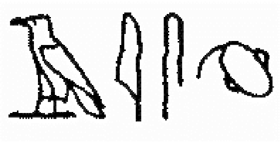
Introduction: Neural dynamics underlying complex behavior in a songbird



--from the Edwin Smith Surgical Papyrus, (Egypt, 1700 BC)
the oldest written record of the word "brain"

The brain. It has fascinated man throughout history, but its secrets have remained largely untapped, and its mysteries unsolved. From 1700 BC to 1500 AD, the first written records about the nervous system (Edwin Smith Surgical Papyrus, ancient Egypt) were expanded into a coarse description of its basic anatomy (e.g., 1561 AD, publication of the first description of the cranial nerves, G. Falloppio). Despite the many accomplishments over this span of 3000 years, the tools needed to begin understanding the physical mechanisms that underlie thoughts and behaviors would require nearly another 500 years of development. It was not until almost the twentieth century that nerve cells were discovered to be independent elements (Ramon y Cajal, 1889), the fundamental computational units of brain function. Progress since then has been rapid, and our understanding of the brain has advanced tremendously. Yet the core questions that have haunted us from the beginning remain unanswered; the neural mechanisms by which thoughts and behaviors emerge are nearly as opaque to us as they were 3000 years ago. The brain remains a riddle that will never lose its luster, because it represents everything that we are, and all that we can ever become.

After the discovery of independent nerve cells by Cajal, much of neuroscience research in the first half of the twentieth century was characterized by studies of the electrical properties of neurons in anesthetized animals. The culmination of this work was the collective realization, made by many researchers and in a number of species, that neurons could be thought of as detectors of behaviorally relevant stimuli. It is worthwhile to consider two representative examples which clarify why these discoveries revolutionized the study of brain and behavior. Hubel and Wiesel (1959) found that neurons in the primary visual cortex of the anesthetized cat discharged maximally when presented with edges of a particular orientation. They further realized that the complex receptive fields of these neurons could be accounted for as a sum of the simpler receptive fields of neurons in the lateral geniculate nucleus, which sends input to the primary visual cortex. At nearly the same time, Lettvin et al. (1959) showed that visual ganglion cells in the frog's retina were tuned to small dark objects moving intermittently in their receptive fields – they were effectively bug-detectors. The implications of these discoveries were staggering: the visual scene was being decomposed, sliced into a series of smaller pieces which could be manipulated, transformed, and used for computation by the rest of the brain.

Remarkable observations such as those described in the previous paragraph demonstrated that the information processing capabilities of the brain could be measured, understood, and correlated with behavior. Over time, however, it became clear that the response properties of neurons in behaving animals were often dramatically different and more complex than those in anesthetized animals. Furthermore, behaviors are generated not by individual cells, but rather by the activity of large numbers of interacting neurons. These collective network dynamics are not easily understood when considering the activity of single neurons. In the last twenty years, the measurement of this network activity and its relation to behavior has become of a problem of paramount importance in systems neuroscience. This shift, from the study of single cells in anesthetized animals to many cells in behaving animals, has brought with it a number of challenges. First, technology is needed to allow the measurement of the activity of large ensembles of neurons in a manner that is robust to the movements of a freely behaving animal. Second, techniques are needed to observe the behavior of an animal, to manipulate that behavior in a controlled manner, and to compensate for behavioral variability. Finally, analytical methods are required to quantify the dynamic activity of many neurons, and to correlate this activity with the behavior of the animal.

The work described in this thesis has touched on all of these problems, but in particular has focused on the question of how the activity of large numbers of interacting neurons is involved in the generation of behavior. The animal in which I have done this research is the zebra finch (*Taeniopygia guttata*), a small Australian songbird (Figure 1.1). As juveniles, these birds memorize a tutor's song and produce an accurate copy of it by the time they reach adulthood (Immelman, 1969; Price, 1979). Once learned, a bird will sing only this song for the remainder of his life. The song contains spectral and temporal structure over a wide range of time scales and is thus a complex learned behavior. It may at first seem misleading, to hope that the lessons learned from the brain of a bird can be generalized in any manner to the brain of a man. But the differences in complexity between humans and bird are no more than that – differences, variations on a common theme. Evolution has generated a single class of neural information processing systems, and while the details of each individual system are unique, the general principles that govern their function appear to be the same across countless species, from insects to primates. Thus by studying the neural control system underlying birdsong, one hopes to gain insight into the general types of neural dynamics evolved by the brain to produce complex behaviors. In the sections which follow, I briefly review the relevant behavioral and anatomical literature on the generation of birdsong, and then summarize the progress I have made towards understanding the neural dynamics underlying complex behavior in a songbird.

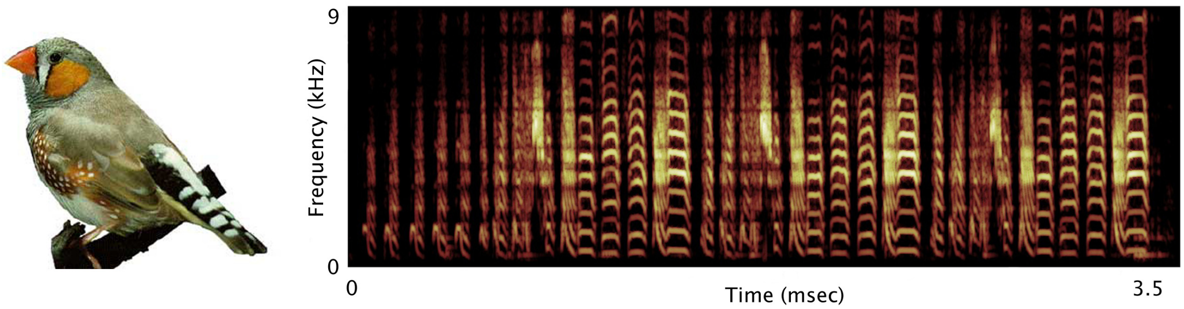


Figure 1.1. A zebra finch and his song.

1.1 A brief history of birdsong

Zebra finches are small, weighing only 10-15 grams; the zebra finch brain is on the order of 500 mm^3. Of this, the combined volume of all of the nuclei responsible for generating birdsong is probably less than 20 mm^3. The individual brain areas studied in this thesis range in size from 400 um to 700 um spheres, each containing a few thousand neurons which are roughly 10 um in diameter. The total number of neurons in the song control system is probably less than 500,000, and is certainly less than 1,000,000. Yet despite these seemingly formidable size constraints, the zebra finch does something remarkable. When he is very young, only 20-30 days old, a male zebra finch will memorize the song of a tutor. The song is a complex pattern of different sounds, arranged in a sequence of about 1 second in length (Immelman, 1969). At around day 45 he begins to sing, first producing scratchy, ill-formed squawks, and then later gradually refining them into a nearly perfect copy of the tutor song. Finally, at roughly 90 days of age, the learning process is complete and the bird will sing only this single crystallized song for the remainder of his life. This sophisticated behavior, central in the life of zebra finches as a courtship mechanism and a mode of territorial defense, is generated by a tiny collection of neural circuits, a biological machine so small it can only be seen with a microscope.

Equally remarkable is how the behavior responds to perturbations of the normal song learning process. Song has been known to be a learned behavior, dependent on hearing the songs of adult conspecifics, since the work of Thorpe (1958), who showed that young birds isolated from tutors develop highly abnormal songs. Interestingly, the songs of isolate birds do contain some recognizable species-specific components, most notably the number and length of individual sound units (syllables), and the separation between them (Marler and Sherman, 1985). Thus some species-typical components of the song are innate. In contrast, the complex modulations of spectral structure, and the stereotyped temporal patterning of different sounds are all components of the song that must be learned. The amount of tutoring required for memorization of the song can be incredibly brief. In some species, hearing the tutor song approximately 10 times is sufficient for accurate reproduction when the bird reaches adulthood (Todt and Hultsch, 1985, Konishi, 1985). Immelman (1969) showed that birds tutored by males of a different species will still attempt to copy the song of the tutor, and will generally be fairly successful at this, despite the unusual sounds that they are generating (see also Price, 1979). After the template song has been memorized, but before the young bird begins to sing, the tutor may be removed without any effect on quality of song eventually produced by the adult bird (Marler, 1976).

Auditory input is critical to song learning in two ways. First, as discussed above, it is vital for the process of memorizing the tutor's song.. The second role of audition in song learning was first demonstrated by Konishi (1965), who showed that birds deafened after

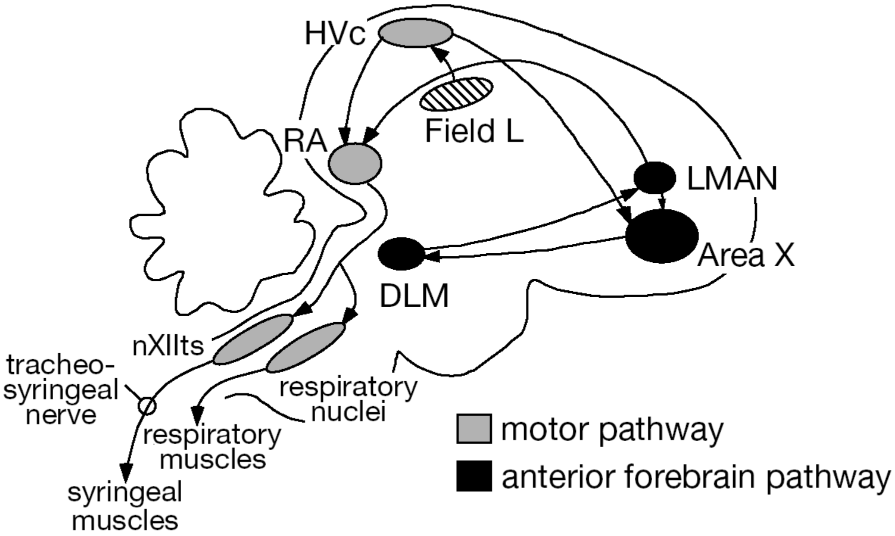


Figure 1.2. Schematic diagram of the zebra finch brain. The motor control pathway, from HVc to the syrinx, is shown in gray. The anterior forebrain pathway is shown in black. *Figure kindly provided by Allison Doupe, UCSF.*

they memorized a tutor song but before they began to sing developed highly abnormal songs and never successfully reproduced the tutor song. Auditory feedback therefore plays a second role in learning, during the sensorimotor process of transforming the bird's initially noisy vocalizations into an accurate copy of the tutor song. It was proposed very early on that in this second stage of learning, auditory feedback is used in an error-correction process to refine the bird's own vocalizations into a progressively more accurate copy of the tutor song. Attempts to identify the neural locus of the song template, and the neural circuits whereby the error-correction process takes place, continue to this day and form a significant part of this thesis.

The zebra finch has become a model system for studying of the neural basis of natural behaviors (i.e., neuroethology), because the richness of the singing behavior is generated by a discrete set of brain nuclei which are amenable to neurophysiological investigation. Nottebohm et al. (1976) first identified the major brain nuclei responsible for the control of song. HVc (the high vocal center) sends a projection to RA (robust nucleus of the archistriatum), which in turn projects to the tracheosyringeal portion of the hypoglossal nucleus, and from there to the muscles of the syrinx, the vocal organ (see Figure 3). Subsequent work by Vicario (1991) demonstrated that the pathway from RA to the syrinx is myotopically organized, with different subregions of RA innervating different syringeal muscles. There are also additional projections from RA to the midbrain, and from there to hindbrain respiratory centers which are also intimately involved in the production of song (Wild, 1993). The syrinx is composed of several pairs muscles, which are intricately controlled during song generation (Suthers, 1990; Goller and Suthers, 1996). There are further intrinsic nonlinearities to the syrinx, such that simple variations in a few muscular control parameters can produce highly complex sounds (Fee et al., 1998). Lesions of HVc or RA result in immediate loss of singing ability or in substantial degradation of song structure (Nottebohm et al., 1976). This first song control pathway, from HVc through RA and to the vocal organ, is thus involved in the production of song.

Nottebohm et al. (1976) also observed a second pathway from HVc to Area X in the anterior neostriatum. Work by Arnold et al. (1976), Bottjer et al. (1989), and others demonstrated that this anterior forebrain pathway traveled from Area X, to the thalamic nucleus DLM, and then back to the anterior neostriatum to nucleus LMAN (lateral magnocellular nucleus of the anterior neostriatum). LMAN projects directly to RA, returning to the original song control pathway described by Nottebohm. A feedback pathway further links LMAN back to Area X (Vates and Nottebohm, 1995). Lesions of any of the nuclei in the anterior forebrain pathway in the juvenile bird have devastating effects on song learning (Scharff and Nottebohm, 1991). Lesions of these nuclei in the adult bird have few consequences for song production (Scharff and Nottebohm, 1991; Bottjer et al., 1984, Nordeen and Nordeen, 1993) unless a further manipulation is done which artificially induces the song to change. In these cases, lesions of nucleus LMAN prevent song deterioration (Williams and Mehta, 1999; Brainard and Doupe, 2000b). Finally, LMAN has been found to make NMDA synapses onto RA neurons, and disruption of these connections during song learning by infusing LMAN or RA with NMDA blockers results in the bird developing a highly abnormal song (Basham et al., 1996; Aamodt et al., 1996). Based on these observations, the second song control pathway, from HVc through the anterior forebrain and back to nucleus RA, is thus involved in song learning and song maintenance.

1.2 Summary of work

This thesis is composed of three major chapters; the first is concerned with the neural mechanisms underlying the generation of song structure, and the subsequent two with use of feedback by the neural control system to maintain song stability.

Chapter 2

In many models of neural population coding, similar sensory or motor states are represented in the brain by similar neuronal ensembles (Georgopoulos et al., 1999; Lewis and Kristan, 1998; Wilson and McNaughton, 1993). In Chapter 2, I explored this issue by measuring the activity of large numbers of single neurons in the pre-motor nucleus RA of the singing zebra finch. During singing, individual RA neurons generate precise bursts of action potential spikes (Yu and Margoliash, 1996). The active set of RA neurons at any time point in the song constitutes a neural ensemble. I found that highly similar song elements were typically produced by stereotyped but uncorrelated ensembles of RA neurons. Small changes in acoustic structure between two syllables were frequently subserved by entirely different patterns of RA neural activity, despite the strong correlations that are known to be present in the vocal muscles during the production of similar sounds. This provides an explanation for the behavioral observation that zebra finches learn syllable structure and sequence simultaneously (Tchernikovski et al, 2001), instead of first learning syllable primitives and then syllable order. There is no neural representation of syllable primitives in RA, such that similar sounds are generated by similar sets of neurons. Furthermore, the neural activity in RA often changed on a time scale an order of magnitude faster than the acoustic structure in the song, causing constant acoustic outputs to be produced by sequences of rapidly changing neural ensembles. Finally, the addition of neurons to the RA neuronal ensemble did not yield a steady increase in predictability about what type of sound was being generated. Most models of neural population coding follow a sum-of-the-parts principle, in which the individual neurons provide considerable information about what is being represented, and additional neurons provide progressively more information. The neural code used within RA is substantially different from these models, and individual neurons provide little unambiguous information about song structure. These observations suggest that within the neural control system of RA, network interactions are the dominant feature, and it is only by reading out sufficient activity to represent the entire state of the network that decoding is possible. The spike patterns observed in RA thus represent a new type of neural code underlying complex learned behaviors.

Chapter 3

Young birds learn to sing by using auditory feedback to compare their own vocalizations to a memorized or innate song pattern; if they are deafened as juveniles they will not develop normal songs (Konishi, 1965; Nottebohm, 1968). The completion of song development is called crystallization. After this stage, song shows little variation in its temporal or spectral properties. However, the mechanisms underlying this stability are largely unknown. In Chapter 3, I present evidence that auditory feedback is actively used in adulthood to maintain the stability of song structure. I found that perturbing auditory feedback heard during singing in adult zebra finches caused a slow deterioration of song. This "decrystallization" consisted of a dramatic loss of the spectral and temporal stereotypy seen in crystallized song, and included stuttering, creation, deletion and distortion of song syllables. After normal feedback was restored, these deviations gradually disappeared and the original song was recovered, indicating that a memory of the original song pattern remained stable, despite the destabilization of the behavior. Thus, the brains of adult birds that do not learn new songs nevertheless retain a significant amount of plasticity. Despite its seeming stability, song is maintained throughout the bird’s life by an active control process, and somewhere in the song control system there must be neurons that are sensitive to perturbations of auditory feedback.

Chapter 4

Nucleus LMAN of the anterior neostriatum is believed to be crucial for the auditory feedback-based plasticity seen in birdsong, and is thought to convey an error-correction signal to the motor control system based on the degree of match between the bird's vocalizations and a memorized song template (Brainard and Doupe, 2000a). In Chapter 4, I measured the activity of individual LMAN neurons while simultaneously manipulating the auditory feedback that birds heard during singing, thus controlling the level of error they could detect in their songs. LMAN neurons were found to produce spikes locked with millisecond precision to specific acoustic features in individual song syllables. This timing precision is comparable to that seen the motor control neurons that generate the song itself (Chi and Margoliash, 2001). Furthermore, using information theoretic methods, I show that perturbation of the auditory feedback heard by singing birds had no effect on LMAN spike patterns. There are four possible signals that could produce the song-locked spike trains observed in LMAN: an auditory feedback signal, a proprioceptive feedback signal from the vocal muscles, a motor command signal, or an efference copy. An efference copy is a record of the commands used to generate a motor output (Sperry, 1950; von Holst and Mittelstaedt, 1950; Bridgeman, 1995). Our results clearly show that auditory feedback does not generate the spike patterns in LMAN. There is no known afference from the syringeal muscles back to the song control system (Bottjer and Arnold, 1984), eliminating the possibility that LMAN spike patterns were driven by proprioceptive feedback. LMAN can be lesioned without effecting the production of song (Bottjer et al., 1984), indicating that it does not generate a motor command. Therefore, the only hypothesis that is consistent our measurements is that LMAN activity during singing is driven by an efference copy of the bird's song. No other signal could cause LMAN activity to have such a high degree of correlation to song structure while being immune to changes in auditory feedback. This conclusion represents a significant departure from the classical view of LMAN being primarily a processor of auditory feedback. The neural mechanisms of song learning and maintenance are substantially more complex than we have realized. Rather than being used in real-time, the influence of auditory feedback on song plasticity appears to occur offline, when the bird is not singing.

Taken together, these three projects significantly expand our knowledge of the neural mechanisms responsible for the generation of birdsong.