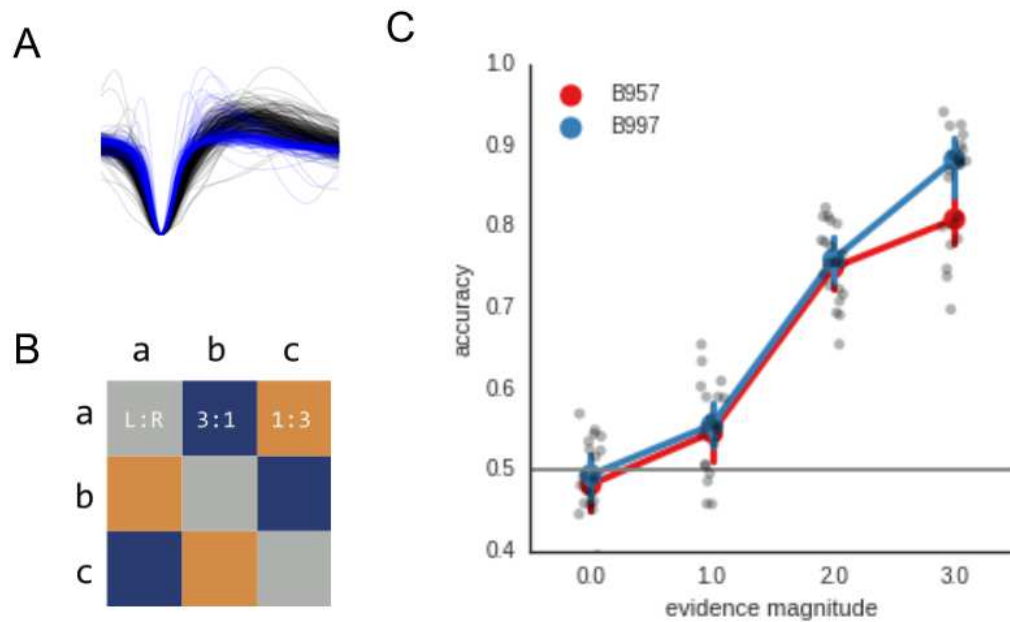


Figure 3.3. Awake behaving single unit population recordings during decision-making.

A: Example waveforms of Regular-Spiking (RS) neurons (black) and Fast-Spiking (FS) neurons (blue). B: Example transition diagram for an awake-behaving recording block. C: Example performance across awake behaving blocks. Color indicates subject identity, averaged across awake behaving blocks. Points in grey indicate individual blocks.

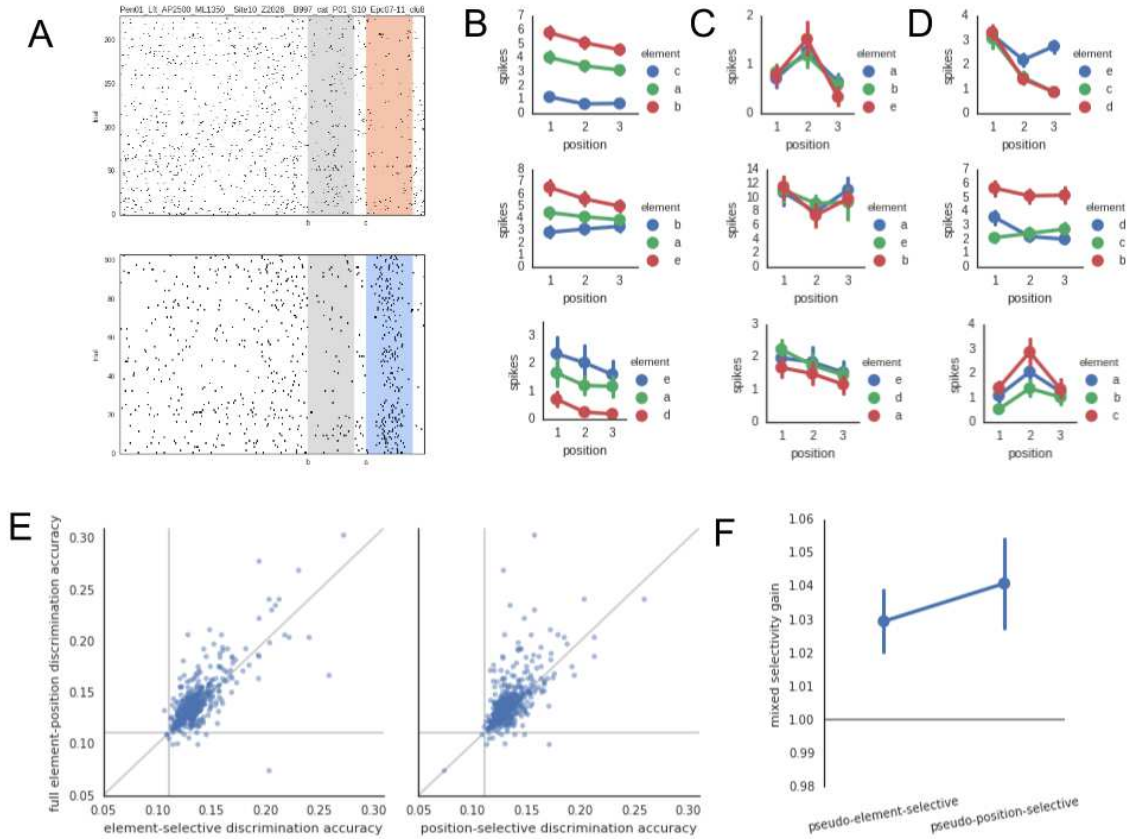


Figure 3.4. Mixed selectivity for element identity and position.

A: Example response of a single neuron to the elements 'a' and 'c' (background shaded in blue and red) when preceded by the element 'b' background shaded in grey). B,C,D: Example responses of neurons which are highly element-selective (B), highly position-selective (C), or mixed-selective (D). Mean (+/-95%CI) number of spikes elicited by the element (hue) in a given position (x-axis). E. Population analysis of mixed selectivity. Accuracy decoding the nine unique element-position locations for each neuron is plotted against the same discrimination when the same neuron has been simulated to be exclusively element-selective (left) or position-selective (right). F: Improvement in position-element discrimination compared to pseudo-element-selective and pseudo-position-selective responses.

3.4.3 Population Encoding of Element Identity

To make decisions based upon sequence information, it is critical that subjects properly identify the identities of sequence elements. Decoding element identity from populations of mixed-selective neurons has the potential to enable greater capacity for

element encoding than single neuron responses (Rigotti et al., 2013). Therefore, we characterized the coding capacity of populations of neurons by training linear classifiers to decode element identity from simultaneously recorded neurons. For each awake behaving block, we created populations of simultaneously recorded neurons of various sizes by sampling populations with sizes ranging from 1 to n , where n is the size of the full simultaneously recorded population. Then, for each population, we fit linear classifiers (see Methods) to discriminate between pairs of elements embedded in sequences. This allowed us to observe how encoding accuracy increases with population size and estimate the upper bound of the recorded population by extrapolating the change in decoding accuracy to the population's asymptotic performance. In general, we found that encoding capacity increased with population size and that both FS and RS populations encoded element identity (Figure 5A; T-test, $p < 0.001$), though RS populations were better able to encode element identities than FS populations (T-test, $p < 0.001$).

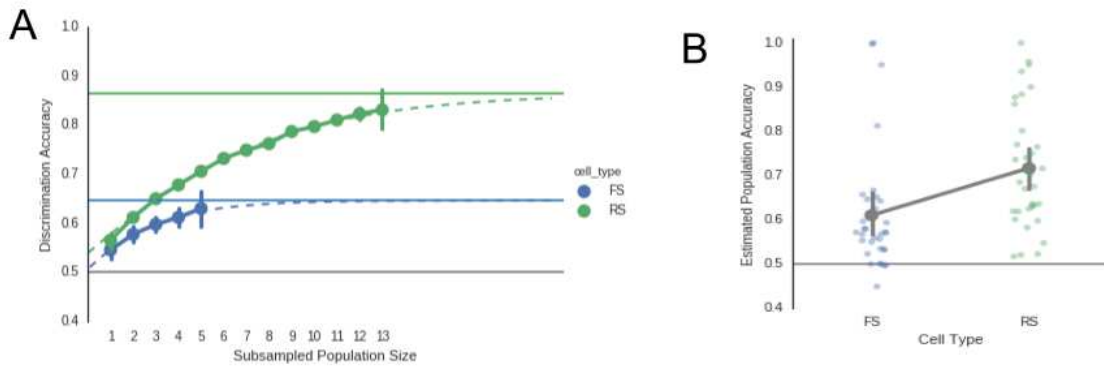


Figure 3.5. Encoding accuracy improves with larger populations.

A: Example performances of two simultaneously recorded RS (green) and FS (blue) populations as linear decoders are trained on increasingly large subsamples of the whole population. In order to estimate the upper bound of the population's encoding potential, we fit a generalized logistic function to obtain the asymptote of the logistic function. B: Estimated Population Accuracy for FS (blue) and RS (green) populations.

3.4.4 Role of Coordinated Activity in Element Encoding

Prior work in CLM has shown that coordinated population activity can be beneficial for encoding behaviorally meaningful stimuli. To assess the role of coordinated population activity in element encoding we repeated the above binomial classification procedure but decorrelated the neuronal activity by shuffling each neuron's elicited spiking response to a given element. This procedure decorrelates the population response to individual elements, while ensuring that the mean population response does not change and that the marginal distribution of each neuron's responses to the elements does not change. This simulates a scenario where the neurons in the population respond completely independent of one another (Jeanne et al., 2013; Seriès et al., 2004).

To measure the contribution of coordinated activity to encoding element identities we compare the performance of the linear classifiers with intact interneuronal

correlations with those trained on uncorrelated activity. For each classification, we divide the performance of the intact classifier to the performance of the uncorrelated classifier to yield the accuracy gain due to the coordinated activity of the sampled population. Values greater than 1 indicate a that coordinated activity improves element encoding, while values less than 1 indicate that coordinated activity impairs element encoding. Then, for each cell type and population size, we then measured the correlation between accuracy gain and intact accuracy. Positive correlations indicate that coordinated activity is beneficial and tends to improve the encoding of elements.

In general, we found that accuracy gain due to coordinated activity predicts the coordinated population accuracy (Figure 5A) for both FS and RS neurons. This effect is stronger for FS populations, improves with increasing population size, and the strength of the improvement with population size is greater for FS populations (ANCOVA, main effect of cell type: $p < 0.0001$, main effect of population size: $p < 0.0001$, interaction: $p < 0.01$)

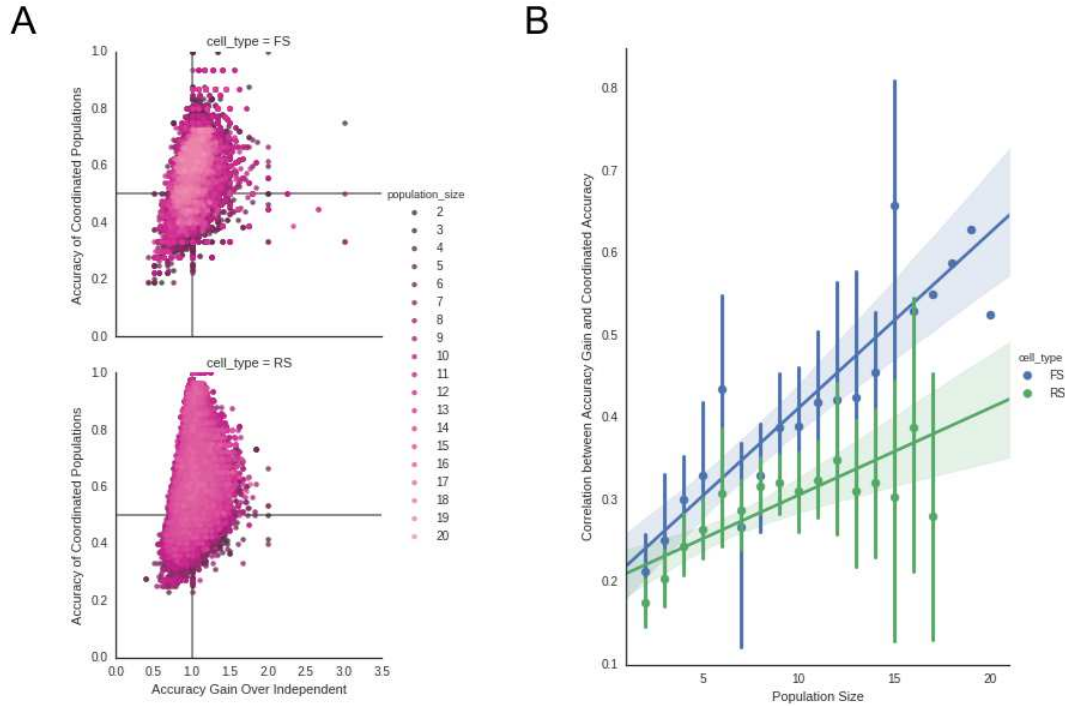


Figure 3.6. Coordinated population activity improves element encoding.

A: Accurate decoding of element identity with coordinated activity intact is predicted by the gain over independent activity. Top: FS populations. Bottom: RS populations. Color indicates population size. B: The correlation between the coordinated activity gain and the decoding accuracy increases with population size and is greater for FS populations (blue) than RS populations (green).

3.4.5 Inter-population Facilitation of Element Encoding

Sequences unfold in time such that the representation of an element embedded in a sequence is due to an interaction between the element and the state of the network when the element is presented (Buonomano and Maass, 2009). We predicted that element representations in FS and RS populations would be dependent upon earlier states of the network. To analyze this, we asked whether accurate encoding of the element identity among FS and RS populations in the past predicted the accuracy of element encoding

either within the same population or across the two populations. We limited our analysis to the 3-element sequences which were provided the most L or R evidence (e.g. `abc`).

For each combination of population identity (FS and RS) in each target and predictor position (1, 2, or 3), we calculated a prediction index as follows:

$$\frac{P(\text{target} = \text{accurate} \mid \text{predictor} = \text{accurate})}{P(\text{target} = \text{accurate} \mid \text{predictor} = \text{inaccurate})}$$

Where *target accuracy* is the accuracy of the target population and *predictor accuracy* is the accuracy of the predictor population. Values greater than 1 indicate that the accuracy of the target population increases when the predictor population accurately encodes elements. We observed that both FS and RS populations more accurately encoded element identity when the other population accurately encoded element identity (Figure 5C). On the other hand, intra-population prediction was impaired (ANOVA for prediction index: no main effect of target population or predictor population. Interaction between target population and predictor population: $p < 0.000001$, post hoc T-tests: FS $\Rightarrow$ RS: $p = 0.042$, RS $\Rightarrow$ FS: $p = 0.022$, FS $\Rightarrow$ FS: $p < 0.0001$, RS $\Rightarrow$ RS: $p < 0.01$).

How does past element encoding affect the representation of subsequent elements in order to improve element encoding? There are a number of ways that element representation could change to facilitate encoding, but it's important to note that each population is fit to a single classifier, so the improvement in discrimination performance that we observe is not due to a drastic change in encoding, but rather a change which improves decoding for the same classifier. One possibility is that element representations could move farther away from each other. While this might improve decoding with this

classifier, it would be a poor general purpose mechanism, as the representations might encroach upon the stimulus space of (a) the other two elements that compose sequences in the original training or (b) other acoustic material that is of relevance to the subject. Alternatively, the covariance structure of the population activity could change in order to improve discrimination (e.g. inverting the canonically positive relationship between signal and noise correlations, as in (Jeanne et al., 2013)). We did not observe any changes in the correlation structure (data now shown), but we had relatively little power to detect such a change, as we had limited numbers of trials from which to calculate pairwise correlations or the covariance matrix. A simpler prediction is that accurate decoding within one population drives a more precise representation in the other population in order to facilitate decoding. To test this hypothesis, we assumed a fixed covariance matrix for the population representation of each element (using the same set of observations that the linear model was trained on) and for each element presentation, we calculated how close it was to the mean representation of the element by calculating the Mahalanobis distance between the element's population response and the mean population representation. Then, for each prediction of target accuracy contingent upon predictor accuracy, we calculated the difference between the mean Mahalanobis distance in the target population when the predicting population was accurate and when inaccurate. Negative values indicate that the predictor population increases the precision of the target population when the predictor population accurately encodes elements. We found that in the cross-population predictions (FS $\rightarrow$ RS & RS $\rightarrow$ FS), the Mahalanobis distance is smaller when the predicting population was accurate, meaning that the representation was closer to the center of the population representation distribution (Figure 7B, T-test:

$p=0.018$). On the other hand, we saw no systematic change in the Mahalanobis distance for intra-population predictions. Importantly, smaller Mahalanobis distances do not generally predict the accurate encoding of a given element presentation (T-test, $p = 0.86$). Similarly, while accurate encoding in the target population also “predicts” accuracy in the predictor population, it does not drive smaller Mahalanobis distances in the past (data not shown).

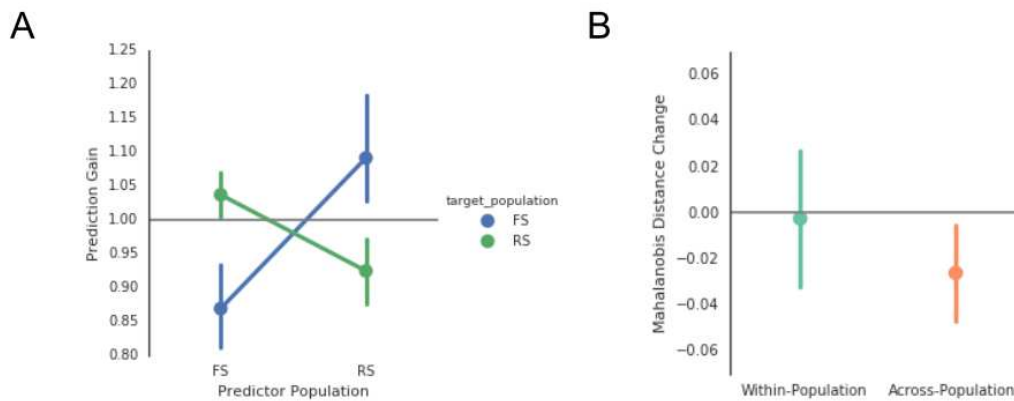


Figure 3.7. Accurate decoding of element identity by FS and RS populations predicts accurate encoding across-, but not within-populations.

A: Accurate decoding of element identity in the FS (blue) and RS (green) populations are predicted by accurate decoding the RS (left) and FS (right) populations, respectively. However, accurate decoding predicts poor decoding within the same population. B: Accurate element decoding within one population results in element representations in the other population which are closer to the mean representation than when the predictor population is inaccurate (orange). Accurate element decoding does not systematically affect fidelity within the same population (green).

3.5 Discussion

In a probabilistic sequence task, the fidelity of the neuronal representation of sequence elements is influenced by the accuracy of classification of element representations in other cell types. Accurate encoding of element identity in FS and RS

populations increase the reliability of population responses of subsequent elements in RS and FS populations, respectively.

This work follows on prior work showing that starlings exhibit exceptional faculty perceiving sequences (Comins and Gentner, 2010). We've extended this work to develop a probabilistic sequence discrimination framework which allows for investigating decision-making processes in awake behaving animals. This two alternative choice task requires subjects to classify sequences according to which of two markov models generated them. Each new element in a sequence is generated according to the transition probabilities of one of two generating markov models. Each model is associated with a L or R responses, such that the subject must respond by pecking L or R, effectively selecting which model generated the sequence. Subjects can infer the appropriate response based on the evidence afforded by transitions between elements. We show that subjects' decisions are predicted by the log-odds that the sequence was generated by one model or the other, consistent with subjects accumulating evidence from a sequence. This task structure (inferring which markov process generates a sequence) provides a valuable starting point for further work into sequence perception, as it can be readily modified to explore other statistical cues, sequence generation models, or adaptation to changing cues.

The full probabilistic sequence protocol generates far too many unique sequences to be used in electrophysiological recordings, where an experimenter must sample each unique sequence with sufficient repetitions to characterize responses in the population while maintaining quality isolation of neurons. Therefore, we designed the markov

models used in this experiment so that we could also generate static blocks for awake behaving neural recording sessions. This allowed us to train subjects, adjusting parameters of the generative task until subjects reached criterion, then create static blocks of trials that conformed to the critical aspects of the task without having to comprehensively sample the entire set of possible sequences during awake behaving electrophysiological recording blocks. These blocks used only a subset of the elements of the full training protocol, but maintained key statistics within each block: for example, each element was equally likely and the log-odds of subjects receiving reinforcement for L versus R responses to a given sequence were consistent with the full task. During awake behaving recording blocks, subjects performed consistently with their prior performance on the full task.

Importantly, this approach also allowed us to select blocks according to the tuning of neurons. For example, if a neuron only responded to element 'a', there would be little use in presenting blocks comprising elements *b*, *c*, and *d*. Instead, we were able to select sequences that showed some promise in modulating recorded neurons responses. This “closed loop” approach of training on a large set of stimuli then constraining the stimulus set based on the tuning properties of the neurons under investigation has been invaluable in other fields of cognitive neurophysiology. For example in many studies of spatial attention, the spatial specificity of a neuron’s tuning is determined first, then the attention task is limited to stimuli within the spatial receptive field of the neuron in order to identify how attention then modulates the response (Desimone and Duncan, 1995). In our task, we attempted to characterize the “element receptive field” by ensuring that at least

one neurons responded to at least one of the three elements, then limiting the stimulus set used for neuronal recordings to 3 of the 5 possible elements. As improved recording technologies (Berényi et al., 2014; Scholvin et al., 2016; Sofroniew et al., 2016) allow the field to interrogate the role of increasingly large populations of neurons in complex cognitive tasks, it will be imperative to develop methods for rapidly characterizing population activity with the respect to the primary experimental parameters of interest. Improved methods for rapid spike sorting from dense microarray recordings (Pachitariu et al., 2016) and automated source identification from calcium imaging (Pnevmatikakis et al., 2016) will be critical in automating data processing steps to allow more robust closed-loop stimulus selection protocols.

Our observation of mixed-selective neurons is consistent with prior work regarding sequence selectivity in the avian auditory forebrain. In prior literature, “sequence selectivity” has typically be defined by responses to combinations of elements which could not be explained based on the responses to individual elements. These neurons, however, could also be described as exhibiting “mixed selectivity” for elements and position or prior elements. A population of neurons with mixed selectivity to sequence parameters is a powerful way for a system to adapt to changing goals, as different parameters can be readily accessed from the same population (Raposo et al., 2014; Rigotti et al., 2013). We were interested in understanding how sequence elements are encoded in CLM and how this is affected by the learned statistics of sequences. To do this, we took a basic approach of assumed a fixed decoder of element identity, then

explored how decoder performance depended on coordinated population activity and whether expectations established by learned sequence statistics influenced coding fidelity.

First, we asked to what extent decoder performance was sensitive to coordinated activity. That is: to what extent do observed correlations among neurons impair or improve read-out? We find that coordinated activity improves element encoding. One way in which neuronal activity may be “coordinated” is through noise correlations (Seriès et al., 2004), which are the correlations that exist between neurons among repeated presentations of the same stimulus. These are typically positive (e.g. when one neuron responds more strongly to a stimulus, the other does as well) and are thought to reflect common inputs (Seriès et al., 2004). Noise correlations can be broken by shuffling neuronal responses among observations but within element identities, simulating neurons which maintain their respective stimulus-tunings, but act independently. This procedure is, however, agnostic to what unobserved factors might drive noise correlations. While it is commonly assumed that noise correlations are the results of common “noise” in the system, it is important to note that in our experimental design, position may also drive correlated activity. In our analysis, shuffling to break noise correlations also breaks any correlations that are to position sensitivity as well. So the effects observed in Figure 5C and D could be attributed to abolishing the benefits of mixed selectivity by forcing our decoders to rely only on the element-selective component of mixed selective neuronal responses. To our knowledge, no one has studied the interactions between mixed selectivity and noise correlations on stimulus encoding, however it is worth noting that the two phenomena interact. A covariance structure that benefits decoding of one factor

may impair decoding of another factor. While it may be intuitive to attribute noise correlations to common inputs (especially in primary thalamorecipient populations), it's important to recognize that noise correlations are not an intrinsic property of the population, but are a function of the factors that are being decoded. For example, a positive noise correlation within a given class (say, element `a`) could be driven by a positive signal correlation between subclasses (element `a` in positions 1, 2, and 3) while the subclasses themselves all exhibit negative correlations. This is effectively a neuronal population coding version of Simpson's paradox (Simpson, 1951). The way that correlation structure affects encoding is therefore highly sensitive to what the experimenter considers to be "signal" (the factors that they are decoding) and what is "noise" (everything else). Mixed selectivity is powerful precisely because the diversity of responses allows flexible partitioning between different "signals", but further work is needed to understand how this interacts with inter-neuronal correlations that can affect optimal coding. In the current work, recording blocks were designed to emphasize key comparisons rather than comprehensively sample every combination of covariates that could influence correlation structures, so our analysis lacks the power to inspect how noise correlations might be influenced by other covariates. Nonetheless, our results emphasize that simultaneous recordings of neuronal populations are important to be able to address these questions.

Secondly, we asked how decoding is influenced by the network state. Theoretical work on state-dependent networks highlights that the response of a network to a stimulus (e.g. a sequence element) is due to the interaction between the stimulus and the network

state (Buonomano and Maass, 2009). We found that prior states of the FS and RS networks influenced element encoding within trials: when FS or RS populations accurately encode the identity of an element, the fidelity of the complementary population representation is improved: the reliability of the complementary population response increases and its encoding accuracy improves. We would not expect such a result from a naive subject being presented random sequences. Rather, this effect seems to reflect expectations that are established by the behavioral training of the subjects. The sequence discrimination task is defined by first order statistics, wherein every event establishes a prediction of the upcoming events. One of the primary predictive cues is that elements are not repeated, so the occurrence of a given element reduces some of the uncertainty in the subsequent element. Our results suggest that this information about the set of possible upcoming events is leveraged to improve the system's ability to discriminate between upcoming events. There are many ways that expectation is established in the behavior. As noted previously, the fact that elements cannot repeat means that the likelihood of element a occurring after element a is zero: $P(x_i=a|x_{i-1}=a) = 0$. On the other hand, the likelihood of element b occurring after element a is higher than the marginal likelihood of element b occurring: $P(x_i=b|x_{i-1}=a) > P(x_i=b)$. However, there are also related statistics that may be encoded: the likelihood of a given element following a sequence $P(x_i|x_{i-n} \dots x_{i-1})$, or the likelihood of an element following a sequence for a given markov model $P(x_i|x_{i-n} \dots x_{i-1},m)$. The probabilistic nature of the sequence discrimination task used for this experiment affords a rich behavioral basis from which to ask how these statistics might differentially contribute to the expectation effects we observed, however we did not have sufficient power to tease apart these models from

one another. Future work will be necessary to understand more precisely how expectations established by learned sequence statistics influence element encoding.

While our results indicate that statistical expectations are leveraged to improve coding fidelity, it is not clear what mechanism improves the reliability of population responses. One possibility is that element identity is decoded from CLM responses by a higher region such as NCL and a top-down signal then biases responses in CLM to improve the fidelity of expected elements. While efferent projections from CLM to the song production system have been confirmed (Bauer et al., 2008; Vates et al., 1996), the connectivity between these CLM and multimodal areas involved in decision making have not yet been identified. Another possibility is that the effect is largely due to local interactions between the FS and RS populations. The latter possibility is especially intriguing, as the interactions we observe are between RS and FS populations which were recorded on the same silicon microprobe, and therefore proximal to each other. Pharmacological manipulations of inhibitory activity (Thompson et al., 2013) or cell-type-specific optogenetic manipulations (Boyden et al., 2005) in CLM and NCL (Roberts et al., 2012) may allow us to better understand how the FS and RS populations are interacting to improve coding fidelity and the role their interaction plays in driving behavior.

Chapter 3, in part, is currently being prepared for submission for publication of the material. Kiggins, Justin T.; Gentner, Timothy Q. The dissertation author was the primary investigator and author of this manuscript.

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CHAPTER 4

Conclusion to the Dissertation

4.1 Major Findings

Language and music pose some of the most significant challenges to neuroscience. One such challenge is that the meaning of an utterance often depends upon the ordering of elements in a sequence. This challenge is shared with many animals, and in the work presented here, we focused on characterizing the capacity of European starlings to discriminate between sequences and understanding how neural representations support sequence discrimination.

We showed in Chapter 2 that starlings can be trained to discriminate between probabilistically generated sequences composed of vocal elements using only the relationship between elements: identities of individual elements and their absolute positions were irrelevant to the task. Subjects readily learned this task, but the probabilistic nature of the task meant that subjects were faced with uncertainty. We showed that subjects performed in a way which was consistent with weighing the evidence afforded by individual transitions. Following behavioral training, we anesthetized subjects and recorded from the caudal nidopallium (NCM) in response to pairs of elements. We showed that neurons in NCM are sensitive to sequences and that, for neurons which are tuned to familiar elements, their capacity to encode sequences is higher than their capacity to encode elements. Neuronal responses to sequences are shaped by a combination of reward-association and behavioral demands: reward-association facilitated sequences, but only if such facilitation improved sequence discrimination.

In Chapter 3, we developed a new probabilistic sequence discrimination task which was optimized for awake behaving recordings. This task provided additional evidence that starlings made decisions based upon the evidence available in sequences. We recorded from populations of fast-spiking putative interneurons and regular-spiking neurons in the lateral caudal mesopallium (CLM). We show that neurons in NCM exhibit mixed sensitivity to element identity and position and that coordinated population activity is important to element encoding. We find that element identities are more resolvable from population activity when their activity is coordinated, an encoding benefit that could be attributable to either mixed selectivity or beneficial correlations among neurons. Finally, population encoding of elements during a sequence is dependent upon accurate decoding of prior elements. We observed an inter-population prediction effect, where accurate decoding by one population predicted the other population was more likely to be able to accurately discriminate elements. This improvement in the fidelity of element representations is a result of improved reliability of the target population representation.

4.2 Future Directions

These results point to interesting questions for future work in the areas of behavior, physiology, and experimental design. First, there are open questions regarding how birds learn these probabilistic sequence discrimination tasks. For example, it may be that starlings learn the statistical associations of these tasks in a stepwise manner, as has been shown in motor learning (Lipkind et al., 2013). The experiments presented here may not be appropriate for such an analysis, however, as shaping strategies (adjusting reinforcement schedules and difficulty parameters) to train subjects up to criterion were

employed ad hoc. A related question, however, is how readily they can accommodate new statistical contingencies and what strategies they might employ to do so. Such an experiment would involve, once subjects have learned the task, cycling through blocks with different statistical contingencies. Not only would such an experiment offer insight into the stability of the sequence associations and contingencies they've learned, but whether they learn the new contingencies in a stepwise or global manner could be answered with other experimental variables held constant.

A second set of questions related to behavior involve the strategies that, once learned, subjects use to solve these probabilistic sequence discrimination tasks. First, although we note that their behavior is consistent with evidence accumulation, the task does not explicitly require subjects to accumulate evidence during the task, but rather allows them to wait until the end of the task to evaluate the evidence that was presented. We partially addressed this in Chapter 3, by implementing a hazard function such that, once subjects heard three elements, there was an equal likelihood that they would hear one more or the sequence would terminate. We observed increased accuracy for longer sequences, indicating that subjects were taking advantage of the additional evidence present in longer sequences. Nonetheless, a more robust test of evidence accumulation would be to allow subjects to respond at any point in the sequence, terminating the sequence when they peck. This design would necessitate continuous integration of evidence during the sequence and would allow us to explicitly test subjects' evidence threshold for decisions. Secondly, we note that an ideal observer which is trying to maximize reward would consistently select L with only one unit of left evidence, but we

don't observe this strategy. Among our best performing subjects in Chapter 2, they never pushed beyond matching the statistics of sequences. This tendency for animals (and humans) to match the statistics has been widely noted (Herrnstein, 1961) and our starlings appear to be no different. However, there are multiple factors might push subjects off an ideal-observer response curve, such as low motivation or poor discrimination of sequence elements (Churchland and Kiani, 2016), making it difficult to characterize deviations. Nonetheless, careful analysis of the influence of recent trials may reveal that starlings are relying on heuristic strategies to perform these types of tasks (Gigerenzer and Goldstein, 1996; Herrnstein, 1970; Sugrue et al., 2004). In general, a more complete understanding of strategies starlings use will help to inform hypotheses about the neural mechanisms underlying these decisions. In addition to questions about behavior and decision making, we also note future directions for inquiry into the physiology underlying these decisions. Our behavioral results indicate that starlings' decisions are well predicted by differentially weighing the evidence in a sequence, which is consistent with dominant models of evidence accumulation. However, it is not clear where or how sequential evidence accumulates. Further anatomical work needs to be done to identify the regions which CM and NCM project to in order to identify candidate regions for electrophysiology, however, a good candidate might be the shell of HVC. The other set of physiological questions concern the relationship between FS and RS neurons. Although we showed in Chapter 2 that accurate decoding of element identity in FS and RS populations could improve the fidelity of the population response in the complementary population, the mechanism of this is not clear. One way to address this question would be to combine population recording with local application of GABA

antagonists to block local inhibition or optogenetic manipulations of FS or RS neurons to disrupt activity. Finally, while we provide evidence in Chapter 2 that fidelity in CLM is modulated by expectation, further work is necessary to more explicitly test this hypothesis. In particular, a behavioral design that generates a greater range of expectations (e.g. where a given element is highly likely, moderately likely and unlikely based upon the prior sequence) would allow a more direct test of how closely the improved fidelity we observe is tied to statistical expectation.

A final future direction for research is into improved methods for closed-loop stimulus selection. Choosing stimuli which are tuned for the neurons that are being recorded from is important. As we showed in Chapter 1, improved sequence discrimination was only observed for neurons which preferentially responded to familiar elements. In Chapter 2, we designed a behavior that could be run on a smaller stimulus set than the training set in order to ensure that we were presenting blocks composed of elements that would drive the neurons we were recording from. However, this was a fairly subjective process, and more principled methods for closed-loop stimulus selection will be necessary to design experiments so that specific hypothesis (e.g. that the reliability of the population response to an element is inversely correlated to the surprisal of the element in the sequence) can be tested within the practical limitations of an experimental session. Optimizing stimuli for the system of interest is not a novel idea in behavioral psychology or neuroscience. Adaptive stimulus selection methods, such as staircase procedures, were developed to efficiently and adaptively identify discrimination thresholds in psychophysical studies (Treutwein, 1995). In neurophysiology, spatial

attention recording paradigms are a good example of the utility of closed-loop stimulus selection. For single-neuron studies, this approach has been fairly straightforward: if a neuron is non-responsive to spatial region, then there is clearly little utility in having a subject attend to that area. We identify are two criteria that are necessary to take advantage of closed-loop stimulus selection in awake behaving experiments. First, the experimenter must be able to quickly characterize the neural response to primary tuning parameters. Secondly, the behavioral training must be invariant to those same tuning parameters. Using visual spatial attention as a case study, we can demonstrate why these two criteria are important. First, in visual spatial attention, one can very easily (in an experimental session) characterize the spatial receptive field of a single neuron. Put more specifically, one can easily optimize 2 stimulus parameters (the x and y coordinates of the stimulus) in order to maximize the 1-dimensional response of a single neuron's firing rate. Second, in visual spatial attention tasks, subjects have been trained to attend to targets at multiple locations in space. Again, put more specifically, subjects have been trained such that their behavior is invariant to the x and y coordinates of the stimulus. This is critical to being able to then vary the location of the attentional target to match the spatial receptive field of the neuron under the electrode. This criteria also limits the utility of closed-loop stimulus selection to behaviors that have major parameters for which the behavior is invariant, such as visual spatial attention or match-to-sample tasks. The first criteria becomes more challenging to meet when the "system" of interest is not a single neuron, but is instead a population of neurons. In this scenario, one will need to define another metric or set of metrics of the population response to optimize. This metric will depend on the experimental question at hand, but there are natural extensions of tuning

sensitivity that may lend themselves to optimization. For example, one might be able to extend the idea of a staircase procedure to identify the set of parameters that define the sensitivity threshold for the population or utilize genetic algorithms to minimize population sparseness. These approaches, however, will require characterizing population neuronal activity in near real-time, necessitating improvements in automated methods for multichannel spike sorting for electrophysiology and cell-identification for calcium imaging. As neural recording technology increases our capacity to record from large populations of neurons, this will be critical for understanding the neural dynamics underlying perception and cognition.

4.3 References

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