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A bird's eye view of neural circuit formation

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Abstract

Neural circuits underlying complex learned behaviors, such as speech in humans, develop under genetic constraints and in response to environmental influences. Little is known about the rules and mechanisms through which such circuits form. We argue that songbirds, with their discrete and well studied neural pathways underlying a complex and naturally learned behavior, provide a powerful model for addressing these questions. We briefly review current knowledge of how the song circuit develops during learning and discuss new possibilities for advancing the field given recent technological advances.

Introduction

Our understanding of neural circuit formation has advanced rapidly in genetic model organisms with well-defined innate circuits (e.g. retina [1] and spinal cord [2], see also Kiehn [3] and Fetcho, in this issue). This success owes much to the stereotypy of the specialized and highly evolved networks that, in turn, reflect the robust developmental programs underlying their organization. Given the predictable structure and function of these circuits, manipulations — genetic and otherwise — can be analyzed and interpreted as deviations from the norm. For circuits underlying complex learned behaviors things get murkier. They are often ill-defined and their development results from genetic programs interacting with the environment in ways that we may not fully appreciate. Yet getting a handle on how such learning circuits are formed is essential for understanding the development and neural basis of complex behaviors. Songbirds offer an exceptional opportunity for addressing this in an experimentally, behaviorally, and recently also genetically [4••,5••], tractable model system.

Like humans, songbirds have an innate predisposition for learning their vocalizations in a process that is subject to species-specific constraints and shaped by sensory experience [6]. Already a formidable model system for many branches of neurobiology [7], much is known about the structure of the discrete circuits underlying song (Figure 1). The picture emerging from this cumulative work is of a neural substrate that is, in a given species, as stereotyped and predictable as the behavior it implements, a prerequisite for evaluating the effects of various manipulations on circuit formation. Principles of how the circuit operates to implement the process of song learning are also emerging [8], allowing us to correlate form with function and meaningfully interpret the results of developmental perturbations.

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How are genetic constraints on learning and behavioral output instantiated in neural circuits? By what mechanisms does the environment and experience influence the organization of developing circuits underlying robust species-specific behaviors? The songbird has already contributed significantly to our understanding of these questions. Recent advances in our ability to modify the expression of targeted genes and deliver genetically encoded constructs for controlling and measuring neural activity will further increase the power and sophistication with which we can address how genes and environment interact in the formation and refinement of complex neural circuits.

This review has two main aims. The first is to highlight the songbird as a powerful model system for the study of neural circuit formation; the second is to review recent pertinent literature.

Quick tour of the song circuit and its development

There are over 4000 species of songbirds, each with its own constraint on song structure and the song learning process. This diversity presents an opportunity for comparative studies on how variations in the rules and mechanisms of circuit formation give rise to the diversity in behavioral outputs and learning trajectories [9]. Neurobiologists have barely begun to exploit this comparative richness, focusing mostly on one species, the zebra finch, by far the best studied songbird and the primary focus of this review.

Development of the song system in zebra finches involves a series of processes, many of which overlap to significant degrees (Figure 1c). We mainly focus on the sensorimotor phase of song learning and the formation of circuits involved in generating the learned motor output. We briefly review the main developmental milestones, and discuss recent work that adds mechanistic insight into how the song circuit is established. Readers interested in more in-depth treatment of the neural basis of song learning should consult some recent excellent reviews on the topic [8,10].

Behavioral outline of song development

Zebra finches are driven to sing — in community, in isolation, and even in the absence of auditory experience. Development of fully formed song requires exposure to conspecifics during a critical period in development. Juvenile birds listen to and memorize the song of a tutor, typically their father. This memory influences song development through processes not yet understood, although present data and models suggest a mechanism of trial-and-error learning [11,12,13•,14]: the bird explores a variety of song forms, and better than average matches to the memory of the tutor song get reinforced. During the initial phases of vocal practice (~25–50 days post-hatch, dph), the song takes a highly variable and unstructured form, called subsong, that lack any reproducible acoustic elements. This slowly evolves into plastic song, where recognizable units of sound — syllables —emerge, and after about two months of vocal practice, the song crystallizes into adult song: a stereotyped sequence of syllables that resembles the tutor song (Figure 2a).

Development of the song circuit

Early development

Only male zebra finches develop a functional song circuit, a sexual dimorphism in brain development that is under genetic, neural, and hormonal control [15]. The development of the song circuit starts in the embryo [16], and continues after hatching with neurons being added to the circuit (Figure 1), albeit at different pace in different nuclei. Lateral Magnocellular Nucleus of the Anterior Neostriatum (LMAN) and the Robust Nucleus of the Arcopallium (RA) have most of its neurons in place before song learning starts (25 dph)

[17,18]. Neurogenesis in HVC and Area X continues throughout life [17,19], although the rate of neuron addition slows dramatically after ~50 dph. Details of the intrinsic developmental programs that drive early wiring of the song circuit are scarce, though the specificity of the circuit suggests that these processes will be tractable subjects for investigation. In this regard, genes known from other systems to be involved in vertebrate circuit formation can be narrowed down by high-throughput functional genomics screens in zebra finches [20], to yield promising targets for genetic manipulations.

Transfer of song control from a basal ganglia circuit to a 'cortical' motor system

Vocal learning is associated with further development and refinement of the song circuit (Figure 2b). At the onset of song learning, HVC and RA, the cortex analogue nuclei that control song in adult birds, are connected only via the Anterior Forebrain Pathway (AFP), a basal ganglia loop (Figure 1). Even though the basic architecture of the AFP is established by 20 dph, it continues to develop during song learning, establishing topography [21] as well as auditory selectivity for the bird's own song [22] (for more on the development of the AFP see [23]). LMAN, the output nucleus of the AFP, innervates RA as early as 15 dph [24]. LMAN neurons show highly variable activity patterns from song to song [13•,12] and drive the motor program in RA during the subsong phase [25•].

LMAN projections to RA stay largely intact during sensorimotor learning and into adulthood [26], but LMAN's role in driving vocal variability diminishes with learning [14]. Since there is little change in the song-related firing patterns of RA-projecting LMAN neurons throughout song development [13•,27], this decrease in vocal variability is likely due to an increased influence of HVC on the motor program. If HVC, the premotor input to RA, is lesioned in adult birds, LMAN once again becomes the principal driver of the motor program, and vocal output reverts to unstructured sounds akin to juvenile subsong [25•].

HVC axons start making functional connections in RA at 30–35 dph [17,24] and continue to increase in number until ~50 dph [28]. These connections mature during development [29,28], and it is the experience dependent refinement of synaptic connections within the motor pathway that is thought to underlie the gradual convergence to imitated song sequences [11].

Thus early in song learning, the variable activity in the AFP drives vocal exploration required for trial-and-error learning. As learning proceeds, HVC gradually assumes control of the motor program. The extent to which this switch in control from the basal ganglia circuit to the motor pathway is driven by a developmental program and to what degree experience (auditory, social, motor practice, etc.) plays a role remains to be fully worked out.

Organization of the HVC circuit

In adult birds, the firing patterns of premotor neurons in HVC are very sparse and precisely locked to song [30]. Given that LMAN inactivation in juvenile birds (which leaves HVC as the dominant input to RA) results in song-locked RA firing patterns [14], it is plausible that HVC provides a stereotyped temporal sequence to RA already early in learning [31–33].

Models that produce HVC-like sequences consist of feed-forward networks in which activity is propagated from layer to layer, setting up a chain of synchronously firing neurons ('synfire' chains) [34]. While we do not yet know the precise connectivity in HVC, recent models indicate that spike-time dependent plasticity coupled with additional normalizing constraints on synaptic connectivity can lead to the emergence of networks with HVC-like properties [33,32]. If indeed the HVC sequences are self-organized without need for an instructive signal, then adaptive learning within the motor pathway may be restricted to the

sequential ordering of sub-sequences (corresponding to notes or syllables) within HVC [35] and to the fine-tuning of synaptic connection in RA.

Organization of the RA circuit

RA is a motor cortex analogue that is organized topographically with respect to vocal muscles, an organization that, crudely at least, is in place before sensorimotor learning starts [36]. Positioned at the confluence of inputs from premotor area HVC and the basal ganglia loop (AFP), RA is likely an important site of learning. The adaptive refinement of connections from HVC to RA, which transform the temporal sequence in HVC into motor commands for song-related muscles, has been modeled as 'synaptic reinforcement learning' [11]. In this scheme LMAN 'explores', by providing excitatory drive to RA neurons at random times, whether HVC-RA synapses active at those times 'improve' the song. This algorithm requires an evaluation signal to guide synaptic reorganization in RA. While no neural correlate of such a learning signal has been found, recent experimental work has refined our view of LMAN, showing that it can contribute an instructive signal to the motor pathway in the form of an adaptive bias in the structure of the variability it contributes to RA [37•]. It is conceivable that this bias alone is able to shape the HVC-RA synapses without reference to an additional evaluation signal. Going forward, it will be important to more closely examine the rules and mechanisms by which LMAN and HVC inputs combine to guide plasticity at the HVC-RA synapse.

Neural mechanisms of song circuit formation

Neural circuits underlying complex learned behaviors are shaped through the interaction between genetic programs and environmental influences. *Developmentally regulated gene expression* defines the basic neural architecture, which gives rise to dynamic patterns of *neural activity* that again influence form through a recurrent process (Figure 2c). At various stages of development, *environmental influences* come on-line and alter activity, refining neural connections. By experimentally perturbing environment, genes, or targeted circuit elements and mechanisms, we can assay the effects these factors have on the form and function of circuits, and dissect the mechanisms involved in complex circuit formation.

Hormones trigger rapid song reorganization

An important way in which genes, environment, and neural dynamics interact is through hormones. Hormone levels are developmentally regulated, but can also be modulated by a variety of factors including social events and the sensory environment. Androgens, in particular, have profound effects on the organization of the nervous system, including the development and maturation of the song circuit [38].

While the focus of this review is on zebra finches, some of the most dramatic demonstrations of androgen effects on song development have come from work in canaries. Exposing juvenile canaries to premature elevation of testosterone levels compresses sensorimotor learning to only a week or two, rather than the six month-long process typical in this species. The result of this artificially accelerated development is a canary song that contains fewer syllables than typical, but is otherwise structurally normal [39]. Similarly, female canaries that normally do not sing, rapidly develop a masculinized song systems and male-like songs after androgen exposure [40,41]. The rapid time-scale of the vocal transition precludes a significant role for neurogenesis [41].

This rapid testosterone-induced maturation of song largely eliminates the environmental component of vocal learning, providing an experimental means of examining how innate rules are expressed in the nervous system to produce species-typical complex behaviors.

Effects of genes and molecular pathways on circuit formation

The creation of the first germ-line transgenic songbird [4••], and other recent progress in avian transgenesis [42,43] is making the study of song circuit formation amenable to genetic perturbations. The first approach, which has already begun [44,45], involves over-expression or knock-down of gene products known to be involved in mammalian neural development. In the zebra finch, effects of these perturbations can be examined in detail through the advanced understanding of behavior and underlying neural processes.

Haesler *et al*. [44] used lentivirus-mediated RNA interference to decrease Foxp2 levels in Area X of juvenile birds, a manipulation that resulted in abnormally variable song that was a poor imitation of the tutor song. This phenotype can be compared with the language impairments that result from FOXP2 mutations in humans [46], an intriguing parallel that may open the path to understanding the link between basal ganglia dysfunction and vocal pathologies. One clue is offered by a recent follow-up study, revealing reduced spine densities in Area X as a result of FOXP2 knock-down [47].

Two other studies targeted retinoic acid, a molecule involved in cell proliferation and patterning of the early embryo. Interfering with normal developmental levels of retinoic acid either in HVC [45] or systemically [48], produced, as with the FOXP2 knock-down study, abnormally variable songs in adults. That all three studies produced essentially the same phenotype could be due to shared mechanisms — or not. A challenge for the future will be to refine song quantification methods to increase the specificity of phenotype measures, and to use this to address the functional causes that underlie disruptions in song development.

Effects of neural activity on circuit formation

Changes in neural activity can profoundly affect the development and formation of neural connections [49]. Given that song nuclei are anatomically distinct and easily targeted, numerous studies have perturbed their activity, mostly through lesions, pharmacology, or electrical stimulation. LMAN, in particular, has received much interest. The nucleus is essential for song learning, but the mechanisms by which its activity shapes the developing circuitry in RA are not understood.

Lesions of LMAN early in development (~20 dph) cause profound cell death in RA [50], presumably by depriving RA neurons of the BDNF released from LMAN terminals [51]. Later lesions (at 40–50 dph) do not cause RA cell death [50], but trigger significant changes in HVC-RA circuitry [29] and behavior: birds prematurely crystallize song, and the synaptic circuitry becomes adult-like, with fewer, but stronger synapses from HVC to RA. Infusing BDNF into LMAN in adult birds, on the other hand, causes an increase in RA synaptic density [52], and makes the song more variable, suggesting that BDNF is involved in regulating the plasticity and stability of the RA network.

To what extent normal AFP-generated LMAN activity is important for the maturation of HVC-RA synapses is not known, but this can now be tested by disrupting normal firing patterns of LMAN with viral or transgenic delivery of exogenous channels or by interfering with the function of the AFP through lesions or inactivations.

Another question concerns how activity in inhibitory networks affects neural circuit development. In mammalian sensory systems GABAergic circuits regulate the critical period for plasticity [53]. Yazaki-Sugiyama *et al.* [54] administered diazepam, a GABA agonist, systemically to juvenile zebra finches and demonstrated a premature closure of the sensory acquisition phase of learning, indicating that the mechanisms regulating the critical period are, to some extent at least, shared between birds and mammals. Sensitive period closure was associated with a premature increase of calretinin, a marker for inhibitory

interneurons, in HVC, suggesting that plasticity may be regulated by inhibitory networks in HVC. It will be interesting to examine whether the inhibitory circuits in HVC and elsewhere are affected also in response to other manipulations [55] that affect the critical period for sensory acquisition.

Effects of sensory influences on circuit formation

Another, more natural, means of affecting activity in the song circuit is by altering the auditory environment. Songbirds rely on auditory input to guide two critical phases of learning: memorization of the tutor song, and processing of auditory feedback during singing. Thus establishing how auditory inputs affect neural activity and ultimately circuit formation is important for understanding how instructive sensory signals shape neural circuits.

Deafening zebra finches early in life severely affects all aspect of song structure [56], yet the basic architectural framework underlying song, as assayed by the size of song control nuclei [57] and the topography within the AFP [58], remain unaffected. Recent studies have gone beyond the basic anatomy to trace the effects of auditory manipulations on the structure and function of the microcircuitry in the motor pathway.

Roberts *et al.* [59••] used two-photon time-lapse imaging of HVC neuron dendrites to examine how auditory experience affects the dynamics of spines. In their studies, juvenile zebra finches that had been acoustically isolated from a tutor had more spine turnover than normally tutored age-matched birds. Exposing these isolate birds to a tutor for the first time, led to rapid stabilization, accumulation and enlargement of dendritic spines. Moreover, *in vivo* intracellular recordings made immediately before and after the first day of tutoring also revealed robust enhancement of synaptic activity in HVC, consistent with strengthening of synaptic input.

Thus the neural activity triggered by tutor song exposure stabilizes the microcircuit in HVC. Interestingly, the rate of spine turnover in HVC at the time of first tutor exposure was positively correlated with how well birds learned to imitate, suggesting that juveniles with more labile and functionally weaker synapses are better learners. These findings are consistent with studies in mammals showing that critical period for sensory map formation is associated with increased spine turnover [60]. However, at 90 dph, coinciding with sexual maturation, there was no longer any difference in spine turnover between tutored and untutored birds; the older isolates were equally poor at learning from a tutor, indicating that both intrinsic and experience-dependent mechanisms contribute to sensitive period closure. In normally reared birds there was also no significant difference in HVC spine dynamics between days 45 and 90. The ‘adult-like’ stability in HVC spine dynamics already early in the sensorimotor learning phase is consistent with the HVC network being shaped, to a large extent, prior to onset of vocal practice [11].

Delayed tutoring also brings about changes in the downstream nucleus RA, as assayed by the activity patterns of RA neurons during sleep [61•]. A significant increase in the spontaneous burstiness of RA projection neurons is seen on nights following the first tutoring session of previously isolated juvenile birds. To what extent this change in neural activity is driven by changes in the HVC circuit [59••], an increase in LMAN burstiness, or whether exposure to tutor song triggers synaptic reorganization in RA, is not known.

Hearing a tutor song may also trigger plasticity in the AFP, as evidenced by increased phosphorylation of Cam-KII in Area X [62]. Curiously, the rate of phosphorylation was significantly higher if the bird had already been exposed to the tutor compared to when it heard it for the first time.

Thanks to new resources available for analyzing zebra finch genes and proteins [63], we can now examine how sensory stimuli and other behavioral events impact gene expression. Dong *et al.* [64] examined the expression of genes in zebra finches that were exposed to new songs, and saw that this sensory event increased expression of thousands of genes in the auditory forebrain alone. A similar study screening for genes regulated by singing yielded more than 800 hits [5••]. The breadth of gene expression changes highlights both the state dependence of cells in the brain, and also the inherent challenges in translating these high-throughput data sets into a deeper understanding of how neural systems function.

Conclusions

Revealing the principles of neural circuit development in the vertebrate CNS is one of the great challenges for present day neuroscience. The process involves the dynamic interplay between gene expression, neural activity, circuit geometry, and environment, to build the most complex information processing system we know of. Even very small variation in genetic make-up or environmental structure can significantly alter this assembly process and produce severe mental disorders, and also explain rapid evolutionary events leading to qualitative transitions in the capacities of the nervous system (e.g. humans vs chimps).

Advances in genomics and transgenesis promises accelerated returns in understanding the mechanics of circuit formation, but a careful choice of animal models must be made. Establishing experimental paradigms for understanding the neurobiology of complex learned behaviors has been particularly difficult. Attempts to do so in the mouse, arguably the most powerful vertebrate genetic system, have had limited success. Yet the need for progress is pressing. Complex mental disorders, such as schizophrenia and autism, are recognized as disorders of brain development [65,66], having both genetic and environmental risk factors, yet very little is known about the neurobiology of the underlying pathologies. Other phenotypes, such as deficiencies in speech and language production caused by FOXP2 mutations [46], will also be hard to understand without a more detailed understanding of the underlying neural mechanisms, including basal ganglia-cortical dynamics, the role of sleep, and the function of neuromodulatory systems.

In this review, we have argued for the songbird as a powerful model system for addressing how genes and environment interact in the formation of complex neural circuits, reasoning that the stereotyped behavior, the dedicated and well delineated circuits for singing, and the increasingly well understood brain dynamics, form a unique base from which to examine effects of various developmental and circuit manipulations.

Another feature which makes the zebra finch stand out among current animal models for learned behaviors is the nature of information transfer within the research community. Experiments on zebra finches are performed in relation to a very specific behavior, where interpretation is not subject to variations in experimental design. The learning trajectory and experimental paradigm is innate, occurring spontaneously in the laboratory much as it happens in nature. Thus results from labs pursuing very different questions add to the absolute understanding of one underlying process, namely how the bird learns and generates its song.

This cumulative effort has made the song circuit of zebra finches one of the best understood neural circuits underlying a complex learned behavior. Perturbations to genes, environment, and neural activity can now be read out through phenotypic changes to stereotyped behavior, circuit structure, and physiology. Whether the goals are framed in terms of understanding the interplay of nature and nurture in circuit formation, or examining the neural mechanisms underlying vocal learning, songbirds offer a great entry point.

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Figure 1.

(a) The zebra finch is the experimental system of choice for neuroscientists interested in a wide range of phenomena, making its vocal control system arguably the best understood neural circuit implementing a complex learned behavior. **(b)** Schematic diagram of the main neural pathways comprising the song circuit. The descending motor pathway (red) controlling the learned song is comprised of HVC (proper name) and the Robust Nucleus of the Arcopallium (RA), two interconnected cortical analogue nuclei, as well as brainstem nuclei that control the avian vocal organ (the syrinx) and respiratory function. Song learning also requires the Anterior Forebrain Pathway (AFP), a circuit homologous to mammalian cortico-basal ganglia-thalamo-cortico loops. Sensory input and efference signals close the sensorimotor loop through various feedback circuits (green). For a more complete circuit diagram please see [7,67]. Other abbreviations — DLM: dorsolateral nucleus of the medial thalamus; DM: dorsomedial intercollicular nucleus; Uva: nucleus uvaeformis of the thalamus; Nif: nucleus interfacialis; Av: Avalanche; nXIIts: the tracheosyringeal portion of the twelfth cranial nerve; VRG: ventral respiratory group. **(c)** Basic timeline for song circuit development.

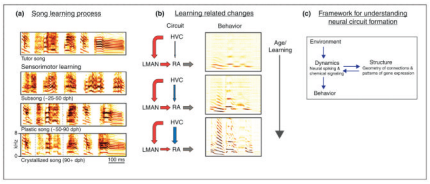

Figure 2.

Song learning is correlated with changes in the underlying circuits. **(a)** Environmental exposure to a tutor defines a template to be imitated. Over the course of sensorimotor learning the juvenile bird's song approaches that of its tutor. **(b)** A hallmark of the song learning process is a gradual increase in the stereotypy of the song. (right) Time-frequency distributions were calculated [68] at three different time points (51, 57, and 90 dph) for one thousand renditions of an identified syllable (delineated with dashed lines in (a)) and superimposed. Amplitude information was removed prior to superposition, resulting in a measure of probability density in time and frequency. Over the course of development, distinct high contrast regions emerge, indicating a progressive increase in stereotypy. (left) This developmental change is accompanied by a reorganization of the song circuit (blue and red arrows denote the motor and anterior forebrain pathways respectively; see Figure 1b). **(c)** Insights from the different levels of analysis can be combined into a common framework for understanding the dynamic process of circuit formation.