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REVIEW

Gene manipulation to test links between genome, brain and behavior in developing songbirds: a test case

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ABSTRACT

Songbird research has made many seminal contributions to the fields of ethology, endocrinology, physiology, ecology, evolution and neurobiology. Genome manipulation is thus a promising new methodological strategy to enhance the existing strengths of the songbird system to advance and expand fundamental knowledge of how genetic sequences and regulation of genomic function support complex natural learned behaviors. In zebra finches (*Taeniopygia guttata*) in particular, a rich set of questions about the complex process of developmental song learning in juvenile males has been defined. This Review uses one area of zebra finch song learning to demonstrate how genome editing can advance causal investigations into known genome–brain–behavior relationships. Given the number and diversity of songbird species, comparative work leveraging genome manipulation would expand the influence of these birds in additional fields of ecology and evolution for song learning and other behaviors.

KEY WORDS: Zebra finch, Critical period, Sensitive period, Learning and memory, CRISPR, Brain development

Introduction

Genetic manipulation is a powerful tool for elucidating processes by which regulated genome function influences biology. It is especially valuable when deployed in models with questions ripe for causal inquiry. Songbirds are one such animal model.

Songbirds, order: Passeriformes, suborder: Oscine, have a long history of ethological and neurobiological discoveries. Behavior is the obvious target for genetic manipulation in songbirds, which are half of the ∼10,000 extant species of birds in the world. All songbirds share the ability to learn song, but they also represent a great diversity: they occupy nearly every ecological niche from the tropics to the poles, they have a medley of behaviors that accompany a variety of life histories and social structures, and they display a range of song-learning strategies. For example, there are songbirds that learn once and those that learn continuously, some species that show a sex difference in singing and some in which both males and females sing, some that require social interactions to learn song and those that can acquire vocalizations in non-social situations, even from inanimate objects. Collectively, songbirds therefore provide a rich biological substrate for building mechanistic interrelationships between chromatin, brain and behavior (Clayton et al., 2009; Murphy et al., 2017).

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The technical capacity to perform effective genetic manipulation is nascent in songbirds; thus, this short review describes major elements that need to come together to meaningfully apply genetic manipulation to causal questions of brain and behavior. The focus will be on a ‘case study’: song learning in one songbird species, the zebra finch (*Taeniopygia guttata*), centered on an extreme trait because it provides an experimental framework to build a comprehensive set of data linking chromatin, brain and behavior. The idea is to use this line of research as an example for how genetic manipulation can be applied to more diverse questions of songbird behavior. The Review is organized into five elements that combine to enable effective genetic manipulation of songbird behavior: (1) defining a behavioral question of song learning; (2) identifying the underlying brain substrate; (3) linking behavior to chromatin to identify potential targets for gene editing; (4) devising an effective strategy to deliver genetic manipulation constructs; and (5) manipulating genes to test the contribution to brain function and behavior.

Defining a behavioral question of song learning

Types of learning involved in song acquisition

For song, there are three types of learning that each contribute to the ultimate performance. (1) Sensory learning is how an individual acquires behavioral patterns from the environment. It is the process of forming a memory of the ‘tutor’ bird or object that the individual’s vocalization will then emulate. Sensory learning is the foundation of the song structure. Individual birds are capable of making a variety of sounds in various order; the precise structure emerges largely from the ‘template’ memory formed via sensory learning. (2) Sensorimotor learning is how the individual re-shapes its own starting vocalizations to match more accurately the memory of the ‘tutor’ song that it is copying. It is accomplished via an ‘error correction’ process that involves comparison of the vocal output with the tutor template. (3) Motor learning results in the highly stereotyped production of song across renditions, via many iterations of rehearsal that fine-tune the motor pattern. Ultimately, the final song structure of a songbird reflects the combination of all of these learning elements.

Song learning in zebra finches

There are many reasons why zebra finches have emerged as a commonly studied songbird. Some are practical. Zebra finches, which are native to vast stretches of arid Australia, live and breed well in lab colonies, and they retain their natural behavioral repertoires in captivity (Zann, 1996). Zebra finches are capable of breeding all year because their reproductive cue is rain, not day length, and they are robust to manipulations. The multitude of contributions they have made to physiology, neurobiology, endocrinology, genomics and behavior is due in large part to their physiological robustness and experimental tractability (Clayton et al., 2005; Drnevich et al., 2012; Replogle et al., 2008).

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Studies using the zebra finch have launched several valuable lines of inquiry. For example, work with zebra finches uncovered novel mechanisms by which the neural song system for sensorimotor and motor learning is organized differently in males and females (males sing, females never sing; Agate et al., 2003; Burek et al., 1994; Gahr and Metzdorf, 1999; Jin and Clayton, 1997; London et al., 2009b; Simpson and Vicario, 1991; Wade and Arnold, 2004), revealed how shifts in anatomical connections explain transitions between innate and learned vocalizations (Aronov et al., 2008), found molecular mechanisms underlying the process of sensorimotor error correction (Mori and Wada, 2015b; Nordeen and Nordeen, 2004), and informed on how context manifests in subtle yet meaningful alterations in stereotyped motor production (Gadagkar et al., 2016).

One extreme feature of zebra finch song learning is a 'critical period'. Critical periods are restricted life phases when a specific experience – which outside this time would have little to no effect – has profound and lasting effects on a particular brain system and patterns of resulting behavior (Knudsen, 2004). Male zebra finches sing all day every day, and they are part of a colony composed of hundreds of birds (Zann, 1996). Thus, availability of a potential tutor cannot explain why sometimes juvenile males can acquire song and other times they cannot. Instead, properties of the brain must be changing such that the ability to learn from these singing males switches, essentially to 'on' and then 'off'. As such, the fluctuations in learning ability across the critical period present a framework for investigations aimed at identifying potential genetic manipulation targets to test neural properties that promote or limit the ability to learn, using the sex, age and effects of prior experience to examine birds in distinct, naturally occurring states of learning.

Focusing on the type of learning defined by critical period fluctuations

The sensory, sensorimotor and motor learning processes that combine to support song occur in distributed, specialized nodes of a neural network (Fig. 1). To take advantage of the natural switches in the ability to learn for mechanistic investigations, it is therefore important to determine which type of learning is regulated by critical period mechanisms so that the appropriate brain areas are targeted with genetic manipulation. It is likely that it is tutor song memorization, the sensory learning component of song acquisition that undergoes critical period fluctuations in learning function, rather than sensorimotor and motor learning processes.

Notably, critical period learning ends because of prior experience, not age. Juvenile males exposed to an adult male tutor between the ages of post-hatching day (P)30 and P65 were no longer able to incorporate elements from a second tutor after P65. In contrast, isolated males ('isolates') prevented from hearing song during that phase, even when they lived with females, which produce calls that have song syllable-like acoustic structure (Fig. 1), can learn the song of a tutor male presented after P65 (Eales, 1985, 1987; Morrison and Nottebohm, 1993). Notably, male isolates produce innate, song-like vocalizations even in the absence of tutoring, and start vocalizing at the same age as tutored males, sing at the same rate as tutored males, and show no overt systemic

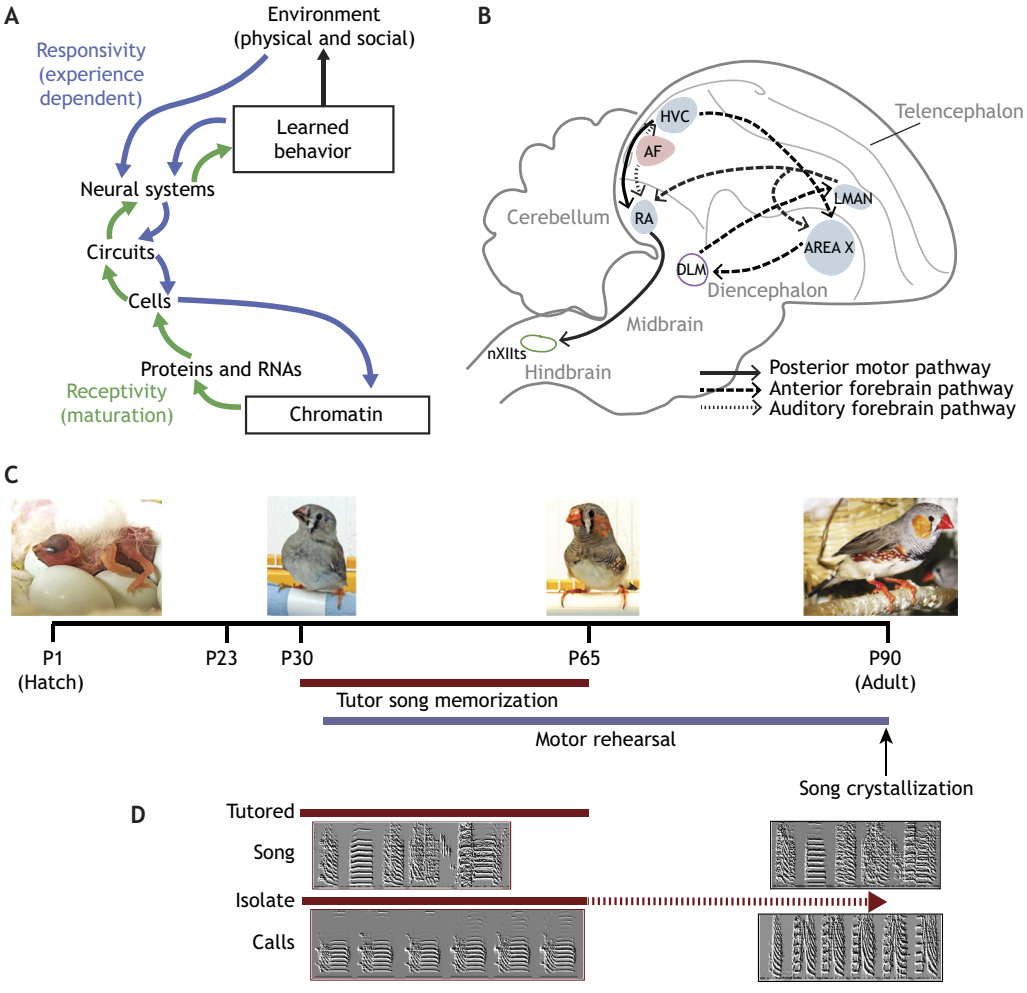

Fig. 1. Connections between genome function, brain and behavior in the context of developmental song learning in the zebra finch. (A) Conceptualization of chromatin as the biological unit connecting maturational processes of brain 'receptivity' (intrinsic properties) and experience-dependent mechanisms of 'responsivity' that together give rise to learned behavior. (B) Schematic diagram of a sagittal section through an adult male zebra finch brain showing major components of the neural network required for learned song. Light blue nodes are telencephalic areas essential for sensorimotor and motor learning; light red is the auditory forebrain (AF). (C) Timeline of zebra finch development post-hatching, with photos showing males at four ages (post-hatching day, P), and the relative positioning of tutor song memorization and motor processes. (D) Males that experience song (tutored condition) between P30 and P65 cannot memorize an additional song after P65 and as adults sing a crystallized song. In contrast, 'isolates' – males prevented from hearing song during this phase – even if they are exposed to a female's calls, display an extended age for tutor song memorization, but produce abnormal song-like vocalizations even without tutor experience.

alterations in hormone physiology compared with tutored males, and no significant alterations in the patterns of gene expression in sensorimotor and motor components of the neural song system in comparison to normally tutored males. These findings hold even for deafened males (Mori and Wada, 2015a). These behavioral observations indicate that tutor experience has no direct effect on the function of motor components of the neural song network, but does change whether or not sensory learning of tutor song occurs. In short, critical period mechanisms likely regulate tutor song memorization, and can be leveraged to investigate neural and genomic properties that control it.

Identifying sensory learning brain areas to target with genetic manipulation

Identifying the neural locus for tutor song memorization is advantageous because it can be manipulated without directly affecting the brain areas for sensorimotor and motor components of song (Fig. 1). Further aiding neuroanatomical localization of manipulations in songbirds is that brain areas are visible with the naked eye, increasing the reliability, consistency and interpretability of measures and manipulations. To test the effects of genetic manipulations specifically on the ability to memorize tutor song, it is thus important to neuroanatomically localize sensory song learning.

Tutor song memorization is not mimicry resulting from simple repetition and dependent solely on primary auditory perception. For instance, passive playbacks are not effective for tutor song copying (Derégnaucourt et al., 2013). Further, social interactions coincident with hearing song, even if the contact is not with the tutor himself, enhance the fidelity of tutor song copying (Adret, 2004; Böhner, 1983; Clayton, 1987; Derégnaucourt et al., 2013; Mann and Slater, 1995). In combination with the fact that complex vocalizations such as song, but not acoustically similar yet simpler calls (Fig. 1), end the critical period, higher-order sensory association areas were predicted to be essential for tutor song memorization.

Indeed, based initially on induction properties of immediate early genes such as *egr-1* (also called *zif268*, *ngfi-a*, *krox24* and ZENK) in adults, two higher-order processing regions of the auditory forebrain, the caudomedial nidopallium (NCM) and caudal mesopallium (CM), emerged as likely candidates for loci of tutor song memorization (Mello et al., 1992, 2004; Mello and Clayton, 1994; Vignal et al., 2005; Phan et al., 2006; Woolley and Doupe, 2008; Gobes et al., 2010). The essential role for genomic function in the auditory forebrain was then established by demonstrating that juvenile males could not copy tutor song if *egr-1* transcription in the auditory forebrain was disrupted during their tutor experience (London and Clayton, 2008). These findings have been confirmed with other methods (Yanagihara and Yazaki-Sugiyama, 2016); the auditory forebrain, and NCM and CM specifically, are thus key loci for genetic manipulation aimed at testing neural mechanisms of sensory song learning.

Linking sensory song learning to chromatin to identify specific genomic targets for editing

Learning and memory depend on genomic function; experience-dependent transcription and translation are required for the formation of long-term memories across taxa (Kandel, 2001). In the case study here, the key is to identify which auditory forebrain genomic elements influence the ability to memorize tutor song across the critical period transitions. This includes mechanisms that permit coordinated transcription upon tutor experience, or 'responsivity' processes, and characteristics that set intrinsic properties of brain cells, which determine how they work alone and together to create a 'receptive' neural circuit capable of responding to experience in the first place (Fig. 1). Considering both intrinsic and experience-dependent mechanisms is especially relevant because zebra finch song learning occurs across development.

cDNA spotted microarrays provided the first evidence that genomic regulation within the auditory forebrain changes across the critical period for tutor song memorization (London et al., 2009a). Within 30 min of adult males hearing song playbacks, hundreds of transcripts either increased or decreased in abundance in the auditory forebrain, compared with levels in males left in silence. Those same transcripts regulated in adults were present at high levels even in the silence condition in P20 males, and they were not changed after P20 males heard song playbacks. Additionally, nearly 1000 transcripts were expressed at different levels when baseline, silence profiles were compared between P20 and adult auditory forebrain. Each of these transcripts is a potential target for functional testing. However, subsequent studies have selectively parsed genomic process associated with intrinsic 'receptivity' and experience-dependent 'responsivity' mechanisms, using the switches in learning across the critical period as an experimental framework to identify more specific targets for genetic manipulation studies.

Identifying targets to test neural mechanisms promoting onset of tutor song memorization

The general prediction for processes that promote tutor song memorization is that they will be responsive to hearing song at, but not prior to, the age of critical period learning onset. The target of rapamycin [TOR, termed the mechanistic target of rapamycin cascade (mTOR) in mammals and birds] molecular cascade was a plausible mediator of this transition. mTOR signaling is initiated in learning and memory contexts in other animals, and occurs after convergent activation of multiple receptor types and signaling cascades, suggesting it may be particularly central in complex learning situations (Hoeffer and Klann, 2010). Increased mTOR signaling is also implicated in the neurodevelopmental autism spectrum disorders characterized by social and communication deficits (Sato, 2016). Further, a branch of the mTOR cascade controls the initiation of protein synthesis by regulating the phosphorylation of ribosomal protein S6, a requisite component of the 40S subunit of the eukaryotic ribosome (Biever et al., 2015; Gressner and Wool, 1974; Hoeffer and Klann, 2010).

To test whether mTOR signaling was consistent with the general prediction, young males at the onset of the critical period for tutor song memorization (P30) and 1 week prior (P23) were either played songs or left in silence (Ahmadiantehrani and London, 2017a; Roper and Zann, 2006). The density of cells containing phosphorylated S6 (pS6) in NCM and CM was quantified, and compared between song and silence conditions. If hearing song had no effect on mTOR activation, the pS6 density would be the same in song and silence conditions. Indeed, this was the case at P23 (Fig. 2B). At P30, however, males that heard song had a significant increase in the number of cells with pS6, and thus experience-dependent protein synthesis. Additionally, hearing song did not increase pS6 cell density in either P23 or P30 females, which do not sing, and the sex difference was absent in adults, when the auditory forebrain functions equivalently for song recognition learning in males and females (Ahmadiantehrani et al., 2018). These findings are consistent with the conclusion that there is a sex difference in tutor song memorization mechanisms and raise the question of how

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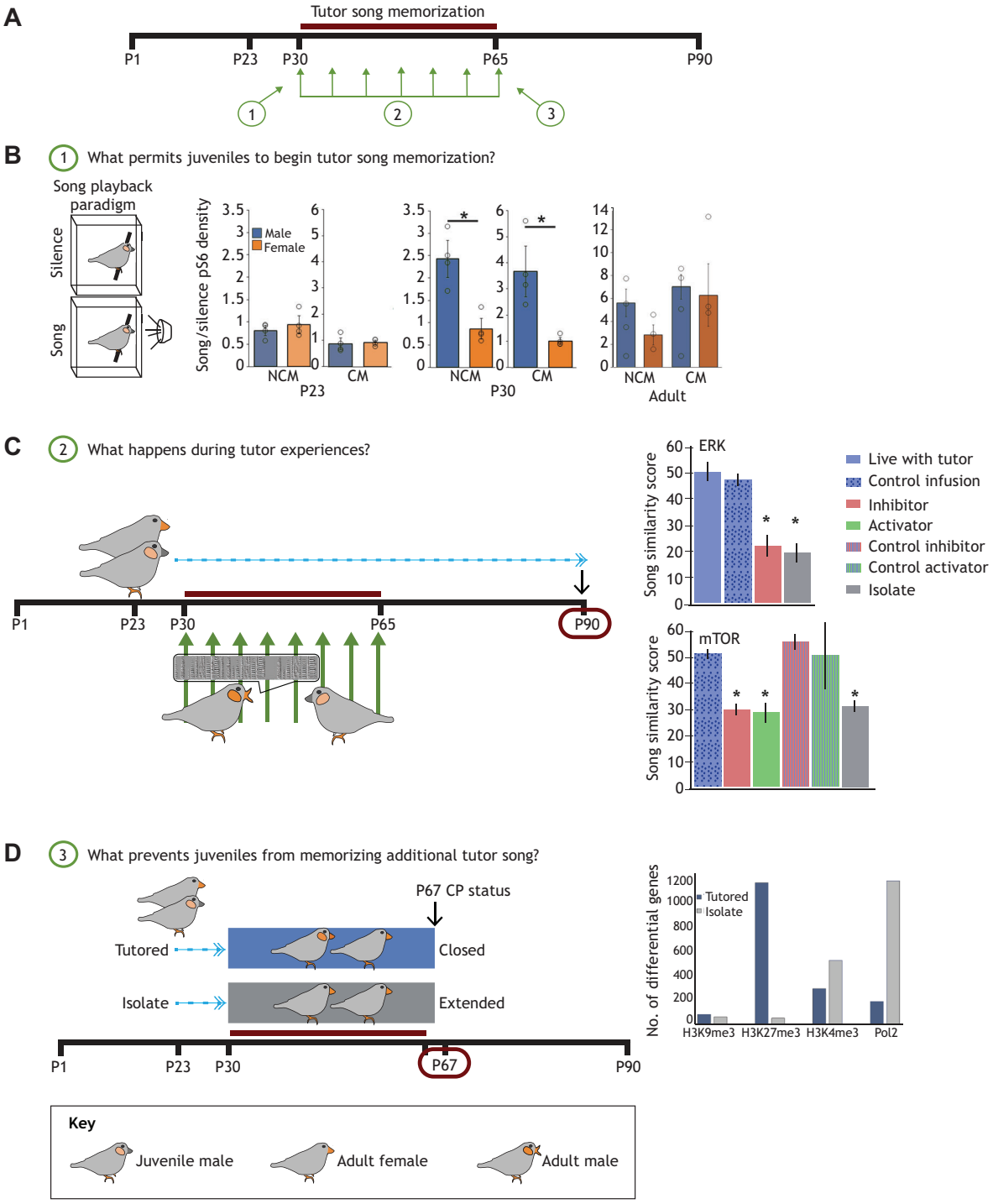

Fig. 2. Potential targets for genetic manipulation in this case study emerging from the 'critical period' framework testing genomic properties of tutor song learning. (A) Timeline showing the relative position of the onset and offset of critical period transitions where tutor song memorization is promoted (1) or limited (3), and when mechanisms of tutor song memorization can be assayed (2). Vertical green arrows (here and in C) indicate multiple tutor experiences. (B) To discover a mechanism that promotes tutor song memorization, levels of pS6 in the caudomedial nidopallium (NCM) and caudal mesopallium (CM) were compared between song playback and silence conditions at P23 and P30, and in adults. Both males and females were examined. The only age at which there was a sex difference in pS6 density was P30, and at this age only males showed a molecular response to hearing song. (C) To test whether genomic activation was required for tutor song memorization, controlled tutor song experience in combination with drugs that interfere with either ERK or mTOR cascade activation was deployed. If ERK or mTOR signaling is disrupted during tutor experience (green and red bars), then males grow up to sing songs that are no more similar to the tutor's song than an isolate's is (gray bars), i.e. significantly less than control birds (blue bars). *P<0.05. Dashed blue arrows (here and in D) indicate the time period during which the juvenile male lives with an adult female. (D) To determine whether there is an epigenetic consequence of accumulated tutor experience on the end of learning ability, males were reared in either tutored (one adult male, one adult female) or isolate (two adult females) conditions until P67, when auditory forebrains were sampled for signatures of repressed and active chromatin. Tutored (blue bars) auditory forebrain had more genes associated with repressive histones (H3K9me3, K3K27me3) whereas isolate (gray bars) auditory forebrain had more genes associated with chromatin signatures of active chromatin [H3K4me3, RNA Polymerase 2 (Pol2)].

genomic regulation of brain properties in males and females may contribute to differences in behavioral learning.

These results revealed that mTOR cascade activation reflects and possibly supports a process that initiates the ability for tutor song memorization, perhaps by synthesizing new proteins required for cellular plasticity underlying long-term memory formation. Individual components of the mTOR cascade that initiate protein synthesis, and genes coding for the proteins synthesized in response to song experience in males of the age that can memorize tutor song are therefore obvious targets for genetic manipulation. Additionally, it is possible that differential activation of mTOR signaling, and therefore pS6, results from distinct populations of membrane receptors in males and females at different ages. These receptor proteins are another informative target for future genetic manipulation studies to test the functional role of individual cellular components rather than molecular cascades.

Additionally, the cells positive for pS6 after song experience were not evenly distributed throughout the NCM and CM in P30 males (Ahmadiantehrani and London, 2017a). Instead, they appeared in clusters that did not follow major anatomical boundaries. This suggests the possibility that these are memory 'ensembles'. Ensemble cells may have distinct intrinsic properties that predispose them to participate in memory processes, and which therefore have predictive power for learning (Cai et al., 2016; Liu et al., 2012; Ramirez et al., 2013). In addition to the experience-dependent mechanisms triggered by hearing song, manipulating more stable genomic elements that characterize cells such as accessible enhancer regions could elucidate cell types that participate in learning and memory, thereby connecting genomic regulation of receptivity and responsivity in learned behavior.

Identifying targets to test genomic mechanisms required for tutor song memorization

Because experience-dependent transcription and translation are required for memory formation, it seemed likely that tutor song memorization required activation of molecular mechanisms that regulated these processes in the auditory forebrain during tutor experience. This hypothesis has been tested for extracellular signal regulated kinase (ERK) and mTOR cascades. ERK activation regulates transcriptional processes, complementing the protein synthesis regulated by mTOR (Cheng and Clayton, 2004; Whitmarsh, 2007). Disruption of either ERK or mTOR signaling in the auditory forebrains of juvenile males when they were interacting with a tutor, but not at times when a tutor was not present, prevented normal levels of tutor song learning (Fig. 2; Ahmadiantehrani and London, 2017a; London and Clayton, 2008). These data indicate that the levels, and possibly the diversity, of RNAs and proteins that are synthesized during tutor experience are essential for tutor song memorization, and are obvious targets for causal testing via genetic manipulation.

Identifying targets to test neural mechanisms that limit tutor song memorization

One of the experimentally valuable components of a critical period is that learning ends as a result of prior experience. Thus age-matched individuals which differ in their tutor experience can be compared to elucidate learning mechanisms that are independent of aging, and identify a set of genomic elements that limit tutor song memorization.

Epigenetic modifications were a plausible mediator of critical period closure because they are relatively stable, regulated by experience, and coordinate transcription of sets of genes (Allis and Jenuwein, 2016; Gibney and Nolan, 2010). Chromatin immunoprecipitation followed by DNA sequencing (ChIPseq) for epigenetic markers revealed that auditory forebrain cells had more genes associated with repressed chromatin in males that had been tutored compared with isolates that had been reared with non-singing females, and more genes associated with active chromatin in isolates compared with tutored males; tutored males can no longer memorize tutor song after P65 but isolates can (Fig. 2; Kelly et al., 2018). These data indicate that more regions of the genome can be transcribed in the isolate than in the tutored auditory forebrain, consistent with the fact that new transcription would be needed for tutor experience to support delayed tutor song memorization in male isolates. Further, gene ontology analysis suggested that differentially regulated genes were functionally associated with transcriptional and translational control, setting up a kind of loop wherein chromatin-level regulation of the transcription of genes involved in transcription and translation is distinct in isolates, which can memorize tutor song, versus tutored males, which cannot. Interestingly, the epigenetic modifications that distinguished tutored and isolate auditory forebrains were not the same as those that characterized male auditory forebrain cells at different ages, opening the possibility of manipulating genomic regions for maturational and experience-dependent properties separately (Kelly et al., 2018).

Devising an effective strategy to deliver genetic manipulation constructs

Genetic manipulation relies on an efficient and effective method to deliver transgene constructs. In zebra finches, there have been some notable successes in delivering manipulation constructs, but this step has historically been a methodological bottleneck, slowing the rate of discovery and restricting the diversity of possible questions to investigate using these animals. Some recent advancements have, however, have enhanced the feasibility of moving forward with genetic manipulation.

Viral infection is the most ubiquitous delivery strategy for genetic manipulation employed in songbirds. For example, lentivirus-mediated delivery was used to generate multiple lines of GFP transgenic zebra finches, CREB transgenic lines and a Huntington gene transgenic line (Abe et al., 2015; Agate et al., 2009; Liu et al., 2015). Unfortunately, the efficiency of creating these lines is often prohibitively low (of the order of 1 viable chick per 150 attempts), especially when a research program is not restricted to studying a single type of genetic manipulation.

An alternative to transgenics is to use viral delivery locally to deliver manipulation constructs to specific brain areas. Lentivirus and adeno associated viruses (AAVs) have been effectively used to knock down individual genes to demonstrate their involvement in song behavior, to express designer receptor exclusively activated by designer drugs (DREADDs) or optogenetic channels to manipulate neural cell firing, and to label cells to make them accessible for tracing and targeted electrophysiological recording (Bauer et al., 2008; Dimidschstein et al., 2016; Haesler et al., 2007; Heston et al., 2018; Xiao et al., 2018; Yip et al., 2012). Even with localized delivery, however, viral infection is not always reliable across birds, labs and constructs, perhaps because of songbird immune system properties (Haesler et al., 2007; Heston and White, 2017; Schulz et al., 2010; Warren et al., 2010; Yip et al., 2012).

In vivo electroporation side-steps issues of viral infection and is highly effective for transgene delivery in zebra finches (Fig. 3; Ahmadiantehrani and London, 2017b). Electroporation takes advantage of the fact that DNA is negatively charged and that

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electrical current disrupts cell membranes so that plasmids can enter cells. Specific brain areas can be targeted by positioning the anode and cathode paddles to pass current in the intended direction. Further, it is possible to co-electroporate multiple constructs simultaneously, and cargo size for effective plasmids is large (up to 2000 kb); thus, the system permits flexible and complex strategies (Woodard and Wilson, 2015). Because songbirds are altricial, the procedure can be performed on post-hatching chicks, which show >95% survival, and of these >95% show transgene expression (Fig. 3; Ahmadiantehrani and London, 2017b). Addition of a transposase such as piggyBac effectively integrates cargo into the genome, and brain cells then express the transgene through to at least P50, permitting genetic manipulation in the context of questions of maturation and tutor song memorization (Ahmadiantehrani and London, 2017b). Of note, piggyBac transposase-integrated transgenes can also be excised from the genome (Woodard and Wilson, 2015). Especially for developmental questions, electroporation is therefore a promising alternative to the more common viral strategies to deliver genetic manipulation constructs.

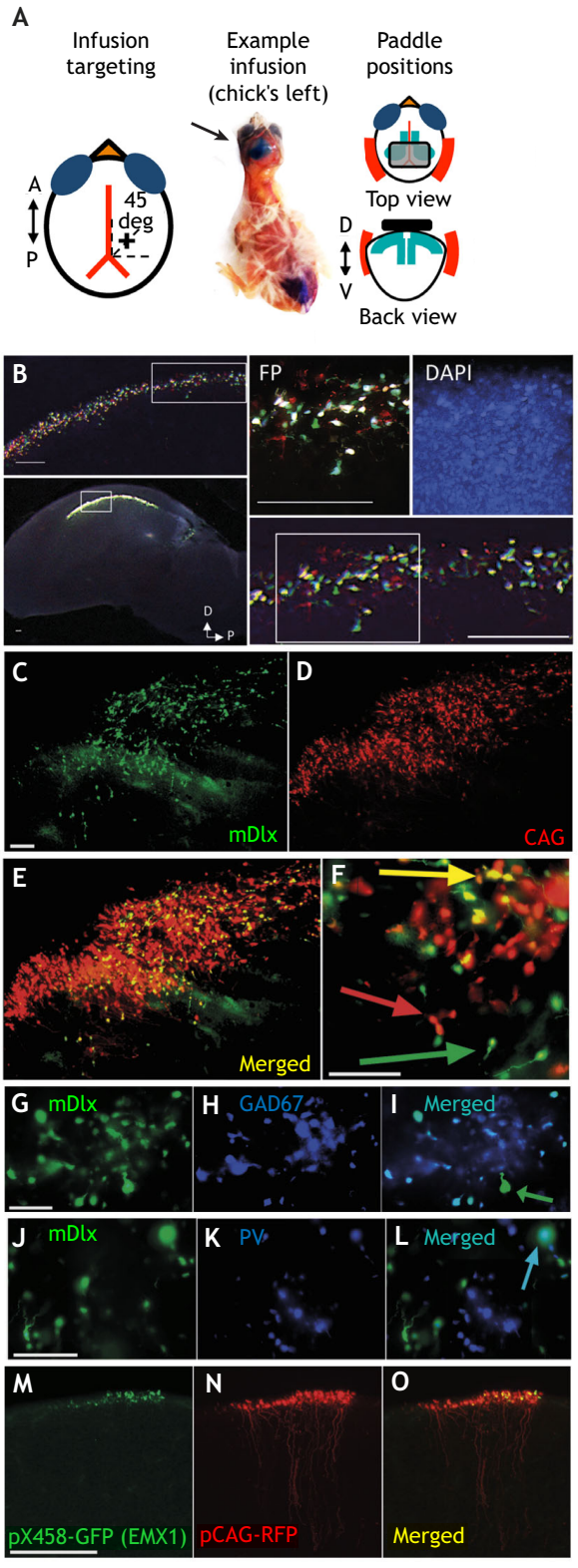

Fig. 3. Promise of electroporation for delivery of ubiquitous and cell type-specific transgene constructs, including CRISPR gRNAs.
(A) Schematic diagrams showing the basic strategy for brain electroporation in chicks post-hatching. A, anterior; P, posterior; D, dorsal; V, ventral.
(B) Electroporation with a mix of RFP, GFP and CPF leads to robust expression of fluorescent proteins, here clustered around the lateral ventricle before they migrate further into the auditory forebrain. Scale bars: 500 µm. (C–F) Co-electroporation with constructs driven by enhancer-specific promoters (C, mDlx-GFP) and ubiquitous promoters (D, CAG-RFP) leads to effective expression from enhancers, but only in a subset of cells, as expected. Scale bars: 100 µm. (G–L) Immunohistochemistry after electroporation with mDlx-driven GFP shows high co-labeling with GAD67 (H,I), a marker for inhibitory cells, but little co-labeling with parvalbumin (K,L), a marker of a subset of inhibitory cells, demonstrating selectivity of enhancer sequences in identifying cell subtypes. (M–O) CRISPR guide RNA driven by a construct also containing GFP and a nuclear localization signal (pX458, Addgene #48138) shows GFP expression localized to the nucleus (M), whereas CAG-driven RFP fills the cells including projections (N,O).

Manipulating genes to test the contribution to brain function and behavior

The final research piece is to combine the data for how genomic function contributes to the ability to learn song with effective and efficient constructs to perform genetic manipulation in the brain. The first gene manipulation experiment in zebra finches demonstrated that diminished levels of a transcription factor, FOXP2, prevented accurate tutor song copying (Haesler et al., 2007). Since then, many of the brain manipulation studies have expressed transgenes such as optogenetic channels (Xiao et al., 2018). In short, there is great untapped potential for genetic manipulation studies to test the contribution of identified genomic regions in various facets of song learning; one such area has been highlighted here.

Leveraging diversity in songbird learning and behavior

Song learning in zebra finches does not represent the full diversity of strategies across songbird species. Their tractability for lab studies does often provide starting points for mechanistic comparative studies to determine the generalizability of genomic regulation in components of learned song. For example, mechanisms that promote and limit the ability to memorize tutor song across the zebra finch critical period noted here may (or may not) also contribute to enhanced learning in parasitic birds, which hatch in the nests of another species but need to learn to sing conspecific song, or in species that can constantly acquire new song elements. Notably, however, the strongest direct evidence of a genetic contribution to learned song comes from work in another songbird species, the Bengalese finch (Mets and Brainard, 2018). Non-songbird species, the sub-oscine passerines, are phylogenetically

related to songbirds but do not learn song and therefore can serve as comparative foils to test genome regions specific to learned song. Song is not the only behavior that varies across species; songbirds also display an extraordinary set of non-vocal social behaviors, including long-term mate bonding, bi-parental care, colonial living, individual recognition, territorial defense and elaborate courtship displays. Genetic manipulation will also be a powerful strategy to understand how the genome gives rise to these complex behaviors. Comparative work can inform mechanistically across shared traits, and leverage specialized biological systems to make new mechanistic discoveries. Songbirds thus represent great potential for the application of genetic manipulations to questions of genome, brain and behavior relationships across species and lifespan.

Summary

Songbird research has made seminal contributions to fields of ethology, endocrinology, physiology and neurobiology. Zebra finches in particular have generated a rich set of defined questions, including those outlined here surrounding the critical period for tutor song memorization, and others centered on sensorimotor and motor components of learned song, that would be immediately advanced by the application of genetic manipulation strategies. Further, given the number and diversity of songbird species and the behaviors they display, comparative work leveraging genetic manipulation would expand the influence of these birds in additional fields. As the possibility for genetic manipulation advances, its application will further illuminate the relationships between genome, brain and behavior, and support the next wave of contributions that the songbird research community makes.

Competing interests

The author declares no competing or financial interests.

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