THE UNIVERSITY OF CHICAGO

DEVELOPMENTAL SHIFTS IN EPIGENETICALLY-DEFINED REGULATORY REGION ACCESSIBILITY REVEAL PUTATIVE CONTROLLERS OF CRITICAL PERIOD LEARNING

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE DIVISION OF THE SOCIAL SCIENCES

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF PSYCHOLOGY

BY

GRANT WILLIAM KUNZELMAN

CHICAGO, ILLINOIS

AUGUST 2023

This dissertation is dedicated to my parents, George, and Holli Kunzelman.

The map was useless

The captain clueless

The crew was hopeless

The code was Rubiks

The cold was ruthless

The wind was music

Fantasizing 'bout diamonds

Shining like Stanley Kubrick

Mud sloshing, swash-buckling

Mop bucket

Clean enough to see your reflection

And not touch it

Clear enough to hear your own mind

And not trust it

Close enough that the goal became dry land

And sirens sang of home

And the boat became an island

- Chancelor Bennett

Table of Contents

List of Figures

List of Tables

Acknowledgements

I owe a great deal of thanks to a great many people. In preparing these acknowledgments, I quickly realized that my grasp of the English language, albeit “proficient”, is inadequate for accurately characterizing the extent of appreciation I feel toward those that have supported me throughout the completion of this dissertation. Thus, to anyone taking the time to read these acknowledgments, please do so with the understanding that these words are nothing more than an echo of the resounding positivity and kindness that these individuals have imparted into my life, both academically and otherwise.

First, I would like to acknowledge my advisor, Sarah London, who taught me what it means to be an independent scientist. Sarah consistently provided me with opportunities to try, fail, learn, and try again. If derived from well-thought-out experiments, such failures were never once met with disdain or disregard. Rather, such failures were met with “What can be learned from this outcome?” and “What are the next steps?”, the answers to which would be instrumental in determining future experimental parameters. At times, this increasingly informed cycle of trial and error felt perpetual and was not without significant frustration. However, these learning experiences and the freedoms that I was afforded in their pursuit, were by far the most crucial aspects of my development as an independent researcher and scientist. These experiences imbued in me a comfort with discomfort, an extreme appreciation for failure, and the capacity to utilize failure as a means of driving forward progress. For that and for all everything I have learned from you about being a well-informed, meticulous, hard-working, and resilient scientist, thank you, Sarah.

Second, I would like to thank my labmates, to whom I owe my maintained semblance of sanity and without whom I surely would have perished in my pursuit of this degree. Through coffee chats, consistent commiseration, and copious cocktail consumption my labmates continuously curated a culture of solidarity and scientific curiosity. Although each aimed to drive forward their own independent research pursuits, they still consistently made time to engage with me regarding my latest coding conundrum or my most recent failed experiment. Rebecca Butler joined the London lab the same year that I did, and while we initially struggled with competitive contentions, throughout graduate school, she became one of my dearest friends. Becca possesses a work ethic on par with few others I have ever met and is absolutely fearless when it comes to admitting when a communicated concept requires further explanation or elaboration, e.g., she regularly asked the questions on everyone else's minds following academic presentations or course lectures (a skill, which in my opinion, is severely lacking amongst early-stage scientists plagued with feelings of imposter syndrome). I am eternally grateful to her for providing me the space to discuss my science, helping me quell my graduate school anxieties, and reminding me not to take myself too seriously. Jo Gogola (They/Them/Theirs) was the senior most graduate student in the lab when I joined, meaning that they were regularly faced with fielding an endless onslaught of questions and curiosities stemming from both Becca and me. With patience and understanding, they were always ready to discuss scientific challenges and troubleshoot methodologies. During the later stages of my graduate school journey, I found great value in discussing the interpretation of my data with Jo, as their familiarity with published literature and field trends, as well as their ability to communicate such, bolstered my analyses and understanding. Kruthika Vm, a research technician in the lab at the time, frequently helped me troubleshoot pilot experiments and think through molecular techniques. Her careful consideration and insights improved both my methodological

processes and experimental outcomes. Furthermore, her general fascination and excitement regarding the scientific process revitalized my feelings toward my own work during periods of hardship and points of difficulty.

Beyond the members of the London lab, there were countless other graduate students to whom I owe a great deal of thanks. While I feasibly cannot thank each and every person, I would be remiss if I didn't acknowledge a few people in particular. Jae Peiso (They/Them/Theirs) took me under their wing within my first few weeks of graduate school. They helped me establish a network of colleagues and friends within the department, assisted me in the preparation my NSF GRFP personal statement, and regularly helped me traverse the interpersonal challenges of graduate school. They introduced an overwhelming amount of joy into the workplace and helped me see value in the work I was performing. Their thoughtful approach to question framing and creative, yet critical, method for solution generation (often in the context of completely non-scientific inquiry) inspired me to strategically think outside the box when problem-solving and troubleshooting. Belonging to one of the only other molecular labs working within the Psychology department, Dana Beach, and Jharnae Love, were always kind to lend me their time and ears as I learned and applied new molecular research techniques. Even if unfamiliar with the technique in question, they each often introduced important experimental considerations or helped me connect with resources necessary to accomplish my goals. To all the peers that supported me throughout my time in graduate school, thank you.

The final members of the university community that I would like to thank not only helped me progress my research in meaningful ways but were also some of the kindest and most caring individuals with whom I have ever worked. Jessica Krupinski regularly went out of her way to ensure that lab supplies deliveries were accurate and timely, even in the midst of the pandemic.

Not only does she have every carried item's catalog number memorized by heart, but she has cultivated a warm and welcoming environment within the lab supplies storeroom through continuous positive personal interactions with every customer. Chris Whaley and Mike McCarthy occasionally helped repair lab equipment and on multiple occasions MacGyvered bird room apparatuses from which my work has benefitted. As building managers, they each went above and beyond on multiple occasions to ensure that research was able to continue uninterrupted. Lucia Sanoval, a custodial staff member at the university, not only maintained lab and building cleanliness but also supported building morale through genuine interactions with researchers. I will always fondly look back on our half-English half-Spanish conversations (as we were both working to increase our comfort with the use of a second language). These conversations would always introduce well-needed perspective and grounding at the end of a long and hard day. To the university staff that showed me the utmost kindness and helped me feel at home at the University of Chicago, thank you.

Finally, I would like to thank my family. My younger sister, Brooke, has always been a role model for perseverance and persistence in my eyes. Her ferocious determination in all that she does inspires me on a daily basis and motivates me to put forth my best effort in all things that I set out to accomplish. My parents, George, and Holli Kunzelman worked tirelessly during my formative years to instill in me a strong appreciation for education and the pursuit of knowledge. They each made tremendous sacrifices to ensure that my sister and myself were afforded opportunities for growth and learning. In far more ways than one, I owe all of my successes to them and am immeasurably grateful to be their son. Lastly, my extraordinary partner, Alayna Vandercar, whose unwavering love, support, and understanding afforded me the space to prioritize

my research and continually inspired me to always put forth my best effort. To the loved ones that supported me throughout this journey, thank you.

Abstract

We do not yet fully comprehend the biological mechanisms through which developmental experiences influence adult behavioral patterns. This is, in part, because maturation- and experience-dependent mechanisms of plasticity intersect dynamically to regulate the neural properties (e.g., cell number, subtype, functionality, connectivity) that constrain developmental learning.

These neural properties are largely determined by the complement of protein-coding and noncoding RNAs transcribed from the genome. Transcription is regulated by transcription factors (TFs), proteins that coordinate the expression or repression of gene sets that orchestrate shifts in functional and structural cell properties through binding regulatory regions of the genome (i.e., enhancers, promoters, and repressors) and interacting with components of basic transcriptional machinery as well as other TFs. Due to their role in influencing cell-type-specific transcription, regulatory region accessibility profiles can be used to differentiate between cellular subtypes with a great deal of specificity. Epigenetic modifications, such as those on histone proteins, modulate regulatory region accessibility. Once such modification, H3K27ac, reliably denotes accessible regulatory regions.

To determine the neural properties and identify the cellular subtypes that support and limit the learning of complex behavior, I performed chromatin immunoprecipitation for H3K27ac and high-throughput DNA sequencing (ChIP-Seq) on auditory forebrains of male and female zebra finches spanning developmental time points (post-hatch (P) day 23, P30, P60, and P67) at which song experience has differing influence on adult behavioral patterns. The first experimental chapter (Chapter 2) of this dissertation focuses on elucidating the biological prerequisites for

developmental experience to be encoded such that it can influence adult behavioral patterns. The second experimental chapter (Chapter 3) of this dissertation focuses on understanding how developmental experience influences existing biology to prevent future experiences from altering established behavioral patterns. In each chapter, I investigate how regulatory region accessibility profiles differ between organisms differing in developmental state of receptivity (i.e. capable and incapable of learning) and age-matched animals of opposite sex. I identify transcription factor binding sites enriched in regulatory regions differing in accessibility between comparisons, identified putative genes under their regulation, and discuss the potential implications of these differences on brain development and function in the context of learning and memory formation. Additionally, in Chapter 3, I use age-matched male birds reared to P67 in environments controlling for experience and degree of social interaction to parse the roles of maturation and experience in establishing regulatory region accessibility profiles. In Chapter 4, I discuss an opportunity to leverage the obtained data to label and manipulate the putative cell populations responsible for memory encoding and present some preliminary data collected with this goal in mind. Finally, in Chapter 5, I discuss some interesting results that emerged from the collective analysis of this data and reflect on observations regarding the nature of bioinformatic analyses.

Chapter I: Introduction

Dissertation Scope

We do not yet understand the biological mechanisms through which developmental experiences influence adult behavioral patterns. This is astonishing, considering the plethora of established adult behavioral patterns that rely on developmental experiences. Neural plasticity is the ability of the nervous system to reorganize in response to intrinsic and extrinsic stimuli. During development, neural plasticity is influenced by both maturation and experience. Maturation brings about the biological features that define a developmental brain state capable of supporting experience encoding. Experience further influences the developing brain, shaping both form and function. In all cases, some level of brain maturation must have taken place such that an experience can further influence brain development. During development, maturation-dependent and experience-dependent plasticity intersect dynamically to regulate brain form and function. As such, it is challenging to attribute observed changes in plasticity relevant for encoding developmental experience to either maturation, experience, or a combination of the two. However, parsing the effects of each is necessary to fully understand how developmental experiences define adult behavioral patterns.

While adult behavior provides a functional readout of encoded developmental experience, defining a biological state at which experience can influence developing brain circuitry has yet to be achieved. This shortcoming is, in part, because we do not fully comprehend how cell number, subtype, functionality, and connectivity give rise to the cellular circuitries capable of encoding experience. These neural properties are largely determined by the complement of protein-coding and noncoding RNAs transcribed from the genome. While all cells within a single organism

possess an identical genome, genomic expression within each individual cell is extremely variable. Expression of the genome is rigorously regulated by the epigenome, a collection of molecular modifications that do not alter gene sequence but regulate gene expression.

One example of epigenetic regulation is post-translational histone modification. Histone proteins (H2A, H2B, H3, and H4) complex in duplicate to form octamers. Histone octamers structure chromatin by assisting in DNA packaging. The tightness with which DNA wraps around histone octamers is, in part, determined by post-translational modifications made to histone proteins around which it is wrapping. Regions of DNA loosely wound around histone complexes possess a higher probability of determining gene expression, while the inverse is true for tightly wound regions of DNA. This is because the degree of DNA accessibility influences whether or not proteins can bind to the DNA and influence gene transcription.

Transcription factors (TFs) are proteins capable of binding DNA and influencing transcription. TFs coordinate the expression or repression of gene sets that orchestrate shifts in functional and structural cell properties. TFs modulate gene transcription by binding regulatory regions of the genome immediately upstream (<=1 kb) of transcriptional start sites (TSSs), known as promoters, and more distal regions of the genome (> 1 kb from the nearest TSS), known as enhancers and repressors. Enhancers and repressors are cis-regulatory elements that spatiotemporally control gene expression through physical contact with the promoter sequences of their target genes. Enhancers are bound by TFs that increase the rate of transcription, while repressors are bound by TFs that decrease the rate of transcription. TFs bind regulatory regions of the genome at transcription factor binding sites (TFBSs), relatively short sequences of DNA recognized and bound by TFs. Through the identification of accessible regulatory regions and the TFBSs enriched within them, we can begin to make determinations about the transcription factors

capable of regulating gene expression within brain cells as well as the neural properties that support learning.

The focus of this dissertation is two-fold. The first experimental chapter (Chapter 2) focuses on elucidating the biological prerequisites for developmental experience to be encoded such that it can influence adult behavioral patterns. Using chromatin immunoprecipitation for H3K27ac paired with high-throughput DNA sequencing (ChIP-Seq), I investigated how regulatory region accessibility profiles differ between organisms differing in developmental state of receptivity (i.e., capable and incapable of learning) and age-matched animals of opposite sex. I identified TFBSs enriched in regulatory regions differing in accessibility between comparisons, identified putative genes under their regulation, and discuss the potential implications of these differences on brain development and function in support of learning and memory formation. The second experimental chapter (Chapter 3) focuses on understanding how developmental experience influences existing biology to prevent future experiences from altering established behavioral patterns. Leveraging the same methodology utilized in chapter two, I again investigated how regulatory region accessibility profiles differ between organisms differing in developmental state of receptivity (i.e., capable and incapable of learning) but focus on how changes in regulatory region accessibility might limit learning and memory formation to promote the stabilization and persistence of established brain biology. Additionally, using age-matched animals reared in environments controlling for experience and degree of social interaction, I attempt to parse the roles of maturation and experience in establishing regulatory region accessibility profiles. In the fourth chapter, I discuss an opportunity to leverage the obtained data to label and manipulate the putative cell populations responsible for memory encoding and present some preliminary data collected with this goal in mind.

The “BIG” problem

Experiences occurring during development are extremely influential in establishing adult behavior patterns. Perhaps the most well-known example of this stems from the pioneering work of Hubel and Wiesel, who demonstrated that suturing shut an eyelid during development leads to functional blindness in that eye, even though the retina remains entirely functional following the reopening of the eye at a later date (Hubel & Wiesel 1964). Further examples highlighting the importance of developmental experience, in arguably more naturalistic manners, arise from studies of maternal stress in rodent models and song learning in birds. In rodents, the development of the HPA (Hypothalamic-Pituitary-Adrenal) axis response to stressful stimuli is influenced by early environmental events, such as maternal care, physical handling, and endotoxin exposure (Meaney et al. 1988; O'Donnell et al. 1994; Liu et al. 1997). In certain songbird species, birds produce a song in adulthood that reflects the song they were exposed to during a restricted period of development (Thorpe 1958; Immelmann 1969; Marler 1970; Zann 1996). Song heard outside the confines of this developmental window has little influence over song produced in adulthood (Roper & Zann 2006). Similarly, it is difficult to overstate the influence of developmental experience in establishing adult behavior in humans, as it is paramount in supporting the acquisition of sensorimotor skills, such as language production, mediating adult manifestations of developmental stress, and regulating sensory systems development (Tierney & Nelson III 2009; Bengoetxea et al. 2012; Korosi et al. 2012; Rowson et al. 2019). Thus, we as a scientific community have cultivated a strong appreciation regarding the influence of developmental experience on behavior acquisition.

Still, there remains a surprising lack of knowledge pertaining to the biological mechanisms through which developmental experiences are encoded such that they are capable of shaping adult

behavioral patterns. For example, there exist countless behaviors, dependent on social interaction during development, that manifest differently based on the age and sex of the organism (Hostinar et al. 2014). However, we do not yet understand why. Further investigation focusing on developmental learning in the context of age, sex, and experience will be crucial in comprehending the mechanisms through which experience is encoded and mechanisms through which that experience influences behavior.

With this goal in mind, two admittedly daunting questions emerge at the forefront: [1] What are the biological prerequisites for developmental experience to be encoded such that it can influence adult behavioral patterns? [2] How does developmental experience influence existing biology to prevent future experience from altering already established behavioral patterns? These questions have been and will continue to be difficult to address. During periods of developmental learning, maturation-dependent, and experience-dependent brain plasticity intersect dynamically to regulate brain form and function. Shifts in brain plasticity result from immediate changes in cell function that occur following each relevant experience, as well as long-term changes resulting from the accumulation of those experiences, all of which is concurrent with generalized maturational programming. Furthermore, it is not clear that the biological mechanisms that promote neural stability are simply the inverse of those that promote neural plasticity, i.e., “on” and “off” may not be controlled by the same switch(s) but rather distinct switches regulating separate neural properties. Thus, attributing developmental shifts in brain plasticity to maturation, experience, or a combination of the two requires carefully designed experimental manipulations aimed at elucidating changes across multiple time scales performed in the context of well-defined developmental learning paradigms.

Neural plasticity, learning, and memory formation

For developmental experience to influence adult behavior, the brain must reach a sufficient state of organization such that mechanisms of brain plasticity can reliably encode the experience via cellular and molecular mechanisms. When the brain has sufficiently organized, applicable experience engages mechanisms of brain plasticity that invoke the establishment and remodeling of neural circuitry. Following a sufficient degree of experience, mechanisms of neural stability predominate, resulting in a loss of plasticity such that the pertinent experience no longer leads to measurable changes in behavior, i.e., the behavior has stabilized. Generally, while mechanisms of brain plasticity are required to support learning and memory formation, not all learned behaviors adhere to the progression described above in that they do not necessarily reach a state of stabilization and remain malleable throughout life. Through careful examination of behaviors dependent on experience occurring within a developmental period possessing a definitive beginning and end, we can begin to elucidate the cellular and molecular mechanisms that regulate transition in learning potential.

While adult behavior provides a functional readout of encoded developmental experience, defining a biological state at which experience can influence developing brain circuitry has yet to be achieved. This shortcoming is, in part, because we do not fully comprehend how cell number, subtype, functionality, and connectivity give rise to the cellular circuitries capable of encoding experience. Despite the lack of consensus regarding a general developmental state necessary to support learning (which almost certainly varies according to the organism and kind of learning in question), there are some general themes of biological development that should be considered. Undoubtedly, cellular proliferation, differentiation, and organization are prerequisites for a state of neural maturation capable of supporting learning and memory formation. For example, the

appropriate balance of excitatory and inhibitory signaling is a general feature of brain maturation, reliant on proliferation, differentiation, and organization, particularly important for fine-tuning neural response properties to specific stimuli (Natan et al. 2017; Wood et al. 2017; Moore et al. 2018; Vickers et al. 2018) and thought to influence the onset of neural responsivity across many systems and species (Zhou & Yu 2018). The prevailing theory is that following sufficient brain development and organization sets of genes and/or molecular cascades become responsive to pertinent experience, and that experience then further influences future responsivity.

The value of genomic and epigenomic investigations

Long-term memory formation and genomic function are inextricably linked. Independent of taxonomic classification, long-term memory formation requires experience-dependent transcription and translation (Kandel 2001), and epigenetics and molecular cascades are central mechanisms of control for functional and structural neural remodeling following experience (Ortuno-Sahagun et al. 2019). Thus, elucidating the biological mechanisms linking developmental experience and adult behavior necessitates investigations of genome and epigenome states.

A genome is the comprehensive list of all of the DNA possessed by an organism organized by chromosomal location. At its most basic level, it is a carefully curated list of As, Cs, Ts, and Gs. Sequences of nucleotides compose individual genes, which are transcribed into RNAs. The collection of RNAs transcribed from the genome is referred to as transcriptome. A small portion of the transcriptome (mRNAs) is translated into proteins, while the vast majority of the transcriptome encodes RNAs that are not directly translated into proteins but do directly regulate gene expression and protein synthesis (e.g., miRNA, lncRNA, siRNA). Furthermore, regions of the genome that do not code for RNA, termed non-coding regions, also exert influence over genomic expression (further detail on non-coding regions of the genome below). Genomic

expression dictates cell survival, growth, and differentiation, as well as cellular features that confer cell type and properties influencing neural plasticity (e.g., receptors, transporters, molecular cascade components, transcription factors, structural proteins, non-coding RNAs). Thus, a complete and error-free genome effectively provides one with a genetic instruction manual for producing all macromolecules required for the organism's survival, development, and function. Additionally, access to a suitable genome supports the analyses of biological organization at a depth unachievable by investigations focused on a single gene or a small population of genes (Clayton & London 2014). Investigations aimed toward understanding coordinated gene networks and gene expression support a more representative interpretation of the cellular and molecular processes underlying complex biological functions, including learning and memory formation. However, simply knowing the code is only an early step in deciphering its significance.

Almost every cell of an organism possesses a complete copy of the genome. However, the manner in which individual cells express the genome is extraordinarily variable. Differences in genomic expression give rise to differences in cell type and subtype, regulate cellular connectivity and circuit formation, and influence how cells respond to endogenous and exogenous stimuli. Expression of the genome is rigorously regulated by the epigenome, a collection of molecular modifications that do not alter gene sequence but regulate gene expression. Epigenetic modifications can be passed from cell to cell, as cells divide, and even from generation to generation as organisms reproduce. Epigenetic modifications dynamically shift across a cell's lifetime, facilitating the activation and repression of various transcriptional programs. Shifts in epigenetic regulation contribute to a cell's ability to adjust the genes it is transcribing in response to changing internal and external environments. As such, investigations focusing on the epigenome are well-suited for examining states of cellular receptivity and predicting responsivity, while

investigations of genomic expression inform cellular responsivity. Albeit gene expression can immediately influence responsivity by regulating the expression of other genes, as well as its own expression, and can regulate future responsivity by altering cellular properties that further influence responsivity. Thus, investigations of genomic expression and epigenetic regulation provide the greatest promise for linking states of receptivity with commensurate measures of responsivity. The work presented in this dissertation focuses on the prior because the experimental aim is to make determinations regarding shifts in neural receptivity rather than responsivity.

Mechanisms of epigenetic regulation

Epigenetic mechanisms regulate the function of the genome without altering genomic sequence, prominently through the regulation of transcription. There are several distinct mechanisms of epigenetic regulation, including DNA methylation, non-coding RNA (ncRNA), and post-translational histone modification, each of which can direct the transcriptional regulation of various suites of genes. Below I provide a brief introduction to the three aforementioned mechanisms of epigenetic regulation, with a greater emphasis placed on post-translational histone modification, as they are more central to the understanding of this dissertation.

To understand how mechanisms of epigenetic regulation influence genomic expression, it is beneficial to know how DNA is organized within the cell nucleus. Within the nucleus, genomic DNA exists wrapped around a complex of eight histone proteins of four different types (two of each type): H2A, H2B, H3, and H4 (Luger et al. 2012). Together, the DNA and histone complex around which it is wrapped compose a nucleosome, the fundamental subunit chromatin. Such organization is required to help fit the entirety of the genome into the nucleus. Chromatin exists in two general states of accessibility – heterochromatin and euchromatin. Heterochromatin is highly condensed, gene-poor, and not typically transcribed. In comparison, euchromatin is less

condensed, gene-rich, and more likely to be transcribed (Huisinga et al. 2006). States of chromatin accessibility and inaccessibility are determined by the epigenetic modification made to the DNA and histone proteins of which it is composed.

Nucleosome positioning, chromatin organization, and access to genomic DNA are largely controlled by histone proteins. Post-translational histone modifications (PTMs) are molecular modifications made to histone amino (N)-terminal tails that have functional implications in chromatin structure and resulting gene expression (Bannister & Kouzarides 2011). At least nine different types of PTMs have been discovered, with the most well-studied of those being acetylation, methylation, phosphorylation, and ubiquitylation (Peterson & Laniel 2004). The various types of histone modifications impact how histones interact with DNA and neighboring nucleosomes. Some PTMs destabilize the interactions between histones and DNA, resulting in an increase in chromatin accessibility. This open chromatin conformation makes the DNA more accessible for binding by transcriptional machinery, thus leading to a greater probability of gene expression. Contrastingly, certain PTMs strengthen the interactions between histones and DNA, thereby decreasing chromatin accessibility and the probability of gene expression. The resulting effect of a PTM is based on PTM type, the histone protein being modified, and the amino acid to which the modification is being made. For example, tri-methylation of lysine, the 36th amino acid from the n-terminus of histone H3 (H3K36me3) increases chromatin accessibility, while tri-methylation of lysine, the 27th amino acid from the n-terminus of histone H3 (H3K27me3) decreases chromatin accessibility. Furthermore, each type of histone modification occurs at specific regions of the genome. H3K36me3 predominately occurs at regions of the genome composing gene bodies, while H3K27me3 predominately occurs at enhancer and promoter regions. PTMs are added, removed, and modified throughout the lifetime of a cell to facilitate the

temporally coordinated activation and inactivation of gene sets important for cell-specific development and function. Since the initial characterization of a PTM by Allfrey and colleagues in the 1960s (Allfrey et al. 1964), countless studies have gone on to establish the importance of PTMs in regulating learning and memory formation (Kim & Kaang 2017; Creighton et al. 2020).

DNA methylation involves the addition of a methyl group to the C-5 position of a cytosine ring, most typically adjacent to guanine (CpG), by DNA methyltransferase (DMNT) (Robertson 2005). These molecular modifications, made directly to the nucleotides composing DNA, have been predominately associated with transcriptional repression, or a reduced likelihood of gene transcription. DNA methylation recruits proteins that reduce chromatin accessibility and interferes with the binding of transcriptional regulators through steric interference (Nan et al. 1998; Klose et al. 2005). Although principally associated with transcriptional repression, there is also evidence to suggest that DNA methylation, if occurring at certain genomic loci, can enhance gene transcription (Harris et al. 2018). DNA methylation is a reversible process. Demethylation, resulting in the removal of a methyl group, is partly regulated by DNMTs and ten-eleven translocation (TET) enzymes (Wu & Zhang 2017), both of which are particularly abundant in neurons where dynamic methylation and demethylation are important for regulating learning and memory formation (Miller & Sweatt 2007; Antunes et al. 2019). Experimental manipulations of DMNT and TET proteins, across multiple brain regions, including the prefrontal cortex, hippocampus, and amygdala, have demonstrated the importance of methylation in regulating long-term memory formation and recall (Miller & Sweatt 2007; Feng et al. 2010; Maddox & Schafe 2011; Rudenko et al. 2013).

ncRNAs are functional RNAs transcribed from the genome that are not translated into proteins. Accounting for approximately 80% of the RNAs transcribed from the genome (Kellis et

al. 2014), common ncRNAs include transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), microRNAs (miRNA), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs). tRNAs serve as adaptor molecules between mRNA and the growing chain of amino acids that compose the protein being synthesized. rRNAs combine with ribosomal proteins to form ribosomes, the cellular organelle responsible for protein synthesis. Thus, both tRNAs and rRNAs directly contribute to the regulation of protein production. miRNAs, siRNAs, and lncRNAs further regulate translation through direct interactions with nucleic acids, DNA and RNA, or proteins, such as RNA polymerase II and transcription factors. While investigations of regulatory roles of ncRNA remain at an early stage, relative to investigations of protein-coding genes, they have already made strong contributions to our understanding of brain function. For example, sets of lncRNAs display region- and cell-specific patterns of expression in brain regions associated with learning and memory formation, such as the hippocampus, prefrontal cortex, and amygdala (Mercer et al. 2008; Kadakkuzha et al. 2015). These suites of lncRNAs are thought to influence learning and memory formation through the regulations of genes involved in synaptogenesis and synaptic plasticity (Bernard et al. 2010; Tripathi et al. 2010; Vance et al. 2014).

Histone post-translational modification H3K27ac

H3K27ac, the acetylation of lysine, the 27th amino acid from the n-terminus of histone H3, is a PTM that reliably denotes accessible regulatory regions of the genome referred to as enhancers, repressors, and promoters (Heintzman et al. 2007; Wang et al. 2008; Creyghton et al. 2010). Promoters are relatively short sequences of DNA (100 bp to 1 kb in length) found directly upstream (5’ to the DNA coding strand) of the gene they regulate. Promoters are responsible for recruiting RNA polymerase to facilitate the initiation of transcription. Like promoters, enhancers and repressors are DNA sequences that modulate gene expression. Enhancers function to increase gene

expression, while repressors function to suppress gene expression. Unlike promoters, enhancers and repressors can be located either upstream or downstream of the genes they regulate and can regulate genes at a great distance (upwards of millions of bps away) (Maston et al. 2006). Notably, enhancers and repressors are cis regulatory elements, meaning they are limited to regulating genes that exist on the same chromosome as they do. Enhancers and repressors regulate gene expression through interactions with promoters. Chromatin folding facilitates interactions between promoters and distal regulatory regions. Typically, a single promoter is under the control of multiple enhancers, and a single enhancer regulates multiple promoters.

Regulatory regions regulate transcription through the binding of transcription factors (TFs). Transcription factors are proteins in possession of DNA-binding domains that facilitate binding to a specific sequence of DNA called a transcription factor binding site (TFBS) (Wingender et al. 2013). Once bound, transcription factors function to either activate or repress the expression of specific genes through interaction with RNA polymerase II and co-regulators, such as histone acetyltransferase (HAT) p300 (Merkulova et al. 2013; Ignatieva et al. 2015). Individual regulatory regions may contain binding sites for upwards of 20 different transcription factors, thus allowing for a relatively high degree of transcriptional regulation (Zhang et al. 2000; Wingender et al. 2013; Wingender et al. 2014). Currently, there are estimated to be approximately 1500 transcription factors encoded in the human genome (Ignatieva et al. 2015), while estimates for other vertebrate species differ, but generally fall within a similar range (Zhao & Kishino 2020). Some transcription factors are expressed only in specific cell and tissue types, while other transcription factors are broadly expressed throughout the organism. For many transcription factors, a consensus binding site sequence and a functional regulatory role have not yet been identified. As such, while a substantial amount of experimentation is still required to facilitate a

comprehensive understanding of all transcription factor regulatory function, investigations aimed at accomplishing just that can be extremely informative in deciphering the complex interplay between the genome and epigenome dictating how cells and cell populations develop and organize.

Investigations of enhancer function have demonstrated their importance in mediating cell-type specific transcriptional responses to intracellular and extracellular stimuli. Transcription factors, induced by cellular signaling cascades, predominantly recognize and bind regions of the genome that already exhibit enhancer-typical PTMs and are bound by other transcription factors (Barish et al. 2010; John et al. 2011). Cells of differing types, as well as individual cells of the same type, possess different compliments of accessible regulatory regions. These findings give rise to the argument that precedent groups of enhancers predominantly control cell-type-specific gene expression in response to internal and external stimuli (Heintzman et al. 2009; Visel et al. 2009; Thurman et al. 2012). Across many different cell types, analyses of epigenetic markers denoting cis-regulatory regions (i.e., enhancers and repressors) by the Encyclopedia of DNA Elements (ENCODE) consortium suggest that an individual genome may contain up to one million cis-regulatory elements (The ENCODE Project Consortium, 2012). Thus, for a given genome, enhancers and repressors drastically outnumber genes and promoters, suggesting that their function may confer greater cell-type specificity. In cortical regions of the brain, researchers have now demonstrated that unique enhancers define individual cellular subtypes with greater resolution than native promoters and individual gene transcripts, each of which have a much greater tendency to span multiple cell types (Blankvoort et al. 2018; Nair et al. 2020). As such, examinations of accessible regulatory regions (with a specific focus on enhancers and repressors), as well as the TFBSs contained within them can inform questions of cell-type-specific responsivity.

Critical periods: a model for investigating developmental learning reliant on maturation and experience

Critical periods (CPs) are restricted developmental phases during which experience has a profound and persistent effect on brain function and resulting behavioral patterns (Hess 1959; Knudsen 2004; Takesian & Hensch 2013). CPs are associated with a particular stimulus (or type of stimulus) that alters the biology of a given brain region. CPs possess an onset, a state of brain maturation at which experience drives molecular and cellular responses that support lasting behavioral outcomes. Within a CP, accumulation of experience, following repeated stable stimulus exposure, reduces neural plasticity until the brain reaches a state of neural stability. When properties of neural stability sufficiently overcome neural properties of plasticity, the CP closes, and future experiences no longer influence the established behavioral patterns. CP closure results from sufficient stimulus exposure, rather than a maturational state. As such, CPs are defined by the concerted effects of both age and experience (Hess 1959; Knudsen 2004; Takesian & Hensch 2013). Such characteristics make CPs uniquely well-suited for investigating developmental behaviors in which maturation and experience both exert influence. CPs add further value in that they allow one to a priori define an organism's state of responsivity. This allows for the comparison of organisms spanning CP transition points to determine how biological processes relevant for the encoding of experience differ between organisms at different stages of development.

Sensory song learning in the zebra finch (*Taeniopygia guttata*)

To examine how regulatory region accessibility profiles change as a function of maturation and experience, I chose to leverage sensory song learning in the zebra finch (*Taeniopygia guttata*). Studies of zebra finch have made impactful contributions to a breadth of research fields, including ecology, physiology, and neurobiology (Clayton et al. 2005). Zebra finches are a particularly well-

suited model organism for investigating how juvenile experience influences life-long behavioral patterns (Figure 1A). Song production in the zebra finch is sexually dimorphic – males sing, and females do not. Females do not sing because they do not develop the brain circuitry required for song production (Nottebohm & Arnold 1976; Nixdorf-Bergweiler 1996). However, both sexes are extremely social and frequently vocalize in the form of calls. Males use song as a mating signal to convey reproductive fitness in the hopes of facilitating life-long mating pairs with females (Figure 1B) (Macdougall-Shackleton 1997; Collins 2004; Tomaszycki & Adkins-Regan 2005; Riebel et al. 2009; Woodgate et al. 2012). Females actively attend to directed song to evaluate the fitness of courting males (Figure 1C). As such, the production and interpretation of song, and its inseparable signals of reproductive fitness, are of great importance for both male and female finches, respectively.

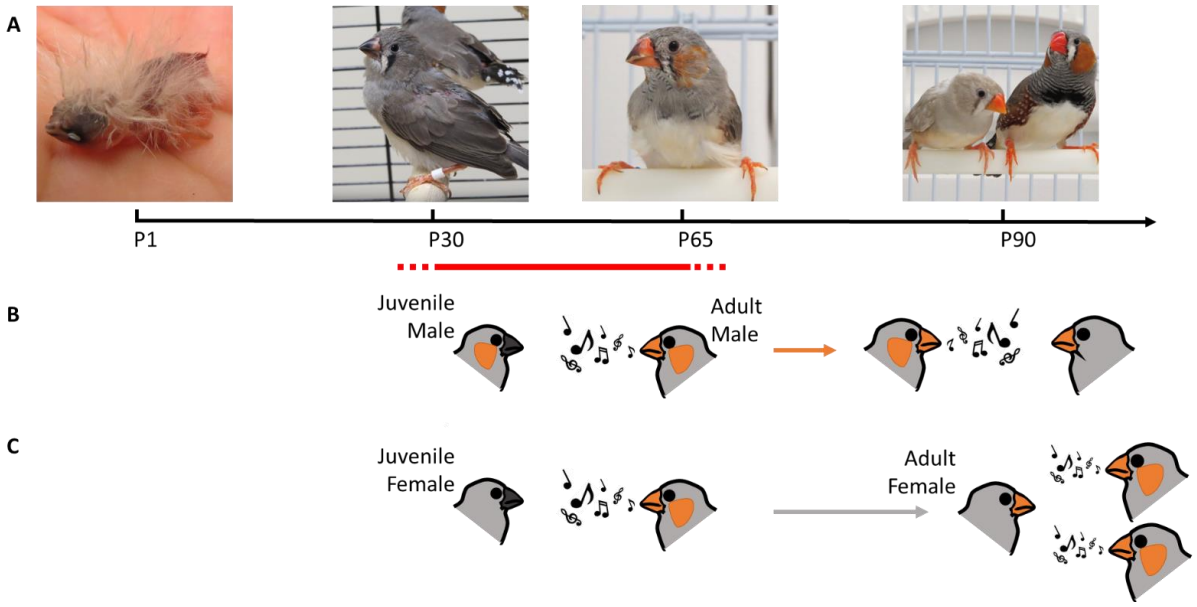

Figure 1. Juvenile song experience influences adult behavioral patterns.(A) Timeline of zebra finch post-hatch (P) development. Photos demonstrate changes in external morphology occurring between P1 (hatch) and P90 (adulthood). Juvenile males and females are influenced by song experience between approximately P30-65 (red bar with dashed ends). (B) In juvenile males (orange cheek patches, black beak), this experience leads to adult song structure mirroring that of his adult male tutor (orange cheek patches, orange beak). (C) In juvenile females (no cheek

Figure 1., continued. patches, black beak), this experience influences song preference(s) and mate choice in adulthood (no cheek patches, orange beak).

Tutor song memorization: a special type of sensory song learning?

In adulthood, each male sings a single unique stereotyped song. Juvenile males most commonly choose to learn the song of their fathers. However, juveniles can also copy the song of an unrelated male with high consistency and accuracy (Williams 1990; Mann & Slater 1995; Adret 2004a, 2004b; London & Clayton 2008; Chen et al. 2016; Ahmadiantehrani & London 2017a; Baran et al. 2017; Ahmadiantehrani et al. 2018). Tutor selection is thought to depend on the adult male with whom the juvenile has the most social interaction, which is most commonly the juvenile's father, but is also thought to be influenced by other features of social interaction, such as degree of paternal care, patterns of aggression, and song availability (Clayton 1987; Böhner 1990; Williams 1990; Mann & Slater 1995; Chen et al. 2016).

Stereotyped song emerges through the coordination of sensory, sensorimotor, and motor learning. Tutor song memorization (TSM), a potentially male-specific type of sensory learning, is the process through which a juvenile male acquires an auditory memory, commonly referred to as a template (Konishi 1965), of an adult male's song. TSM is foundational to support song production later in life (Figure 1B) (Immelmann 1969). Sensorimotor learning, also commonly referred to as sensorimotor error correction, is the process through which the juvenile edits and adjusts his initial immature vocalizations to more closely resemble the song template acquired during sensory song learning. Finally, motor learning is the process through which the juvenile male works to refine his vocal outputs independent of subsequent tutor song experience, such that the song becomes highly stereotyped for the remainder of his adult life. The ability to produce high-quality song directly impacts a male's likelihood of pair bonding with a female (Williams

1990). The body of work presented in this dissertation predominately focuses on the biological regulation of sensory song learning.

TSM meets all criteria necessary to be called canonical CP (London 2019). The CP for TSM opens on post-hatch day (P) 30; tutor song heard prior to P30 is not incorporated in song produced in adulthood, whereas song heard at P30 is (Roper & Zann 2006). However, experimental evidence exists that auditory experience before P30 influences neural properties important for TSM (Braaten 2010; Adret et al. 2012; Chen et al. 2017). Males yet to enter the CP for TSM (P21-P24) possess small populations of song-selective neurons, among which a small fraction preferentially respond to tutor song, while none preferentially respond to familiar or unfamiliar song (Adret et al. 2012). Furthermore, male and female zebra finches discriminate between familiar and novel songs if exposed to the songs between P22 and P30, but not if exposed to the songs between P9 to P17 (Braaten 2010). However, as song heard before P30 is not reflected in adult song production, the robust opening for the CP for TSM is considered to be P30.

Under normal ecological conditions, the CP for TSM closes at P65. However, if a juvenile male is prevented from experiencing song between P30 and P65, the CP for TSM remains open, and the juvenile male is still capable of copying song heard after P65 (Eales 1985, 1987; Morrison & Nottebohm 1993). The maintained state of receptivity beyond P65 in the absence of tutor song experience demonstrates that tutor song experience, not maturation, is the determining factor in the closure of the CP. Furthermore, isolation from hearing tutor song does not affect the onset or frequency of song production, nor does it significantly alter patterns of gene expression in motor control nuclei of the song control system (Mori & Wada 2015). However, song produced by juveniles isolated from hearing song appear abnormal, which can be attributed to the bird not having access to a model for the tuning of his immature vocalizations. For example, males

prevented from hearing song during development produce song with fewer notes and more simplistic frequency modulation than normally reared males (Immelmann 1969; Price 1979; Eales 1987; Slater et al. 1988; Morrison & Nottebohm 1993; Williams et al. 1993; Zann 1996). These findings suggest that TSM, not motor production learning, more heavily relies on tutor song experience.

Sensory song learning in females: discrimination learning and preference learning

Female zebra finches do not sing. However, it is known that females perform sensory song learning during the CP for TSM and that song heard during this period of development influences song preferences in adulthood (Miller 1979b, 1979a; Clayton 1988; Riebel 2000; Riebel et al. 2002; Riebel 2003b, 2003a; Lauay et al. 2004; Terpstra et al. 2006; Riebel et al. 2009; Svec & Wade 2009; Holveck & Riebel 2014; Chen et al. 2017; Diez et al. 2019; Wei et al. 2022) (Figure 1C). At present, it is not clear if TSM in males and sensory song learning in females are distinct processes characterized by separable biological and electrophysiological properties or if they are indistinguishable types of learning under the regulation of identical biological and electrophysiological properties. This gap in knowledge is driven, in part, by the necessity to use different measures in males and females for assaying song memorization in adulthood.

Some of the first evidence of sensory song learning in female songbirds came from the work of David Miller, who showed that adult female zebra finches (P100 ± 3) prefer the song of their father to that of similar and dissimilar sounding conspecifics, even after not having heard the father's song since P35 ± 3 (Miller 1979b). Miller went on to demonstrate that females also distinguish between their mate's song and songs of other males (Miller 1979a). This foundational

work helped spark further investigations into the importance of song experience during development for female song preference in adulthood.

Adult female song preference is influenced by developmental song experience (Miller 1979b, 1979a; Clayton 1988; Riebel 2000; Riebel et al. 2002; Riebel 2003b, 2003a; Lauay et al. 2004; Terpstra et al. 2006; Riebel et al. 2009; Svec & Wade 2009; Holveck & Riebel 2014; Chen et al. 2017; Diez et al. 2019; Wei et al. 2022). Females exposed to tutor song during development preferentially approach speakers airing tutored song over untutored song (Lauay et al. 2004; Svec & Wade 2009). Untutored females do not demonstrate a preference for tutored song, but do prefer conspecific song over heterospecific song, suggesting that aspects of song preference reflect innate biases (Braaten & Reynolds 1999; Lauay et al. 2004). Established preferences are highly consistent across measures, suggesting that early song exposure might lead to a consolidation of adult preference behaviors (Riebel 2000). Finally, studies examining the potential influence of sex on song preference learning indicate that song exposure during the CP for TSM is equally influential in establishing adult preference in both sexes (Riebel 2000). These studies support the notion that song experience during development is just as important for shaping adult behavioral patterns in females as in males.

Does female sensory song learning occur within a critical period?

Unlike TSM in males, it remains unclear whether female sensory song learning adheres to the criteria necessary to be considered a developmental CP (Riebel 2003a). Sensory song learning in females appears to onset around P30, but the experimental work required to determine the precise age of onset has not yet been performed (Clayton 1988). Females isolated from their father's songs at P25 do not show a preference for the song of their father in adulthood (Clayton 1988). However, birds isolated at P35 do (Clayton 1988; Holveck & Riebel 2014). Furthermore,

female zebra finches can discriminate between familiar and novel songs if exposed to the songs between P22 and P30, but not if exposed to the songs between P9 to P17 (Braaten 2010). These findings suggest that some level of brain maturation must occur before developmental song experience can influence adult song preference and support there being an age of onset for female sensory song learning.

The experimental evidence required to determine if juvenile female sensory song learning possesses an experience-dependent closure has not yet accumulated. This is, at least in part, because we do not yet know if juvenile song preference learning is qualitatively distinct from adult preference learning, or to what extent adult preference learning is influenced by preferences established during development (Wei et al. 2022). As adults, females demonstrate reliable preference for song heard during early development (Clayton 1988; Riebel et al. 2002; Riebel 2003a; Lauay et al. 2004; Holveck & Riebel 2014; Chen et al. 2017) and the neuromodulatory effects of juvenile song experience likely persist into adulthood (Terpstra et al. 2006; Hauber et al. 2013). After preference has been established during early development females maintain the capacity to establish new preferences throughout development (Holveck & Riebel 2014), as well as in adulthood (Miller 1979a; Clayton 1988; Woolley & Doupe 2008). Maintained potential for preference formation and alteration may be particularly important for mate selection in adulthood, as possession of overly static preference may be detrimental to reproductive success. Conversely, overly flexible preference can be just as disadvantageous in informing suitable mate selection. Deciding if juvenile sensory song learning in females meets the necessary criteria to be called a CP will require determining if juvenile and adult sensory song learning are distinct processes and to what extent preferences established during development are influenced by adult song experience.

Song control system and the auditory forebrain

Developmental song learning relies on a distributed network of specialized interconnected brain nuclei (Figure 2). These nuclei, and the connection between them, can be subset into pathways with differing functional roles. The anterior forebrain pathway (AFP), is a cortico-basal ganglia-thalamo-cortical circuit that includes the lateral magnocellular nucleus of the anterior nidopallium (LMAN) and Area X. The AFP promotes fluctuation or stabilization of song produced via the motor system. As such, the AFP exerts greater influence during the sensorimotor phase of song acquisition, a period when the singing juvenile is adjusting elements of his song to best match those of the template he acquired during the sensory phase, than stereotyped song production in adulthood (Bottjer et al. 1984; Scharff & Nottebohm 1991; Doupe & Solis 1997; Aronov et al. 2008). The posterior motor pathway, composed of the brain nuclei HVC (proper name) and the robust nucleus of the arcopallium (RA), drives vocalization and song production (Nottebohm & Arnold 1976). HVC serves as a sort of focal hub nuclei for the entire song control system as it is a target of auditory information and projects to both RA and Area X.

The auditory forebrain (also referred to as the auditory lobule (AL)) (Cheng & Clayton 2004), a brain region absent in the initial characterization of the song control system (Nottebohm & Arnold 1976), is now recognized as brain region integral for sensory song learning (Mello et al. 2004; Phan et al. 2006; London & Clayton 2008; Gobes et al. 2010; Yanagihara & Yazaki-Sugiyama 2016; Ahmadiantehrani & London 2017a; Ahmadiantehrani et al. 2018). AL is divisible into three highly interconnected subregions: Field L, caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM). Field L is analogous to mammalian primary auditory cortex and is the initial thalamorecipient of auditory information (Wang et al. 2010). Field L projects to NCM and CMM which are analogous to mammalian secondary auditory and association cortexes

(Vates et al. 1996; Dugas-Ford et al. 2012). Projections initiating in AL travel to HVC and RA via neighboring processing regions HVC-shelf and RA-cup, respectively (Karten 1968; Bauer et al. 2008). These projections are presumed to be the anatomical pathway through which auditory information informs motor production of the song control system (Vates et al. 1996; Theunissen et al. 2004; Bauer et al. 2008).

In females, song control nuclei LMAN, Area X, HVC, and RA are significantly smaller than in males (Nottebohm & Arnold 1976; Nixdorf-Bergweiler 1996; London et al. 2003), which is thought to result in the lack of song production observed in females. Contrastingly, NCM, CMM, and field L appear similar in size and structure in males and females (Krentzel & Remage-Healey 2015; Brenowitz & Remage-Healey 2016). This finding, however, does not preclude the possibility that anatomical similarities between male and female ALs belie prominent differences in neurophysiology (Dagostin et al. 2012; Yoder et al. 2015) and molecular responsivity (Bailey & Wade 2003, 2005; Pinaud et al. 2006; Ahmadiantehrani & London 2017a; Ahmadiantehrani et al. 2018).

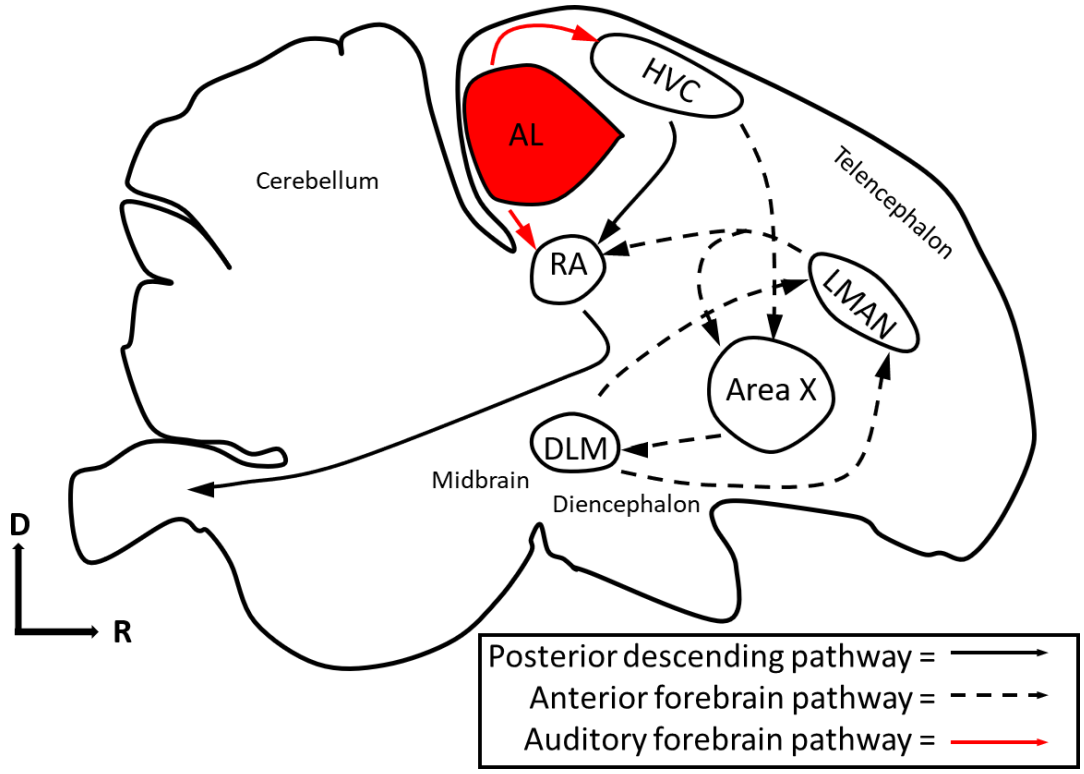

Figure 2. Developmental song learning relies on a distributed network of specialized interconnected brain nuclei. Diagram of the adult male zebra finch brain depicting three modules of the song control system (sagittal plane, dorsal is up, rostral is right). The amorphous labeled shapes represent major brain regions contributing to the system. The auditory forebrain pathway (red arrows) transmits neural signals from the auditory forebrain (AL) to HVC and RA through projections to their adjacent processing areas HVC-shelf and RA-cup (not represented), respectively. The posterior motor pathway (solid arrows), which is required for song production, transmits neural signals from HVC to RA onto further hindbrain regions necessary for motor production. The anterior forebrain pathway (AFP) (dashed arrows) instills plasticity and stability in motor production. The AFP is particularly important for informing sensorimotor error correction during song acquisition. Contributing to both the AFP and posterior motor pathway, HVC functions as a sort of hub nucleus for the entirety of the system. Figure adapted from Figure 3 in London, 2019.

Song control system supports sensorimotor and motor learning

To this day, there remains some contention regarding the neural substrates responsible for sensory song learning and the mechanisms through which the brain acquires and stores a song

template. For some time following its initial characterization, researchers believed that the song control system was the neural substrate for sensory song learning and that it housed the template acquired via TSM. These beliefs were supported by a series of electrolytic lesion studies performed on AFP nuclei LMAN and Area X, demonstrating that these nuclei, when lesioned, do not impact adult song production, but result in abnormal song development in juvenile males (Bottjer et al. 1984; Sohrabji et al. 1990; Scharff & Nottebohm 1991). Young males with LMAN lesions sing stable, yet atypical songs ("monotonous repetitions of a single note complex") (Bottjer et al. 1984; Scharff & Nottebohm 1991), while young males with Area X lesions do not develop crystallized songs ("rambling series of unusually long and variable notes") (Sohrabji et al. 1990; Scharff & Nottebohm 1991). Because song production is disrupted in these birds, one cannot eliminate the possibility that sensorimotor, rather than sensory learning is impaired, as it is difficult to determine the degree to which TSM has occurred (Bolhuis & Gahr 2006). Further work, focusing on the contributions of the AFP to sensory song learning, aimed to bypass the shortcoming of permanent lesioning techniques through the temporary inactivation of N-methyl-D-Aspartate (NMDA) receptors via the administration of an NMDA receptor antagonist amino5-phosphonopentanoic acid (AP5) on days of tutor song exposure, but not days in between (Basham et al. 1996). AP5 administration resulted in a significant reduction in the amount of tutor song copied compared to all experimental controls. However, infusions were administered during a developmental period in which sensory and sensorimotor learning overlap (P32 - P52), once again allowing for the possibility of sensorimotor learning, rather than sensory learning, impairment (Bolhuis & Gahr 2006).

Electrophysiological investigations into song control system have also provided some support for its involvement in sensory song learning. Neurons in LMAN, Area X, HVC, and RA

all demonstrate selective responsivity to song. In adulthood, neurons within these brain regions demonstrate greater selectivity for a bird's own song (BOS) than his tutor's or that of another conspecific (Margoliash & Konishi 1985; Margoliash 1986; Solis et al. 2000). LMAN and Area X contain neurons that are selective for BOS, neurons that are selective for tutor song, and neurons that are equally responsive to both (Solis et al. 2000). Neuron selectivity in HVC shifts across the development (Nick & Konishi 2005). Early in the sensory and sensorimotor learning phases (P35 - P69) HVC neurons respond preferentially to tutor song over BOS, novel conspecific song, heterospecific song, and white noise. Later on in development (≥ P70), when sensory learning has halted, but sensorimotor learning is still underway, HVC neurons respond preferentially to BOS. No correlation was observed between neuron responsivity during the first window of measurement and the similarity between BOS and the song of the tutor from which he learned (Nick & Konishi 2005). Collectively, these electrophysiological studies suggest that the neural representation of learned song exists outside the song control system in adult birds, but that the song control system is critical for supporting sensorimotor and motor learning during development. These studies also highlight the importance of distinguishing between different types of learning as well as designing assays aimed at carefully teasing apart how different types of learning might become integrated into an interconnected network of brain nuclei.

Auditory forebrain supports sensory song learning

Current evidence suggests that the AL, specifically NCM and CMM, contain the neural substrate of TSM and, more generally, sensory song learning. As such, the remainder of this introduction, and the work presented in this dissertation, predominately focuses on AL development in the context of age, experience, and sex.

The potential importance of NCM and CMM in sensory song learning first emerged from studies examining the induction of mRNA coding for the immediate early gene (IEG) EGR-1 (commonly referred to as ZENK in songbird literature, an acronym for, ZIF268, EGR-1, NGFI-A, and Krox-24). David Clayton and colleagues observed that EGR-1 mRNA levels rapidly increase in the ALs of adult zebra finches following the presentation of tape-recorded songs. EGR-1 induction in AL was greater in response to conspecific song than heterospecific song and absent in response to tonal bursts, which, notably, are sufficient to induce an electrophysiological response in the same brain region (Mello et al. 1992; Stripling et al. 2001). This seminal work was the first to demonstrate a genomic response potentially associated with song recognition, discrimination, or the formation of auditory memory in AL. Additionally, this work provided the first indication that an auditory stimulus capable of inducing an electrophysiological response in AL may not be sufficient to induce a molecular response.

Work by the same group shortly thereafter demonstrated that EGR-1 induction was not homogeneous throughout AL, but was isolated to NCM, CMM, and regions L1 and L3 of field L (absent in L2). Additionally, induction was observed in HVC-shelf and RA-cup, targets of AL projections, but not in song control nuclei LMAN, Area X, HVC, or RA (Mello & Clayton 1994). In contrast, EGR-1 is induced in song control nuclei during song production (Jarvis & Nottebohm 1997). These results provided some of the first evidence supporting the hypothesis that AL contributes to the neural substrate of sensory song learning (Ribeiro & Mello 2000; Solis et al. 2000).

Further evidence for this hypothesis originated from subsequent experiments demonstrating that repeated exposure to the same song results in decreased molecular and electrophysiological responsivity in NCM (Chew et al. 1995; Mello et al. 1995; Chew et al. 1996).

EGR-1 mRNA transcript abundance increases in adult male NCM for 30 minutes in response to repeated playback of a single song, after which time abundance will incrementally decrease back to baseline, even in the presence of persistent playback. Re-exposure to the same song the following day fails to induce EGR-1 transcription, while novel song does. The authors interpreted this to mean that individual cells in NCM underwent a selective loss in their EGR-1 responsiveness to the repeated song, but maintained the ability to respond to the novel song (Mello et al. 1995). Multiunit extracellular recording demonstrated that NCM neurons of adult males and females demonstrate fast, long-lasting, and stimulus-specific habituation. Habituation for conspecific calls and songs persisted longer than habituation for other stimuli (heterospecific songs, tone sequences, human speech, and bouts of white noise) and was not influenced by exposure to different stimuli of the same class, suggesting that NCM may support the recall of vocalization from many conspecifics (Chew et al. 1996). Administration of non-selective RNA or protein synthesis inhibitors, actinomycin D or cycloheximide, respectively, into NCM following song experience does not alter initial habituation to song, but impairs long-term habituation, demonstrating that maintenance of habitation depends on the local induction of gene expression in NCM (Chew et al. 1995). Future work revealed that rate of habituation in adult NCM is lower for tutor song than novel conspecific song, potentially reflecting recognition of the learned tutor song (Phan et al. 2006). Furthermore, lower rate of habituation to tutor song, relative to novel conspecific song, correlated with the degree of similarity between the tutor's song and the BOS (Phan et al. 2006).

The hypothesis that NCM is, at least in part, the neural substrate for sensory song learning was further bolstered by a series of experiments demonstrating a relationship between IEG induction and the strength of song learning (Bolhuis et al. 2000; Bolhuis et al. 2001; Terpstra et al. 2004). Adult males, re-exposed to the song on which they were tutored as juveniles,

demonstrate an induction of ERG-1 and c-Fos, an IEG also broadly implicated in learning and memory formation. There was a significant positive correlation between the number of song elements copied from the tutor song and the strength of EGR-1 and c-Fos induction in NCM (but not CMM) (Bolhuis et al. 2000; Bolhuis et al. 2001; Terpstra et al. 2004). This work demonstrates that molecular response in adult NCM is influenced by juvenile experience and that the degree of that response is directly correlated with the amount of song memorized. A similar relationship between EGR-1 induction and tutor song experience has been demonstrated in juvenile males within the CP for TSM (P54-P59). EGR-1 protein synthesis was greater in response to tutor song than to novel song or silence in NCM and was greater in response to tutor song than to silence in CMM (Gobes et al. 2010).

Arguably, the strongest evidence that AL contains the neural substrate for sensory song learning comes from work demonstrating that the inhibition of MEK (Mitogen-activated protein kinase kinase), a kinase that phosphorylates ERK (Extracellular signal-regulated kinase) – also commonly referred to as MAPK (Mitogen-activate protein kinase) – in NCM exclusively during periods of tutor song exposure in juvenile males reduces the similarity between the song they sing in adulthood and that of their tutor (London & Clayton 2008). Phosphorylation of ERK increases in AL following exposure to song, but not to tones or noise, and returns to basal levels shortly thereafter (Cheng & Clayton 2004). Similar to EGR-1 induction, ERK phosphorylation following song experience is localized to AL, attenuated following repeated exposure to the same song, and retriggered by exposure to a novel song (Cheng & Clayton 2004). Inhibition of extracellular-regulated protein kinase kinase (MEK; the enzyme responsible increasing ERK activation through phosphorylation) prevents the induction EGR-1 mRNA transcription following song exposure (Cheng & Clayton 2004). In males otherwise isolated from hearing tutor song during the CP for

TSM, London and colleagues administered the ERK inhibitor U0126, via bilateral cannulation targeting NCM, during tape-recorded tutor song playbacks. Males that received U0126 infusions did not learn to imitate the song of their tutor, but control males that received infusions of U0124, a molecularly similar inactive form of U0126, learned to imitate the song of their tutor. U0126 infusion did not appear to disrupt the general AL function, as males receiving U0126 infusion were still capable of TSM if song exposure occurred after the effects of U0126 administration had dissipated. Furthermore, U0126 administration did not appear to disrupt general auditory processing in AL as treated juveniles could still perform auditory discrimination required for an operant conditioning task (London & Clayton 2008).

Transcriptomic responsivity in the auditory forebrain: beyond immediate early genes

The induction of IEGs (e.g., EGR-1, FOS, and ARC) are thought to participate in the early stages of molecular cascades that link extracellular stimulation to changes in neuronal gene expression and long-lasting alteration in synaptic efficacy. Such molecular cascades influence synaptic plasticity through the induction of other IEGs and genes that influence synaptic vesicle release, neurotransmitter metabolism, miRNA synthesis, receptor protein synthesis, protein phosphorylation, calcium signaling pathways, actin cytoskeleton organization, and proteosome activity (Clayton 2000; Ribeiro & Mello 2000; Duclot & Kabbaj 2017; Clayton et al. 2020). The regulation of such later stage biological processes is consistent with the requirement of a second wave of protein synthesis to support long term song habituation following repeated song exposure (Chew et al. 1995) and the knowledge that some IEGs, including EGR-1, appear to support the maintenance of long-term potentiation (LTP), rather than its induction (Richardson et al. 1992; Abraham et al. 1993; Jones et al. 2001; Knapska & Kaczmarek 2004; Wlodarczyk et al. 2011).

Notably, investigations of transcriptional responsivity in the AL to song have demonstrated the induction of genes associated with just such biological processes. For example, various isoforms of synapsins (syn2a, syn2b, and syn3), a protein family implicated in the regulation of synaptic vesicle storage and release, are induced by conspecific song in NCM of adult males and females and regulated by EGR-1 binding (Thiel et al. 1994; Petersohn et al. 1995; Velho & Mello 2008). Song exposure also leads to bidirectional changes in the degree of expression of microRNAs implicated in the regulation of neural differentiation (miR-124) or that putatively regulate genes that control neuronal differentiation (tgu-miR-2954-3p) in the AL of adult males and females (Gunaratne et al. 2011). Interestingly, one of the conserved microRNAs identified from this study, miR-124, which was down regulated following song exposure, has since been demonstrated to directly binding and inhibit EGR-1 (Yang et al. 2012; Sun et al. 2015; Liu et al. 2016a; Wang et al. 2016).

Thus, while a handful studies have begun to identify individual genes involved the transcriptomic response to song, relatively few studies have performed investigations on the scale necessary to best capture the complex genomic response likely necessary to support sensory song learning, and even fewer studies have performed such assays in the context of development. One study that has accomplished this utilized microarray and in situ hybridization to compare the transcriptomic response to song in the ALs of P20 and adult (>P100) males (London et al. 2009). This identified hundreds of genes that significantly differed in the degree of expression between adults that had and had not been exposed to song. However, under the same statistical stringency, only a single gene differed in the degree of expression between P20s that had and had not been exposed to song. The lack of transcriptomic song responsivity observed at P20 was attributed to a majority of the adult song-responsive genes being expressed at constitutively high levels at P20,

mirroring measures of EGR-1 responsivity made at that time point (Jin & Clayton 1997; Stripling et al. 2001). Furthermore, the experiment identified hundreds of genes differing in expression level between P20s and adults independent of song exposure condition, further highlighting the value of experiments performed in the context of development.

Developmental differences in molecular responsivity to song between males and females

As previously discussed, current evidence suggests that TSM begins in males at P30 and that sensory song learning begins in females at a similar developmental time point. Adding credence to the theory that these may be different types of learning, males and females differ in molecular responsivity within AL following song exposure at P30. At P30, but not at P20, normally reared juvenile male zebra finches, but not males reared in isolation of tutor song, demonstrate an induction of EGR-1 mRNA in the AL (Jin & Clayton 1997; Stripling et al. 2001). The absolute magnitude of EGR-1 mRNA was highest in P30 males, while fold-induction was greater in adults, in part due to a decreased basal expression in adulthood (Jin & Clayton 1997; Stripling et al. 2001). In contrast, P30 females do not demonstrate an induction of EGR-1 protein following exposure to conspecific song, but do demonstrate an induction of FOS, another IEG implicated in the regulation of learning and memory formation and a subunit of the AP-1 transcription factor family (Bailey & Wade 2003, 2005; Alberini 2009). However, at P45, males and females demonstrate similar patterns of induction for both EGR-1 and FOS in response to conspecific song (Bailey & Wade 2005) and in adulthood males and females demonstrate similar patterns of induction EGR-1, c-FOS, and ARC (Mello et al. 1992; Mello & Clayton 1994; Nastiuk et al. 1994; Velho et al. 2005). The observed differences in juvenile IEG induction suggest that

either males and females engage different neural mechanisms for song learning or that they differ in their developmental trajectories but settle at a common pattern of IEG induction in adulthood.

Further evidence supporting differences in molecular responsivity to song between juvenile males and females stems from investigations of the mechanistic Target of Rapamycin (mTOR) signaling cascade. The mTOR signaling cascade integrates environmental signals relayed by multiple upstream receptor systems and influences synaptic communication by regulating down-stream effector proteins eukaryotic initiation factor 4E-binding protein (4EBP1) and S6 kinase (S6K), which each alter the rate of protein synthesis (Hay & Sonenberg 2004; Hoeffer & Klann 2010; Graber et al. 2013; Garza-Lombó & Gonsebatt 2016). mTOR signaling has been shown to influence learned behaviors in rodent models (Garza-Lombó & Gonsebatt 2016; Giovannini & Lana 2016; Kazdoba et al. 2016; Lana et al. 2017) and more recently in juvenile male zebra finches (Ahmadiantehrani & London 2017a). Hearing conspecific song activates the mTOR signaling cascade in the AL of P30 males, but not P23 males or females at either age (Ahmadiantehrani & London 2017a). A direct relationship between mTOR signaling and TSM was established via bidirectional manipulation of mTOR through the infusion of rapamycin, a constitutive activator of mTOR signaling, or SC79, a selective inhibitor of mTOR signaling, targeting the AL. The administration of either rapamycin or SC79 diminished tutor song copying, suggesting that balanced mTOR signaling may also characterize the onset of TSM in males. Furthermore, similar to ERK, mTOR signaling also demonstrates signatures of habituation, suggesting that it, too, is influenced by previous experience (Ahmadiantehrani et al. 2018).

Cellular composition of the auditory forebrain

Substantial progress has been made towards identifying the cell types that compose the AL, yet the exact compliment of cell types required to support sensory song learning within the AL remains unknown, as does the progression of these cell types across development.

Catecholamine synthesizing neurons (specifically those synthesizing norepinephrine and dopamine) project from the locus coeruleus (LC) and ventral tegmental area (VTA) to auditory processing areas of the brain and differentially respond to auditory stimuli based on familiarity (Carcea & Froemke 2013; Sara 2015). Investigations of cell-type specificity within the AL utilized dopamine beta-hydroxylase (DBH) to label noradrenergic fibers throughout the zebra finch brain demonstrate a relatively high degree of noradrenergic innervation throughout the AL, with minimal variation between AL subregions (Mello et al. 1998). Song-responsive neurons in NCM express alpha- and beta-adrenergic receptors near noradrenergic fiber terminals and treatment with alpha- and beta-adrenergic receptor antagonists (phentolamine and propranolol, respectively) during song experience reduces subsequent EGR-1 induction, but does not interfere with spontaneous or evoked electrophysiological activity within NCM (Velho et al. 2012). Alpha-adrenergic antagonism appears to disrupt the maintenance, but not the initiation, of the altered physiological state of the AL, supporting the hypothesis that noradrenergic innervation supports sustained changes to song-responsive neurons through the modulation of gene expression resulting from the electrophysiological response induced by song experience (Velho et al. 2012). Norepinephrine (NE), when infused into NCM during song exposure, enhances auditory response, burst firing, and coding properties of NCM neurons in both males and females (Lee et al. 2018). Interestingly, NE appears to alter song responsivity through different, yet compensatory, mechanisms in males and females. NE application increases NCM neuron response strength in

males by increasing auditory-evoked activity and in females by decreasing baseline firing rates, suggesting that in some respects the influence of catecholamines in evoked song response differs between males and females (Lee et al. 2018). Lastly, there is a correlation between the degree of EGR-1 induction in catecholaminergic neurons within the LC (but not in the VTA, substantia nigra (SN), or periaqueductal gray (PAG)) and degree of song familiarity that mirrors patterns of EGR-1 induction in NCM and CMM following song exposure (Dai et al. 2018). In agreement with this observation, the degree of catecholaminergic release onto NCM and CMM neurons varied in accordance with the degree EGR-1 response in VTA, further supporting a functional role for catecholamines in the processing of song (Dai et al. 2018).

GABAergic and glutamatergic neurons have also been implicated in the processing of song within the AL. GABAergic neurons are abundant throughout the AL and make up approximately half of the neurons composing NCM subregions (Pinaud et al. 2004; Pinaud et al. 2006; Pinaud & Mello 2007). Whole cell patch-clamp recordings in NCM slice preparations suggest that GABAergic neurons are spontaneously active, independent of excitatory input, and inhibit excitatory inputs onto NCM neurons via γ-Aminobutyric acid type A (GABAA) receptors. GABAergic inhibition has been implicated in the modulation of song response, as blockade of GABAA-mediated inhibition in NCM via application of bicuculline (BIC), a competitive GABAA receptor antagonist, alters temporal sequencing of song-evoked responses in NCM (Pinaud et al. 2008). Interestingly, GABAergic cell populations present differently in the NCM of males and females. Immunocytochemistry performed for calbindin, a calcium-binding protein expressed in a subset of GABAergic cells, revealed almost twice as many calbindin-positive neurons in male NCM than females NCM. Notably, calbindin-positive neurons did not demonstrate song-induced EGR-1 expression and neither their density, nor distribution, within NCM appeared affected by

song exposure, but this in no way precludes their potential involvement in modulating sensory song responsivity (Pinaud et al. 2006). Interestingly, work examining how GABAergic cell populations in AL differ as a function of age, sex, and experience, utilizing in situ hybridization for GAD65 and parvalbumin, markers for inhibitory cell sub-types, revealed minimal differences in the densities of GAD65- and parvalbumin-positive cells in NCM and CMM (Gogola et al. 2019). Glutamatergic signaling within NCM has also been implicated in the regulation of song responsivity. In comparison to control birds, song exposure increases the probability of glutamatergic neuron bursting under conditions of GABAergic transmission blockade (achieved via BIC application) (Dagostin et al. 2012). While this effect was observed in both males and females, bursting was more commonly observed in song-exposed males, even in the absence GABAergic transmission blockade, suggesting a sex difference in the responsivity of glutamatergic cell populations within NCM (Dagostin et al. 2012).

Thus, while progress has been made towards identifying the cellular subtypes within the AL and in investigating their relations to molecular measures of song responsivity, the exact complement of cells required to support sensory song learning remains unknown. Why is this? Perhaps this reflects a shortcoming in the relatively gross measures of cell type specificity that have, thus far, been used to characterize the cellular composition of the AL. A more precise characterization of cellular subtypes within the AL, as is achievable through measures of regulatory region accessibility (Heintzman et al. 2009; Visel et al. 2009; Thurman et al. 2012; Blankvoort et al. 2018; Nair et al. 2020), may provide the necessary detail to characterize and distinguish between the cellular subtypes within the AL that do and do not support sensory song learning. Addressing this gap in knowledge is paramount in furthering our comprehension of how

maturation-dependent and experience-dependent plasticity intersect to regulate sensory song learning.

Dissertation Goal

The overarching goal this dissertation is to examine how regulatory region accessibility profiles within the AL change in relation to age, sex, and experience as a means to [1] identify the cellular subtypes composing the AL that distinguish birds capable and incapable of sensory song learning or inform the question of if mechanisms of sensory song learning differ between males and females and [2] further our understanding of the molecular, cellular, and biological processes that support the encoding of developmental experiences that guide adult behavioral patterns.

Chapter II: Developmental shifts in regulatory region accessibility spanning the onset of the CP for TSM

Introduction

Based on existing molecular and behavioral data, there were two major questions regarding epigenetic regulation that I sought to address: [1] Are regulatory region accessibility profiles changing in males as they transition into their CP for TSM, and if so, how? [2] Are female regulatory region accessibility profiles changing similarly, or do females demonstrate different epigenetic profiles for learning at this stage of development? Utilizing ChIP-Seq for H3K27ac, I identified putative accessible regulatory regions spanning the genome in birds differing in age and sex. I compared experimental groups to identify differentially accessible regions, identified TFBSs enriched with those regions, and assigned genes to each differentially accessible region by nearest TSS. Finally, I utilized GO ontology analysis and an in-depth literature search to examine how TFs with enriched binding sites and putatively regulated genes may influence AL function in the context of development and sex.

Methods

Animals and housing

All procedures were conducted in accordance with the NIH guidelines for the care and use of animals for experimentation and were approved by the University of Chicago Institutional Animal Care and Use Committee (ACUP no. 72220). All experimental birds were hatched in the London laboratory breeding colony at the University of Chicago. Animals were housed on a 14:10 h light/dark cycle. Food and water were provided ad libitum.

To test the effect of age and sex on accessible chromatin profiles, males and females were reared in 'Normal' conditions; they spent the entirety of their lives in the communal aviaries in which they hatched. Male and female 'Normal' birds were collected at four ages: post-hatch (P) day 23 (one week prior to the opening of the CP for TSM), P30 (at the onset of the CP for TSM), P60 (towards the end of the CP for TSM), and P67 (at the close of the CP for TSM). To parse the roles of maturation and tutor song experience in establishing regulatory region accessibility profiles related to the known experience-dependent close of the critical period, two independent sets of male birds were reared in 'Tutored' and 'Isolate' controlled conditions. All Tutored and Isolate birds were removed from home aviaries between P21 and P23 and moved to cages within a sound-attenuating chamber coinhabited by two adult foster females and one or two other juveniles of the same age. At P30, each Tutored male was moved to a new cage within a sound-attenuating chamber to live with one adult female bird and one adult male bird. To control for the complexity of social interaction each bird experienced while preventing song exposure from a tutor male, at P30, each Isolate male was moved to a new cage within a sound-attenuating chamber to live with two adult female birds. Tutored and Isolate males remained in these conditions until P67.

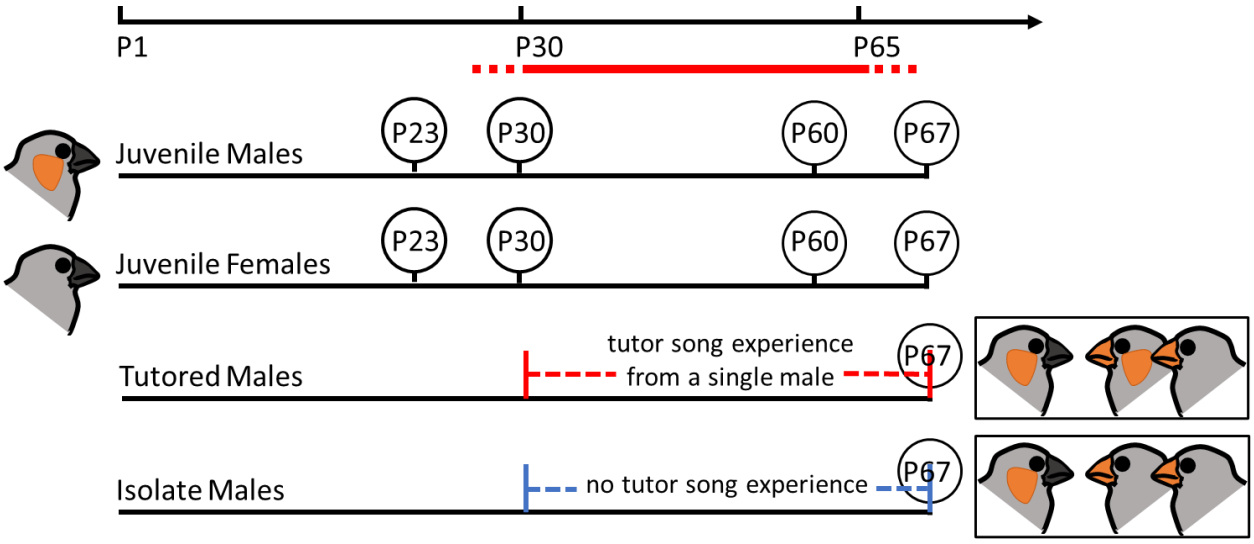

Figure 3. Schematic of experimental group conditions and ages of collection. Juvenile males and females were reared in the communal aviaries in which they hatched until post-hatch (P) day 23, P30, P60, and P67. Tutored and isolate males were removed from the communal aviaries in which they hatched at ~P22 and moved sound-attenuating chamber coinhabited by two adult foster females. At P30, Tutored males were then moved to a new sound-attenuating chamber to live with an adult male-female pair (depicted by the upper rectangular panel in the lower right) when they heard the song of a single male till P67 (red dashed line). At P30, isolate males were then moved to a new sound-attenuating chamber to live with an adult female-female pair (depicted by the lower rectangular panel in the lower right) when they were prevented from hearing any song till P67 (blue dashed line).

Auditory forebrain collections.

Auditory forebrain (AL) collections were performed between 6- and 8-hours post-lights-on. ALs were dissected within 2 minutes of sacrifice, and the bilateral tissues were immediately flash-frozen using dry ice. All tissue was stored at -80°C until further processing. The sex of all birds was confirmed by visual inspection of the testes or ovary. To obtain enough chromatin to perform successful ChIPseq for H3K27ac, each biological sample was a pool of ALs from 3 individuals. To avoid introducing bias based on genetic relatedness or parental rearing behavior, no biological pool contained tissue from siblings. As such, birds were not randomly assigned to

conditions. ALs were distributed between biological replicates so each pool had a similar mass (30-50 mg). There were n=3 pooled samples for all ten conditions: P23 males, P23 females, P30 males, P30 females, P60 males, P60 females, P67 males, P67 females, P67 Tutored males, and P67 Isolate males.

Chromatin immunoprecipitation and sequencing (ChIP-Seq)

ChIP-Seq for H3K27ac histone modifications was performed by the Service department at Active Motif using a previously validated antibody for immunoprecipitation (Active Motif Cat# 39133; Carlsbad, CA). Input was obtained from sequencing a pool of unprecipitated DNA combined in equal proportions from all samples.

ChIP-Seq read quality assessment and genomic alignment

DNAseq reads were quality-checked and trimmed using FastQC (Andrews 2010) and Trim Galore (Martin 2011; Krueger 2012). Sequences of 20 nucleotides or less in length, after poor-quality nucleotide and adaptor removal, were not used for subsequent analysis. Reads were then mapped to the 2021 zebra finch genome assembly (RefSeq bTaeGut1.4.pri) using Bowtie 2 (Langmead & Salzberg 2012; Rhie et al. 2021) set with default sensitive parameters for seeding and multiseed alignment. SAMtools (Li et al. 2009) was used to convert between SAM and BAM file formats, to filter out reads that did not uniquely map to the genome, and to generate corresponding index files.

Peak calling and quality assessment

Regions of enriched read alignment (peaks) were identified using MACS2 (Zhang et al. 2008; Feng et al. 2012a) with an effective genome size of 2.09E+09 and a q-value cutoff of 0.01. Before performing differential analysis, the quality of the obtained reads and peaks was assessed

using ChIPQC (Carroll et al. 2014). One-way ANOVAs were conducted examining the effect of condition on the number of reads obtained, the number of reads removed, the number of reads remaining following read removal, the percentage of reads removed, the percentage of reads mapped to the genome, the number of called peaks, average peak length, the number of nucleotides contained within peaks, average peak length, average number of reads per peak, and average peak signal value. If a significant effect of condition was observed, post hoc Tukey tests were conducted to determine which groups significantly differed from each other.

Differentially accessible region identification

Significant differential regions between experimental groups were identified using DiffBind (Stark & Brown 2011; Ross-Innes et al. 2012). Regions of differential enrichment identified with DESeq2 (Love et al. 2014) with an FDR value less than 0.05 were used for downstream analysis. Called peak sets contained both broad and narrow peaks. Thus, to best capture differences in enrichment between experimental groups, differential regions were called both with and without utilizing the DiffBind summit parameter. Visual investigation in Integrative Genomics Viewer (IGV) (Robinson et al. 2011) confirmed that each method of differential region identification captured different sets of reliable differential regions. The summited and non-summited outputs were merged using bedtools (Quinlan & Hall 2010; Quinlan 2014), maintaining broader peaks at locations of overlap.

Differentially accessible region annotation

ChIPseeker (Yu et al. 2015) was used to annotate differential regions according to the NCBI genome annotation corresponding to the bTaeGut1.4.pri assembly (GCF_003957565.2). Peaks were labeled as putative enhancers if located further than 5 kb from the nearest

transcriptional start site (TSS) and putative promoters if located within 5 kb of a TSS (Blankvoort et al. 2018).

Nucleosome-free region identification

Transcription factor binding sites (TFBSs) are located within nucleosome-free regions (NFRs), stretches of the genome closely flanked, but not directly bound, by nucleosomes. Not only are NFRs the most probable location of TFBSs, but NFR identification also reduces the amount of sequence utilized for TFBS identification, thereby diminishing the potential for the detection of false positive TFBS. NFRs are more accurately identified within broad peaks than narrow peaks. To facilitate the most accurate identification of NFRs, a second round of peak identification was performed using MACS2 (Zhang et al. 2008; Feng et al. 2012a). All peak calling parameters utilized for identifying narrow peaks were maintained, except the broad function, and a broad cutoff value of 0.1 was applied. NFRs were identified from each sample's resulting broad peak profiles using HisTrader (Yan et al. 2020). NFR profiles from each set of biological replicates were merged using bedtools (Quinlan & Hall 2010; Quinlan 2014) to produce a single NFR profile for each condition. Bedtools (Quinlan & Hall 2010; Quinlan 2014) was used to reduce differential regions to their NFR sequences by intersecting the differential regions with the NFR profile corresponding to the condition in which the peaks demonstrated greater enrichment. Finally, bedtools (Quinlan & Hall 2010; Quinlan 2014) was used to convert NFR BED files into FASTA files containing the necessary nucleotide sequences for TFBS identification.

Transcription factor binding site enrichment analysis

Simple Enrichment Analysis (SEA) (Bailey et al. 2015; Bailey & Grant 2021), belonging to the MEME Suite of programs, was utilized to identify TFBSs enriched within each set of differential region NFRs. A background model was generated by shuffling the input sequences,

and an E-value ≤ 10 was used as a statistical enrichment cutoff. The JASPAR CORE (2022) non-redundant vertebrates database (Castro-Mondragon et al. 2022) was used as input for the motifs tested.

GO term enrichment analysis

GO term enrichment analysis was performed on TF and gene sets using ShinyGO (Ge et al. 2020). The human genome (GRCh38.p13) was utilized for all GO term enrichment analyses. Background was composed of all protein-coding genes in the genome. Many enriched TFBSs identified were for protein dimers. GO is not designed to accept protein dimers as input. As such, TF dimers were split into their component proteins when performing GO enrichment analysis. For generating GO enrichment visualizations, terms with the greatest number of contributing TFs or genes were prioritized. For the generation of GO enrichment visualizations pertaining to the function of TFs, terms inherent to the function of all TFs were not displayed (e.g., Positive/Negative regulation of transcription, DNA-templated, Positive/Negative regulation of nucleic acid-templated transcription, Positive/Negative regulation of biosynthetic process), nor were terms entirely unrelated to brain development or function (e.g., Skeletal system development, Reproductive system development, Embryo development ending in birth or egg hatching). The same filtering was not applied when generating GO enrichment visualizations pertaining to the function of genes. All GO terms resulting from enrichment analysis and their corresponding statistical values are maintained in results files.

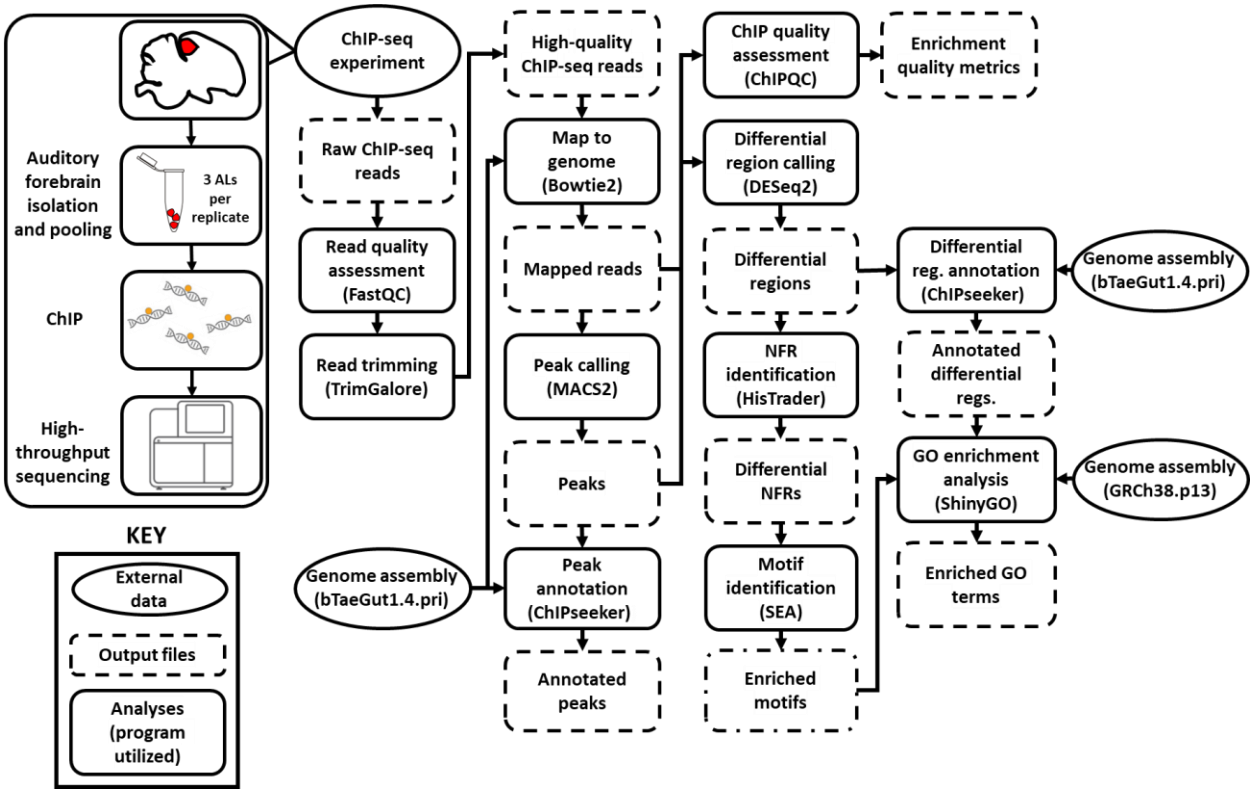

Figure 4. Bioinformatic analysis pipeline. A flowchart depicting the progression of analysis applied to the data obtained from the H3K27ac ChIP-Seq experiment (depicted in a box in the upper left of the figure). Each step of the analysis pipeline is contained within a solid box, and the program utilized to perform the analysis is found in parenthesis in the same box. Outputs from each stage of analysis are contained within dashed boxes proceeding each stage of analysis. Externally obtained data sources are contained within solid ovals. Arrows indicate the directional flow of analyses and the steps at which externally obtained data was utilized. A key is provided in the lower left of the figure.

Results

P23 and P30 ChIP-Seq data meet quality control measures

Sample quality and reproducibility measures indicate that the data are suitable for further downstream analysis. An average of 34,271,218 reads (75 nts in length) were obtained per ChIP, with at least 28,988,336 reads obtained for each sample (Figure 5A). Following read trimming, samples maintained an average of 33,182,950 reads, with at least 28,135,662 reads maintained for

each sample (Figure 5C, Table 1). A one-way ANOVA revealed no main effect ($F(3,8) = 3.14$, $p = 0.087$) of condition on read filtering (Figure 5B), suggesting that there was no overall difference in the quality of reads obtained across samples. At least 95% of reads from each sample aligned to the genome, and a one-way ANOVA revealed no main effect of condition on the proportion of reads mapped to the genome ($F(3,8) = 0.926$, $p = 0.47$) (Figure 5D). An average of 54,549, but no fewer than 48,942, peaks were called for each sample (Figure 5E). One-way ANOVAs revealed no main effect of condition on average peak length ($F(3,8) = 0.496$, $p = 0.69$) or average peak signal value ($F(3,8) = 1.083$, $p = 0.41$) (Figure 5F, 5H). However, a one-way ANOVA revealed a significant main effect ($F(3,8) = 6.58$, $p = 0.018$) of condition on the average number of reads per peak, and post hoc Tukey tests showed that P23 male peaks had, on average, a greater number of reads associated with each peak than P30 males ($p\ adj = 0.045$) and P30 females ($p\ adj = 0.038$) (Figure 5G). The difference in the average number of reads per peak did appear to influence overall peak strength, as measured by average signal value (Figure 5H).

Assessment of peak sets showed that metrics of quality control were similar across samples (Table 1) and consistent with published profiles, peaks were predominately located in promoter, intronic, and distal intergenic regions for all samples (Figure 6A). Correlation values between individual replicates revealed that same-sex samples more closely correlated with each other than samples of the opposite sex, excluding sample ‘P30F-N1’, which demonstrated relatively low correlation with all other samples. Excluding sample ‘P30F-N1’, female, but not male, P23 and P30 replicates were more closely correlated with each other than same-sex replicates of the alternative time point (Figure 6B, 6C).

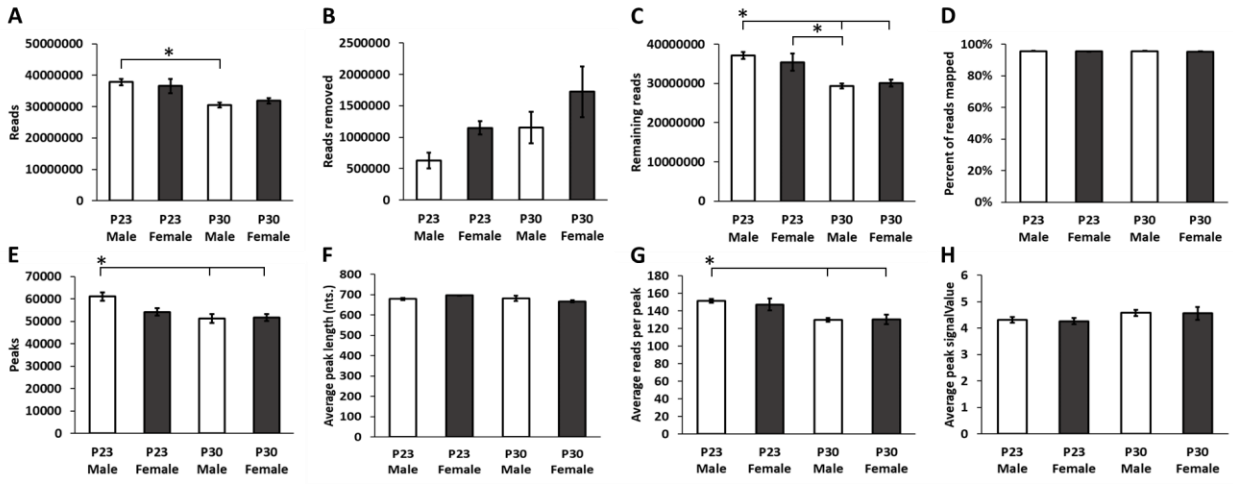

Figure 5. Comparison of read processing and peak characteristics across P23 and P30 experimental groups. Bars and asterisks indicate groups significantly differing from each other with a p-value < 0.05. Asterisks have been placed above the condition significantly differing from the others for significance indicators involving more than two groups. (A) The number of reads obtained from ChIP-Seq. (B) The number of reads removed following QC. (C) The number of reads remaining following read removal. (D) The percentage of remaining reads mapped to the genome. (E) The number of called peaks. (F) Average peak length in nucleotides (nts.). (G) Average number of reads per peak. (H) Average peak signalValue. SignalValue is a measurement of the average enrichment of a genomic region.

Sample ID	Reads	Map%	Filt%	Dup%	ReadL	FragL	RelCC	SSD	RiP%
P23-Male-N1	38973080	100	8.09	0	74	204	2.23	0.795	23.2
P23-Male-N2	36196899	100	7.64	0	74	196	2.69	0.823	26.6
P23-Male-N3	36363108	100	7.27	0	74	204	2.82	0.783	25
P23-Female-N1	32663590	100	8.71	0	74	233	2.18	0.797	21.5
P23-Female-N2	33942434	100	7.71	0	74	203	3.02	0.795	23.3
P23-Female-N3	39685660	100	8.58	0	74	206	2.22	0.858	22.9
P30-Male-N1	30153024	100	8.44	0	73	229	2.29	0.784	21.8
P30-Male-N2	28135662	100	8.02	0	74	233	2.92	0.771	22.8
P30-Male-N3	29805116	100	8.17	0	74	198	2.19	0.779	23.3
P30-Female-N1	30778361	100	9.21	0	74	200	1.88	0.772	19.1
P30-Female-N2	28430304	100	7.79	0	74	230	3.2	0.805	25.4
P30-Female-N3	31207882	100	8.06	0	74	232	3.02	0.808	23.1

Table 1. Summary of ChIP-Seq quality metrics for P23 and P30 experimental replicates. Each row pertains to a single sample. Sample ID indicates the age and sex of the birds from which the sample was derived and the replicate number. Reads indicate the number of reads remaining following read trimming and removal. Map% is the percentage of reads that aligned to the genome. Filt% is the percentage of reads removed from downstream analysis for not meeting the q-value cutoff threshold of 15. Dup% is the percentage of reads for which there was an identical read aligned to the same genomic location. ReadL is the average length of reads. FragL is the estimated mean fragment length determined by systematically shifting the reads on each strand toward each other until the minimum genome coverage is achieved. RelCC is obtained by comparing the maximum cross-coverage peak to the cross-coverage at a shift size corresponding to the read length. Higher scores (generally 1 or greater) indicate good enrichment. SSD (standardized standard deviation) is computed by looking at the standard deviation of signal pile-up along the genome normalized to the total number of reads. RiP% indicates the percentage of reads located in peaks.

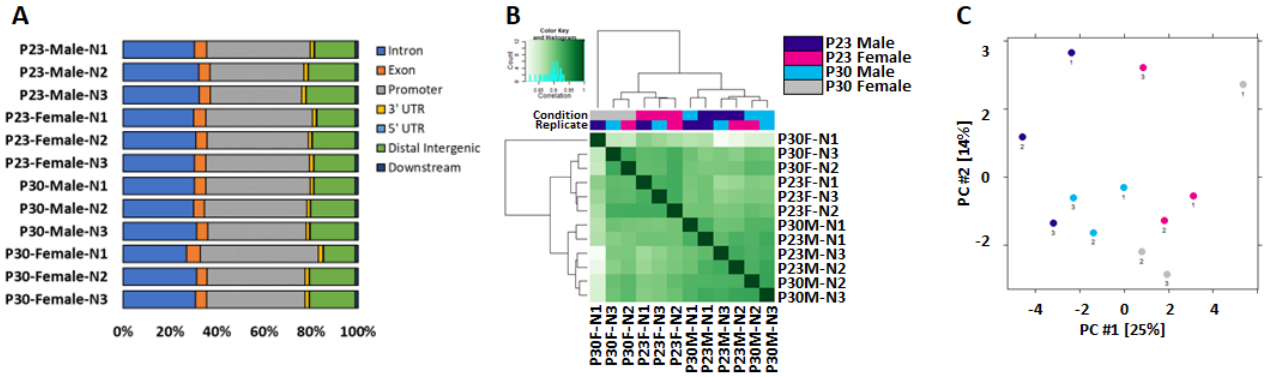

Figure 6. Comparison of replicate peak profiles of P23 and P30 experimental groups. (A) Distribution of peaks across genomic features for biological replicates from each experimental condition. (B) Heatmap of correlation values between experimental replicate peak profiles resulting from unsupervised hierarchical clustering. All samples are listed along the bottom and right side of the figure. Each shaded square indicates the correlation coefficient between two samples. A histogram in the upper left displays the frequency of correlation coefficients and the color of green shading associated with each coefficient. Correlation coefficients ranged from 0.7 to 1. Correlation coefficients of 1 were only obtained when comparing samples to themselves. Identical dendrograms are shown at the top and along the left side of the figure. Solid colored rectangles along the top figure correspond to condition (dark blue = P23 male, pink = P23 female, light blue = P30 male, gray = P30 female) and replicate number (dark blue = replicate 1, pink = replicate 2, light blue = replicate 3). (C) PCA of all replicates colored according to experimental group (dark blue = P23 male, light blue = P30 male, pink = P23 female, gray = P30 female). Principal component 1 is represented along the x-axis and accounts for 25% of the variance in the data. Principal component 2 is represented along the y-axis and accounts for 14% of the variance in the data.

Males had fewer accessible regulatory regions at P30 than at P23

I first sought to determine how the number of accessible regulatory regions changes between P23 and P30 in males. A one-way ANOVA revealed a significant main effect ($F(3,8) = 6.58$, $p = 0.015$) of condition on peak number, and post hoc Tukey tests showed that P23 males had a significantly greater number of peaks than P30 males (p adj = 0.018) (Figure 5E, 7A). The observed differences in peak number between P23 and P30 males appear to result from differences in chromatin accessibility rather than technical variation. A one-way ANOVA revealed a significant main effect ($F(3,8) = 6.79$, $p = 0.014$) of condition on read number, and post hoc Tukey

tests showed that a significantly greater number of reads were obtained for P23 males than P30 males (p adj = 0.022) (Figure 5A). One-way ANOVAs revealed no main effect of condition on read filtering ($F(3,8) = 3.14$, $p = 0.087$) (Figure 5B); thus, following QC and read removal, condition maintained a significant main effect ($F(3,8) = 6.58$, $p = 0.006$) on read number, and post hoc Tukey tests still showed that P23 males had a significantly greater number of reads than P30 males (p adj = 0.011) (Figure 5C). Observing no main effect of condition on the proportion of reads mapped to the genome ($F(3,8) = 0.926$, $p = 0.47$) (Figure 5D), average peak length ($F(3,8) = 0.496$, $p = 0.69$) (Figure 5F), or average peak signal value ($F(3,8) = 1.083$, $p = 0.41$) (Figure 5H), the difference in peak number between P23 and P30 males appear to be the result of differences in chromatin accessibility, rather than technical variation.

Differential analysis revealed regulatory regions with greater accessibility in P23 and P30 males

To investigate the effects of age on male regulatory region accessibility, I compared P23 and P30 male peak sets to identify differentially accessible regions of the genome. I identified 61 differentially accessible regions between P23 and P30 males, 35 regions were more accessible in P30 males ('P30M-over-P23M'), and 26 regions were more accessible in P23 males ('P23M-over-P30M') (Figure 7B).

Differentially accessible regions in males were associated with distinct biological processes

To understand how regions differing in accessibility between P23 and P30 males might influence the neural properties that support TSM, I determined what TFs may contribute to differences in learning potential between experimental groups by identifying TFBSs enriched in the regions differing in accessibility between P23 and P30 males. P23M-over-P30M regions were enriched with TFBSs for a single TF, MEF2A, and P30M-over-P23M regions were enriched with

TFBSs for 13 TFs, including MEF2D, ARX, and EN1 (Figure 7C, 7D). I performed GO term enrichment analysis on TFs with binding sites enriched in P30M-over-P23M regions to determine the biological processes that might be initiating to support TSM at P30 in males. GO term enrichment analysis of P30M-over-P23M TFs highlighted general themes of cellular proliferation, differentiation, and tissue development, but also highlighted themes specifically relevant to brain development, including neurogenesis, neuron differentiation, and neuron projection guidance (Figure 7E).

To further investigate how regions differing in accessibility between P23 and P30 males might influence the neural properties that support TSM, I ascribed the gene with the nearest TSS to each differentially accessible region and performed GO term enrichment analysis for biological process (BP), cellular component (CC), and molecular function (MF). The only terms significantly enriched in the genes associated with P23M-over-P30M regions were Anchored component of plasma membrane and Carbohydrate binding (Figure 8A). Terms significantly enriched in the genes associated with P30M-over-P23M regions highlight processes related to synaptic function, with an emphasis on glutamatergic signaling (Figure 8B).

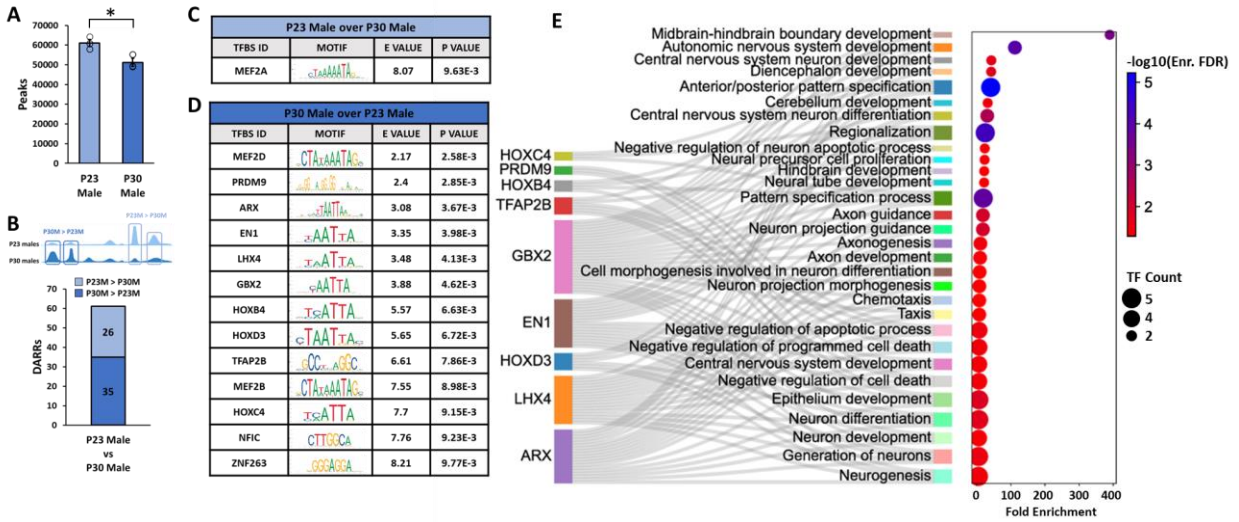

Figure 7. Comparison of P23 and P30 male regulatory region accessibility profiles. (A) The number of called peaks for P23 and P30 males. (B) The number of differentially accessible regions for P23 and P30 males. (C) TFBSs enriched in P23M-over-P30M regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (D) TFBSs enriched in P30M-over-P23M regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (E) Sankey dot plot displaying results from biological process GO term enrichment analysis performed on P30M-over-P23M TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results file. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

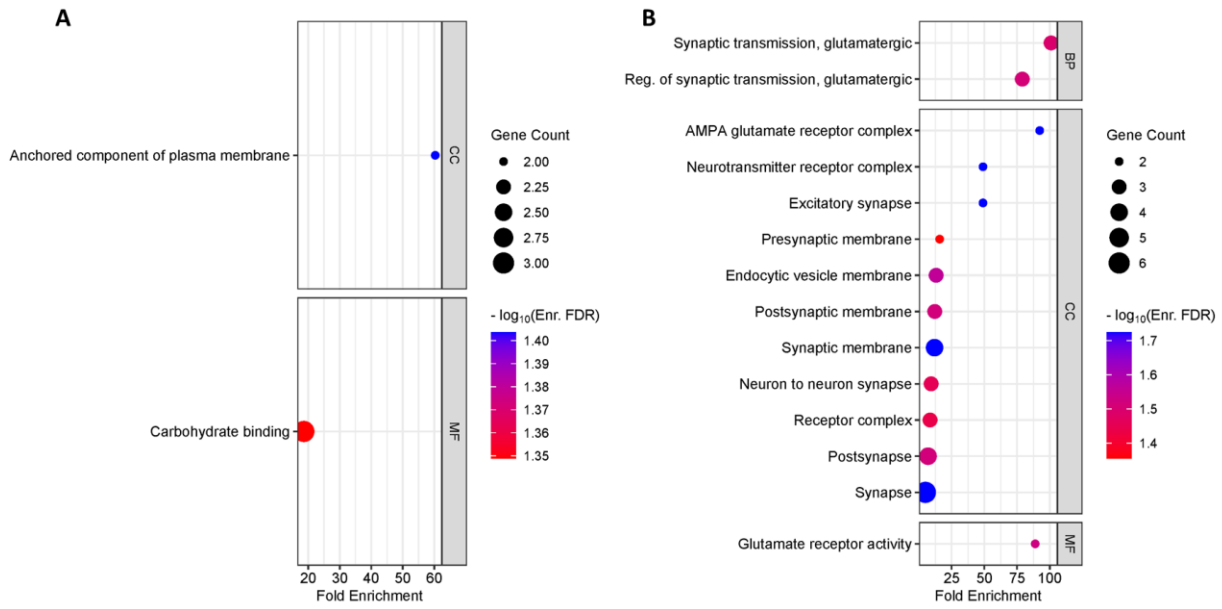

Figure 8. GO term enrichment for P23M-over-P30M genes and P30M-over-P23M genes. (A) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P23M-over-P30M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. (B) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P30M-over-P23M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Females had a similar number of accessible regulatory regions at P23 and P30

To determine if female regulatory region accessibility profiles are changing across development similarly to how they are in males, I applied the same bioinformatic analysis pipeline to compare P23 and P30 females. Unlike males, females do not undergo a reduction in regulatory region accessibility between P23 and P30 (Figure 5E, 9A). Although there was a significant main effect of ($F(3,8) = 6.58$, $p = 0.0149$) of condition on peak number, post hoc Tukey tests showed

that P23 and P30 females did not significantly differ (p adj = 0.77) (Figure 5E). P23 and P30 females did not differ in the number of reads removed ($F(3,8) = 3.14$, $p = 0.087$) (Figure 5B), the proportion of reads mapped to the genome ($F(3,8) = 0.926$, $p = 0.471$) (Figure 5D), the average number of reads per peak (p adj = 0.113) (Figure 5G), or average peak signal value ($F(3,8) = 1.083$, $p = .41$) (Figure 5H), suggesting that peak numbers were not influenced by technical variation in bioinformatic processing. This was the first indication from my experiment that the developmental trajectory of regulatory region accessibility in the AL differs between sexes.

Few regions are differentially accessible between P23 and P30 in females

To investigate the effects of age on female regulatory region accessibility, I compared P23 and P30 female peak sets to identify differentially accessible regions of the genome. There were ten-fold fewer differentially accessible regions between P23 and P30 in females than in males. I identified only 6 differentially accessible regions between P23 and P30 females, 5 of the regions were more accessible in P30 females ('P30F-over-P23F'), and 1 of the regions was more accessible in P23 females ('P23F-over-P30F') (Figure 9B). No regions differing in accessibility between P23 and P30 females overlapped regions differing in accessibility between P23 and P30 males.

Differentially accessible regions in females are associated with distinct biological processes

To understand how regions differing in accessibility between P23 and P30 females might influence the neural properties that support sensory song learning, I determined what TFs may contribute to differences in learning potential between experimental groups by identifying TFBSs enriched in the regions differing in accessibility between P23 and P30 females. Composed of only a single region, I could not perform TFBS enrichment analysis for P23F-over-P30F. P30F-over-P23F regions were enriched with TFBSs for 14 TFs, including ZNF148, HOXD13, and BATF3 (Figure 9C). Excluding PRDM9, there was no overlap in the TFs with enriched binding sites in

P30M-over-P23M and P30F-over-P23F regions, suggesting that different sets of TFs are increasing in their potential to influence transcription at P30 in males and females.

GO term enrichment analysis of P30F-over-P23F TFs highlighted general themes of cellular proliferation, differentiation, and tissue development, but did not identify themes specific to brain development. Instead, this analysis highlighted themes of responsivity (Figure 9D).

To further investigate how regions differing in accessibility between P23 and P30 females might influence the neural properties that support sensory song learning, I ascribed the gene with the nearest TSS to each differentially accessible region and performed GO term enrichment analysis to derive biological function (Figure 10A). The single P23F-over-P30F region was assigned to the gene CCDC43, a broadly expressed cytosolic protein that has predominantly been investigated for its potential role in cancer cell growth and metastasis (Wang et al. 2020; Chen et al. 2022). The three characterized genes associated with P30F-over-P23F regions were DRD5, JAM3, and BTF3. DRD5 encodes a G-protein coupled dopamine receptor shown to regulate hippocampal-dependent memory formation (Sariñana et al. 2014; Sarinana & Tonegawa 2016). JAM3, also ascribed to P30M-over-P23M regions, encodes a junctional adhesion molecule important for both neuron development and cell adhesion (Mochida et al. 2010). BTF3 encodes basic transcription factor 3, a protein that complexes with RNA polymerase II to initiate transcription (Zheng et al. 1987).

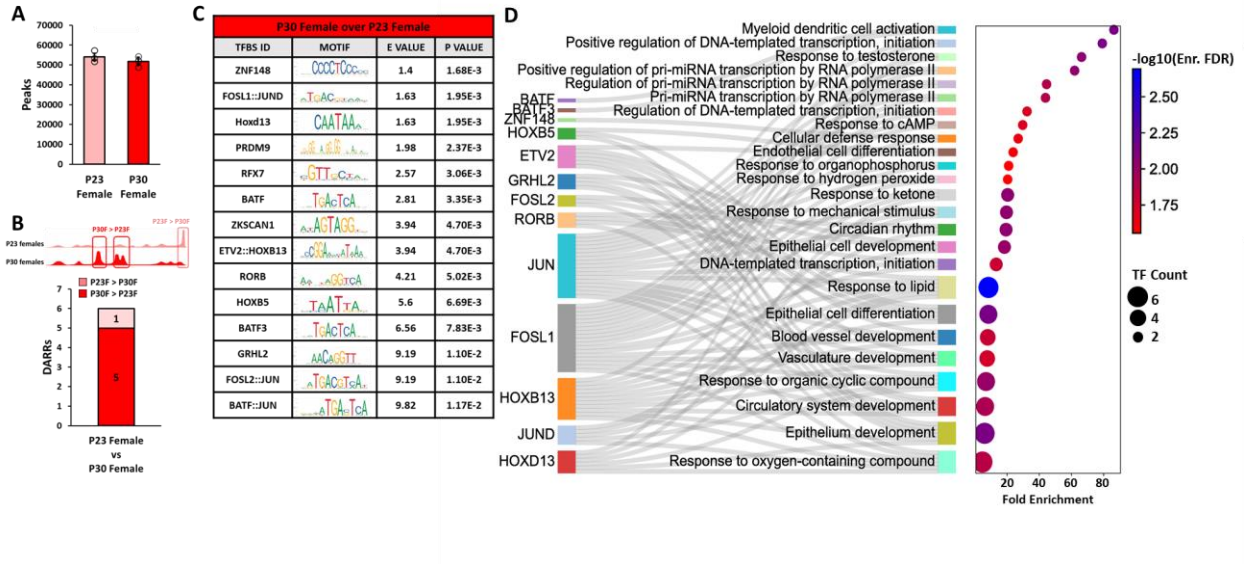

Figure 9. Comparison of P23 and P30 female regulatory region accessibility profiles. (A) The number of called peaks for P23 and P30 females. (B) The number of differentially accessible regions for P23 and P30 females. (C) TFBSs enriched in P30F-over-P23F regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (E) Sankey dot plot displaying results from biological process GO term enrichment analysis performed on P30F-over-P23F TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results file. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

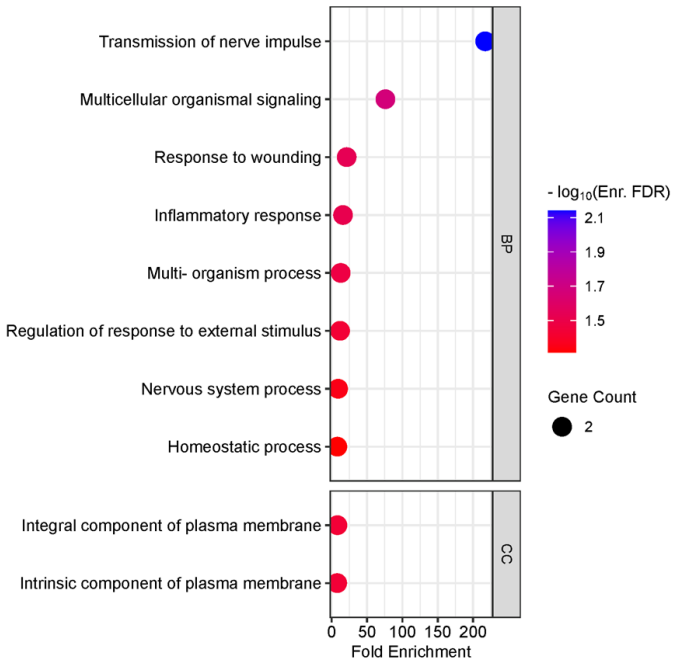

Figure 10. GO term enrichment for P30F-over-P23F genes. Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P30F-over-P23F genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Males and female regulatory region accessibility profiles were more similar at P30 than at P23

To investigate the influence of sex on regulatory region accessibility and explore potential differences in mechanisms of sensory song learning, I compared P30 male and female peak sets to identify regulatory regions differing in accessibility between them. Additionally, to determine how sexually dimorphic regions change across development, I identify regions differing in accessibility between P23 males and females. The results suggest that regulatory region accessibility differs to

a greater extent at P23 than at P30, and at both ages, a large majority of differentially accessible regions were more accessible in males than females (Figure 11A). Comparison of age-matched males and females identified 2486 differentially accessible regions at P23 and 1593 differentially accessible regions at P30. At P23, 1929 regions were more accessible in males ('P23M-over-P23F'), and 557 regions were more accessible in females ('P23F-over-P23M'). At P30, 1294 regions were more accessible in males ('P30M-over-P30F'), and 302 regions were more accessible in females (Figure 11A) ('P30F-over-P30M').

Sexually dimorphic regions were predominately located on sex chromosomes

At P23, 80.1% of sexually dimorphic regions were located on sex chromosomes, and at P30, 94.2% of sexually dimorphic regions were located on sex chromosomes, suggesting that sexually dimorphic regions further consolidate to sex chromosomes between P23 and P30. At P23 and P30, all sexually dimorphic regions located on chromosome W were more accessible in females, and excluding a single region, maintained as differentially accessible and located at the same genomic location at both P23 and P30, all sexually dimorphic regions located on chromosome Z were more accessible in males.

Sexually dimorphic regions were enriched with fewer TFBS at P30 than at P23

To determine how sexually dimorphic regulatory regions might influence the development of neural properties that support sensory song learning, I identified TFBSs enriched in sexually dimorphic regions at P23 and P30 and compared across sets of TFs potentially functioning within differentially accessible regions to investigate how themes of biological regulation may differ between males and females at P23 and P30. P23M-over-P23F regions were enriched with 149 TFBSs, P23F-over-P23M regions with 124, P30M-over-P30F regions with 123, and P30F-over-P30M regions with 78 (Figure 11B).

Biological themes associated with sexually dimorphic TF profiles suggest males and females differ more at P30 than at P23

To understand how TFBSs enriched in sexually dimorphic regions might influence biological function, I compared biological themes associated with the TFs with enriched binding sites in P23M-over-P23F regions, P23F-over-P23M regions, P30M-over-P30F regions, and P30F-over-P30M regions. Intending to identify broad themes of differential regulation, I grouped individual TFs by functional categories defined by high-level GO terms. This approach identified 96 biological themes regulated by at least one TF with enriched TFBS in P23M-over-P23F, P23M-over-P23F, P30M-over-P30F, or P30F-over-P30M regions. At P23, 10 biological themes differed in the number of contributing TFs by 5 or more. At P30, 32 biological themes differed in the number of contributing TFs by 5 or more. These results suggest that similar numbers of TFs regulate the identified biological themes in males and females at P23 but not at P30. The notably greater difference in functional themes observed at P30 is certainly driven by the comparably lower number of TFs with enriched binding sites in P30F-over-P30M regions than the other comparisons (Figure 11D). Thus, to further investigate how TFBSs, enriched in regions sexually dimorphic at P30, might influence differences in sensory song learning at this age, I performed GO term enrichment analysis for biological process on the TFs with binding sites enriched in regions sexually dimorphic at P30 (Figure 12A) and GO term enrichment analysis for all GO groups on the genes associated with those regions (Figure 12B, 12C). The analysis performed on TFs suggests that many of the same biological themes are under regulation at sexually dimorphic regions, but also highlighted themes uniquely enriched in each set of TFs (Figure 12A). In contrast, the GO analysis performed on the genes associated with these sexually dimorphic regions highlighted completely different processes (Figure 12B, Figure 12C), suggesting that TFs, similar

in identity and functionality, may be regulating genes with different biological, molecular, and cellular functions.

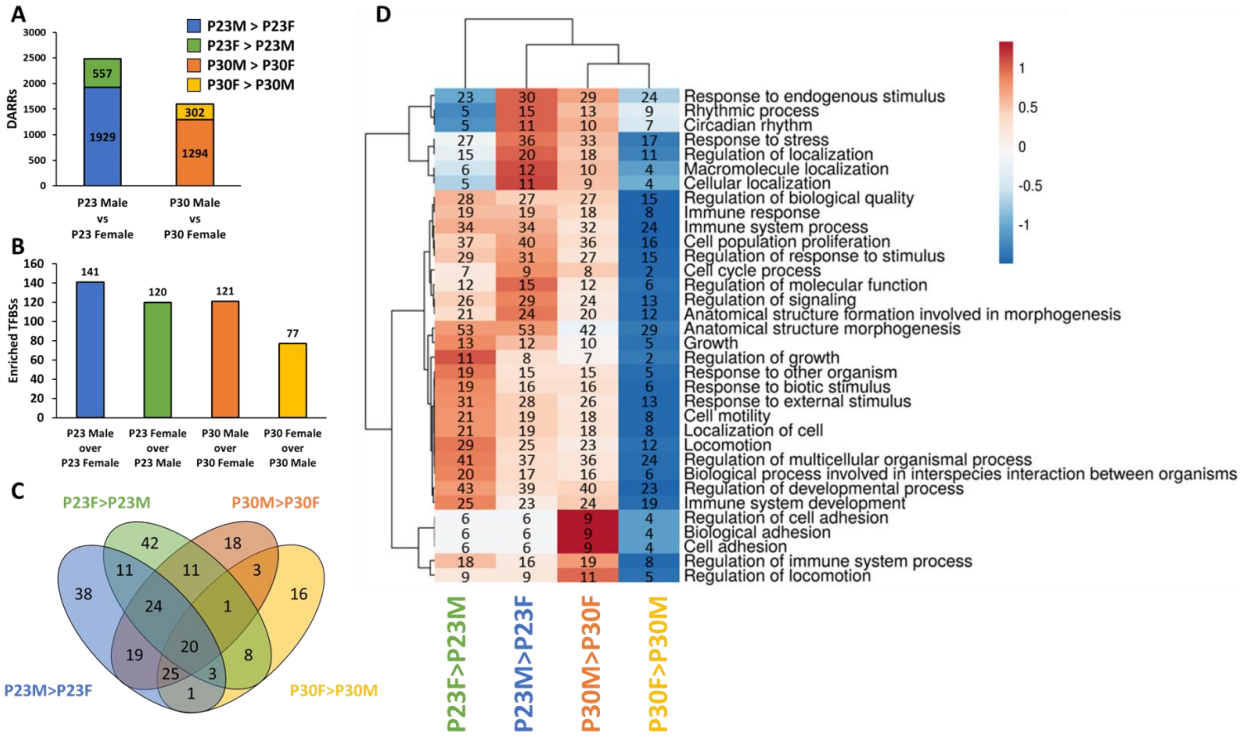

Figure 11. Comparison of sexually dimorphic regulatory region accessibility profiles at P23 and P30. (A) The number of sexually dimorphic differentially accessible regions at P23 and P30. The color of the bar indicated the directionality of greater accessibility. (B) The number of unique TFBSs enriched in sexually dimorphic regions at P23 and P30. (C) Venn diagram examining the overlap between TFBSs enriched in sexually dimorphic differentially accessible regions at P23 and P30. (D) Heatmap of high-level GO terms differing by 5 or more contributing TFs at P23 or P30. GO term values were centered, and unit variance was applied to each set of GO term counts. Rows and columns were clustered using correlation distance and average linkage. Color indicates the relationship between the number of contributing TFs and the center GO term value. The number TFs associated with each GO term is displayed in each cell.

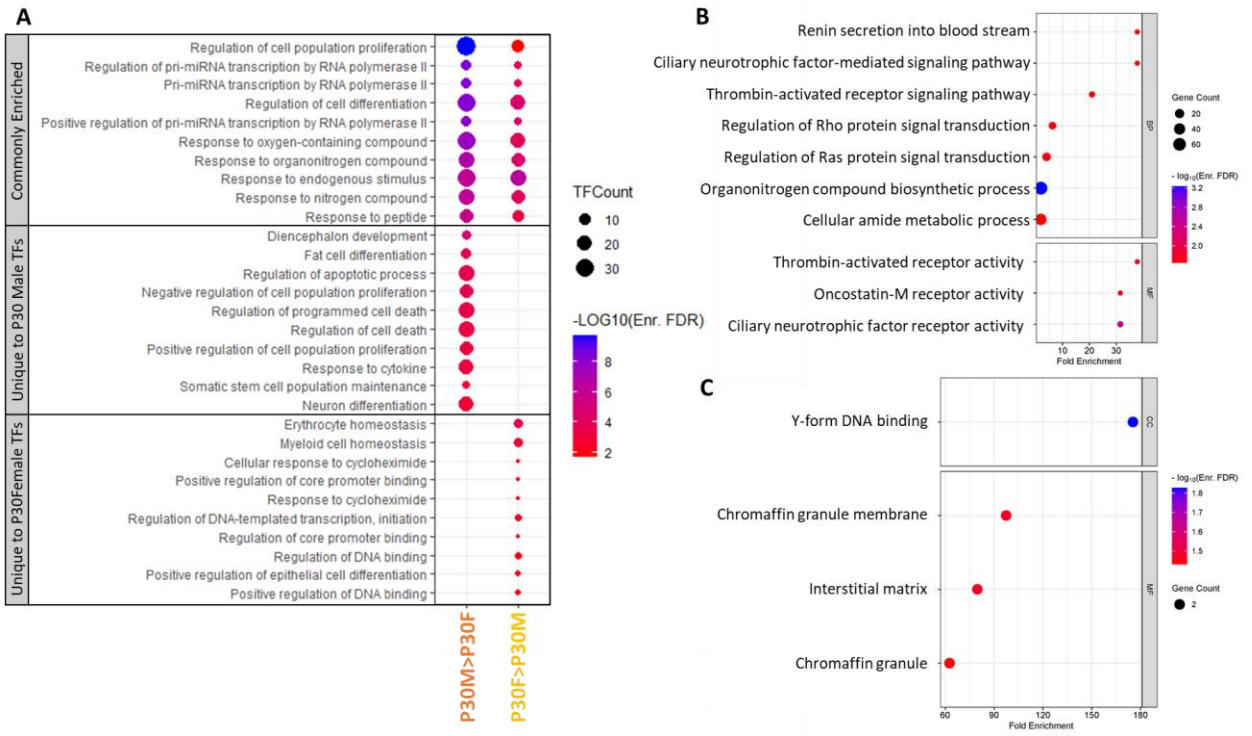

Figure 12. Comparison of GO term enrichment results for P30M-over-P30F and P30F-over-P30 TFs and genes. (A) Dot plot comparing the results from biological process GO term enrichment analysis performed on P30M-over-P30F TFs and P30F-over-P30 TFs. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the comparison(s) from which they were derived. 'Commonly enriched terms' denotes terms with the lowest enrichment FDRs commonly enriched in both P30M-over-P30F TFs and P30F-over-P30M TFs, 'Unique to P30 Male TFs' denotes terms with the lowest enrichment FDRs enriched in P30M-over-P30F TFs, but not P30F-over-P30M TFs, and 'Unique to P30 Female TFs' denotes terms with the lowest enrichment FDRs enriched in P30F-over-P30M TFs, but not P30M-over-P30F TFs. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the comparison from which the enrichment analysis was derived (B) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P30M-over-P30F genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P30F-over-P30M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Comparison of sexually dimorphic TFBS profiles identified 'Core ' and 'Sex-specific' TFBS

I compared the identities of TFBSs enriched in sexually dimorphic regions to identify similarities and differences in the TFBSs enriched in sexually dimorphic regions at both P23 and P30. There were 240 unique TFBSs enriched in sexually dimorphic regions: 114 (47.5%) TFBSs were unique to a single comparison, 53 (22.1%) TFBSs were enriched in two comparisons, 53 (22.1%) TFBSs were enriched in 3 comparisons, and 20 (8.3%) TFBSs were enriched in all four comparisons (Figure 11C). P23M-over-P23F regions (38) and P23F-over-P23M regions (42) contributed the most to TFBSs unique to a single comparison, while P30M-over-P30F regions (18) and P30F-over-P30M regions (16) contributed fewer TFBS (Figure 11C).

At P23, 203 TFBS were enriched in sexually dimorphic regions, of which, 58 (28.6%) were enriched in both sets of regions, 83 (40.9%) were only enriched in regions demonstrating greater accessibility in males, and 62 (30.5%) were only enriched in regions demonstrating greater accessibility in females. At P30, 149 TFBS were enriched in sexually dimorphic regions, of which, 49 (32.9%) were enriched in both sets of regions, 72 (48.3%) were only enriched in regions demonstrating greater accessibility in males, and 28 (18.8%) were only enriched in regions demonstrating greater accessibility in females. As such, at P23 and P30, similar proportions (28.6% and 32.9%) of TFBSs were enriched in sexually dimorphic regions independent of the sex in which the regions were more accessible. Furthermore, between P23 and P30, there was an increase in the proportion of TFBSs solely enriched in sexually dimorphic regions with greater accessibility in males (40.9% to 48.3%) and a decrease in the proportion of TFBSs solely enriched in regions with greater accessibility in females (30.5% to 18.8%).

Lastly, to determine how TFBSs enriched in sexually dimorphic regions change across development, I compared TFBSs enriched in sexually dimorphic regions with greater accessibility in males at P23 and P30 and TFBSs enriched in sexually dimorphic regions with greater accessibility in females at P23 and P30. P23M-over-P23F regions and P30M-over-P30F regions were commonly enriched with 88 (50.6%) TFBSs. In contrast, P23F-over-P23M and P30F-over-P30M regions were commonly enriched with (27.3%) TFBSs, suggesting that there is greater similarity between the TFBSs enriched in sexually dimorphic regions across development in males than in females.

Comparison of TFBSs enriched in sexually dimorphic regions revealed a subset of TFBSs enriched in sexually dimorphic regions independent of age and sex – termed 'core' TFBSs above (Figure 13A). TFs with binding sites enriched in all four comparisons included broad-functioning IEGs prominently involved in learning and memory formation. These included CREB1 and AP-1 TF complex members, JUN, JUNB, JUND, FOS, FOSL1, and FOSL2. Additionally, this set contained binding sites for TFs regulating brain development, including SP4, ZNF148, ZNF281, and RFX4 (Figure 13A). This analysis also revealed TFBSs exclusively enriched in sexually dimorphic regions with greater accessibility in either males or females P23 and P30 – termed 'sex-specific' TFBSs above. 19 TFBSs were exclusively enriched in P23M-over-P23F and P30M-over-P30F regions, and 7 TFBSs were exclusively enriched in P23F-over-P23M and P30F-over-P30M. To understand how 'core' and 'sex-specific' TFBSs might influence differences in sensory song learning, I performed GO term enrichment analysis for biological process on the TFs with binding sites enriched in 'core' (Figure 13b) and 'sex-specific' TFBSs (Figure 14B, 14D).

A

TFBS ID	MOTIF	P23 Male		P23 Female		P30 Male		P30 Female	
		E VALUE	P VALUE	E VALUE	P VALUE	E VALUE	P VALUE	E VALUE	P VALUE
PRDM9		2.58E-19	3.07E-22	6.80E-8	8.08E-11	3.06E-12	3.65E-15	1.70E-3	2.03E-6
RFX5		5.42E-19	6.44E-22	3.45E-5	4.10E-8	1.02E-13	1.21E-16	1.66E-3	1.98E-6
RFX3		3.76E-15	4.48E-18	4.88E-7	5.80E-10	5.59E-9	6.67E-12	3.19E-3	3.81E-6
RFX2		4.67E-12	5.56E-15	1.91E-6	2.27E-9	4.18E-11	4.98E-14	9.97E-5	1.19E-7
RFX1		1.34E-13	1.60E-16	1.36E-6	1.61E-9	2.27E-9	2.70E-12	1.25E-3	1.49E-6
ZNF93		2.30E-10	2.74E-13	7.96E-4	9.46E-7	2.20E-7	2.63E-10	1.38E-2	1.65E-5
RREB1		3.72E-8	4.43E-11	6.73E-4	8.00E-7	3.53E-3	4.22E-6	3.67	4.38E-3
ZNF281		2.24E-4	2.66E-7	4.42E-3	5.26E-6	7.43E-3	8.86E-6	1.18E-4	1.40E-7
SP4		5.40E-6	6.42E-9	2.26E-1	2.69E-4	1.78E-3	2.13E-6	4.30E-3	5.13E-6
PATZ1		4.59E-6	5.46E-9	1.04E-1	1.24E-4	9.82E-5	1.17E-7	2.52	3.01E-3
RFX6		6.95E-8	8.27E-11	2.36	2.81E-3	1.25E-4	1.49E-7	7.11	8.48E-3
NFIC		1.19E-6	1.41E-9	1.29	1.54E-3	2.11E-2	2.52E-5	3.65	4.36E-3
KLF1		7.99E-7	9.50E-10	9.88	1.18E-2	3.49	4.16E-3	1.99E-2	2.38E-5
ZNF148		1.40E-4	1.67E-7	7.94	9.44E-3	9.55E-4	1.14E-6	5.10E-1	6.09E-4
SPIB		4.43E-6	5.27E-9	7.78	9.26E-3	1.42E-1	1.69E-4	4.00	4.77E-3
RFX4		5.04E-3	5.99E-6	1.20	1.43E-3	3.05E-2	3.64E-5	4.47	5.33E-3
FOS		2.31E-2	2.74E-5	7.48E-1	8.89E-4	3.36E-1	4.01E-4	1.60	1.91E-3
CREB1		9.64E-4	1.15E-6	3.72	4.43E-3	1.43	1.71E-3	8.17	9.75E-3
FOSL2::JUND		6.13E-3	7.29E-6	2.87	3.41E-3	6.26E-1	7.48E-4	7.66	9.14E-3
JUN::JUNB		8.06E-2	9.58E-5	7.31	8.69E-3	2.74E	3.27E-3	6.98	8.33E-3

B

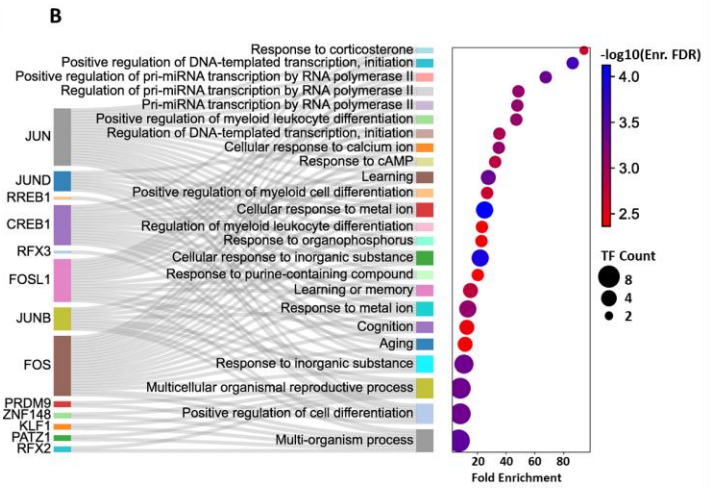

Figure 13. TFBSs enriched in sexually dimorphic regulatory regions in males and females at P23 and P30 (Core TFBS). (A) TFBSs enriched in P23M-over-P23F, P23F-over-P23M, P30M-over-P30F, P30F-over-P30M differentially accessible regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (B) Sankey dot plot displaying results from biological process GO term enrichment analysis performed on core TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results file. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

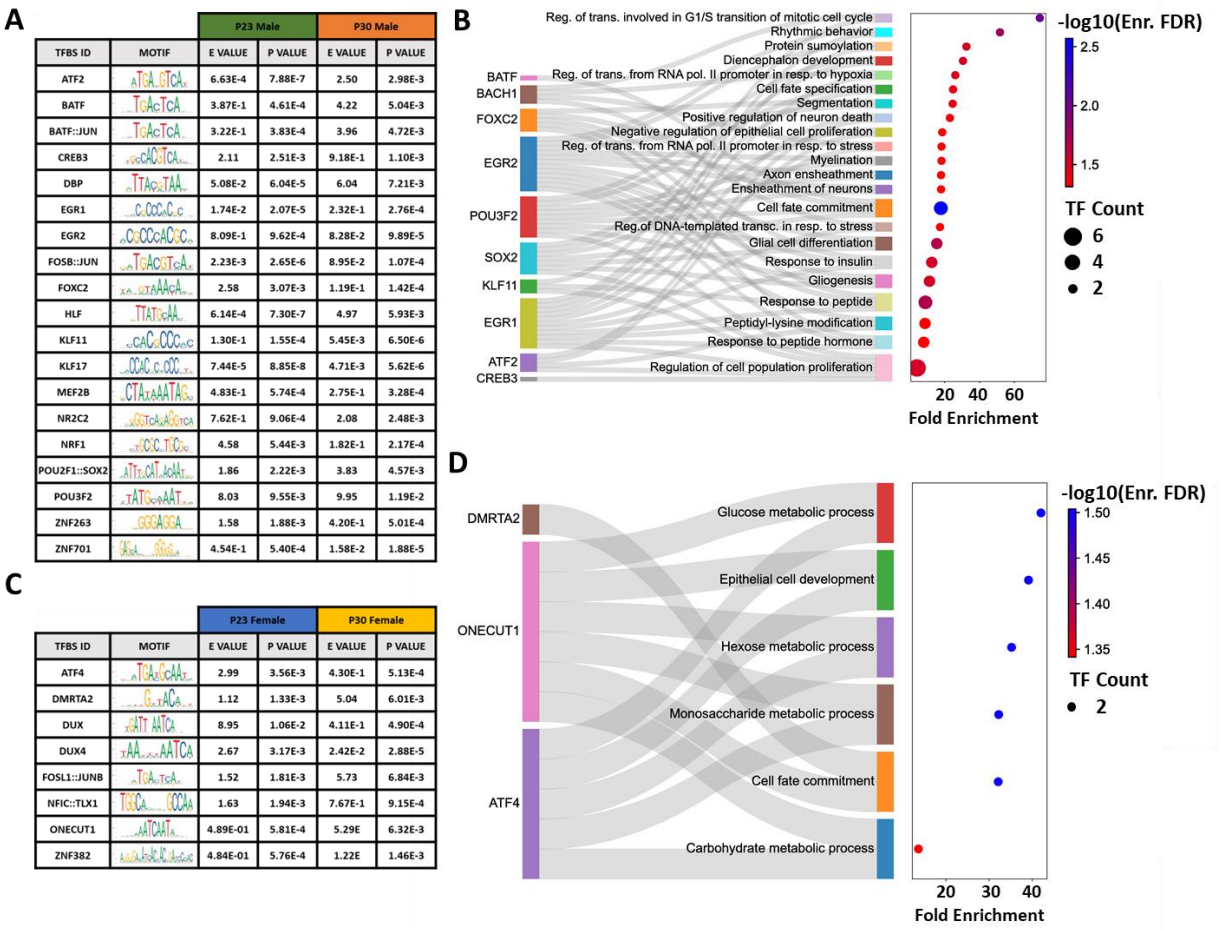

Figure 14. Male and female-specific TFBS enriched in sexually dimorphic differentially accessible regions at P23 and P30. (A) 'Male-specific TFBSs" – TFBSs enriched in P23M-over-P23F and P30M-over-P30F differentially accessible regions and absent in P23F-over-P23M and P30F-over-P30M differentially accessible regions. Corresponding motif logos, enrichment values (E VALUES), and p values and displayed following each TFBS. (B) Sankey dot plot displaying results from biological process GO term enrichment analysis performed of 'male-specific' TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results file. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed. (C) 'Female-specific TFBSs" – TFBSs enriched in P23F-over-P23M and P30F-over-P30M differentially accessible regions and absent in P23M-over-P23F and P30M-over-P30F

Figure 14., continued. differentially accessible regions. Corresponding motif logos, enrichment values (E VALUES), and p values and displayed following each TFBS. (D) Sankey dot plot displaying results from biological process GO term enrichment analysis performed of 'female-specific' TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results file. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

Discussion

Male Onset: Differences in regulatory region accessibility between P23 and P30 in males

TSM is the process through which a juvenile male acquires an auditory memory during development that he will utilize to guide his own song production later in life. Tutor song heard prior to P30 is not incorporated into song produced in adulthood, whereas song heard at P30 is (Roper & Zann 2006), suggesting that at P30, juvenile male brains have sufficiently developed to support the encoding of experiences that meaningfully shape adult behavior. It has been demonstrated that the onset of TSM is characterized by the commencement of responsivity for various molecular cascades within the AL, including ERK and mTOR signaling cascades (Jin & Clayton 1997; Stripling et al. 2001; Cheng & Clayton 2004; Ahmadiantehrani & London 2017a). However, the specific neural properties (e.g., cell number, subtype, functionality, and connectivity) gating the onset of molecular responsivity and the downstream implications of the activation of these cascades within the AL remain largely unknown. To identify the molecular, cellular, and biological processes that might differentiate cell populations capable and incapable

of supporting TSM, I compared the regulatory region accessibility profiles of cells composing the AL in P30 males with those of P23 males utilizing ChIP-Seq for H3K27ac.

I was able to reliably detect differences in regulatory region accessibility between P23 and P30 males. My results indicate that males undergo a reduction in the sheer number of H3K27ac-defined peaks between P23 and P30. However, differential analysis, a more stringent methodology for comparing transcriptomic and epigenetic marks, revealed that a slightly greater number of H3K27ac-defined regions were more accessible in males at P30 than at P23. Unlike the comparison of count alone, differential analysis considers peak strength, location, and reproducibility among biological replicates to identify regions differing in accessibility. Furthermore, differential analysis merges nearby neighboring peaks during comparison, thus giving rise to only a single differentially accessible region, rather than multiple differentially accessible peaks. Thus, it appears that the onset of sensory song learning in males can be characterized by a reduction in the number of H3K27ac-defined peaks and an increase in reproducible, location-specific H3K27ac-defined regions.

Regions demonstrating greater accessibility at P23 were enriched with binding sites for only a single TF, MEF2A. MEF2A has been implicated in a breadth of neurodevelopmental processes, including the survival of maturing cerebellar neurons and activity-dependent excitatory synaptic stability (Lin et al. 1996; Mao et al. 1999; Gaudilliere et al. 2002; Gong et al. 2003; Heidenreich & Linseman 2004; Akhtar et al. 2012; Assali et al. 2019). Work in murine models has demonstrated that MEF2A inhibits dendritic spine growth and restructuring by reducing AMPA receptor trafficking and endocytosis through the regulation of genes including FOS, EGR1, HOMER1, ARC, and BDNF, many of which have been repeatedly implicated in the regulation of sensory song learning (Flavell et al. 2006; Flavell et al. 2008; Pulipparacharuvil et al. 2008; Vetere

et al. 2011; Akhtar et al. 2012; Cole et al. 2012; Rashid et al. 2014). As MEF2A has been implicated in restricting memory formation by decreasing dendritic spine establishment (Pfeiffer et al. 2010; Vetere et al. 2011; Cole et al. 2012; Rashid et al. 2014), there is the potential that MEF2A may function at P23 to promote neuronal survival while limiting memory formation. Interestingly, binding sites for MEF2A have previously been previously identified as enriched in genomic regions demonstrating greater accessibility in P67 Isolates than in age-matched Tutored birds (Kelly et al. 2018). These two findings may be contradictory to one another as P23 males and P67 Isolates possess different capacities for TSM. However, the aforementioned study also demonstrated that the epigenetic landscape of Isolate males was no more similar to that of P32 birds than Tutored birds, suggesting that different epigenetic profiles may support learning at different maturational stages (Kelly et al. 2018). Furthermore, it is likely that MEF2A binding site enrichment was determined from different regions of the genome in each experiment, suggesting that in each context MEF2A may be regulating different sets of genes.

Anchored component of plasma membrane and Carbohydrate binding were the only two GO terms enriched in the genes ascribed to regions demonstrating accessibility in P23 male than P30 males. The genes that gave rise to these terms include CNTN1, RTN4RL1, P4HA1, and ACAN. CNTN1 (Contactin 1) and ACAN (Aggrecan) both regulate neural cell adhesion and organization. CNTN1 regulates cerebellar granule neuron axon guidance and dendritic projection, oligodendrogenesis, and the formation of junctions between neuron axons and neighboring oligodendrocytes at locations interspersing nodes of Ranvier (paranodal axo-glial junctions) (Berglund et al. 1999; Çolakoğlu et al. 2014). ACAN is a constituent and coordinator of perineural net (PNN) development (Matthews et al. 2002; Giamanco et al. 2010; Kwok et al. 2010; Yamada & Jinno 2017; Rowlands et al. 2018). PNNs regulate critical period plasticity by stabilizing

established synaptic connections and can support or hinder learning under differing circumstances (Wlodarczyk et al. 2011; Sorg et al. 2016; Banerjee et al. 2017; Lensjø et al. 2017). PNNs increase in organization in song control nuclei of the AFP throughout maturation, as demonstrated by the comparison of P33 and adult birds, and have been reported within the AL, but isolated to field L (absent in NCM and CMM) (Balmer et al. 2009; Meyer et al. 2014; Cornez et al. 2018). As PNNs are thought to support the stabilization of established neural networks, the observation of increased accessibility of a putative ACAN regulatory region is quite interesting. The regulatory region associated with this gene may be functioning to repress ACAN expression in P23 males, thereby maintaining mechanisms of neural plasticity. RTN4RL1 (Reticulon 4 Receptor Like 1) is a cell surface receptor that restricts dendritic spine development and synapse formation during brain development (GrandPré et al. 2002; Wang et al. 2002). Collectively, a reduction in accessibility at the regulatory regions for these genes could contribute to supporting the onset of TSM in males.

Regions demonstrating greater accessibility at P30 were enriched with binding sites for transcription factors implicated in processes of brain development and organization, including neurogenesis (ARX, LHX4, HOXD3, EN1, GBX2), neuron differentiation (ARX, LHX4, HOXD3, EN1, GBX2), negative regulation of neuron apoptotic process (TFAP2B, EN1), and regionalization (HOXD3, EN1, GBX2, HOXB4, HOXC4). These biological processes are certainly instrumental aspects of maturational programming that give rise to a brain state capable of responding to experience.

Studies on ARX loss of function in humans and mutant mouse lines have demonstrated that ARX disruption results in decreased neuron proliferation (sufficient to result in microcephaly), aberrant neuron migration, disrupted GABAergic neuron development, and neurocognitive deficits (Kitamura et al. 2002; Colombo et al. 2007; Kitamura et al. 2009; Friocourt & Parnavelas

2011; Simonet et al. 2015; Lee et al. 2017; Joseph et al. 2021). Interestingly, TFAP2B has also been shown to be important for the specification of GABAergic interneurons and, like ARX, is expressed in both developing and mature inhibitory cells (Zainolabidin et al. 2017). GABAergic neurons are prolific throughout the AL and contribute to the modulating sensory song response in adulthood, yet normally reared (identical to those used for this project) P25 and P45 males do not demonstrate significant differences in GAD65-expressing cell densities within the AL (Pinaud et al. 2004; Pinaud et al. 2006; Pinaud & Mello 2007; Gogola et al. 2019). Thus, further work is still required to understand the relevance of inhibitory cell populations in regulating the CP for TSM. Perhaps different measures than quantification alone (e.g., measures of functional connectivity to other cellular subtypes, measures of receptor heterogeneity) will be necessary to make such a determination.

GBX2 and EN1 are critical for appropriate midbrain-hindbrain boundary development (Davidson et al. 1988; Wurst et al. 1994; Wassarman et al. 1997; Nakayama et al. 2013; Kouwenhoven et al. 2016; Hagan et al. 2017), in part, through the regulation of complementary gene networks (Liu & Joyner 2001), but also possess unique functions in developing and adult neurons. GBX2 influences cholinergic interneuron tangential migration in developing mouse striatum (Chen et al. 2010), and EN1 has been repeatedly implicated in the differentiation and survival of midbrain dopaminergic neurons (Albéri et al. 2004; Simon et al. 2004; Sgadò et al. 2006; Sonnier et al. 2007; Alves dos Santos & Smidt 2011). EN1 appears to be, at least partly, under the regulation of the mTOR signaling cascade, as the administration of rapamycin prevents EN1 protein induction (Di Nardo et al. 2007). This is particularly interesting as the mTOR signaling is induced by song experience in P30 males, but not P23 males, and is required for TSM (Ahmadiantehrani & London 2017a). Cholinergic innervation is present in the AL, and

experimental manipulations aimed at elucidating its role in sensory song response support its involvement in the regulation of molecular and electrophysiological response properties of the AL (Mello et al. 1998; Velho et al. 2012; Dai et al. 2018; Lee et al. 2018). I hypothesize that GBX2 and EN1 may be functioning to regulate developing connections between cholinergic neurons projecting from catecholamine-synthesizing brain regions such as the LC and VTA to the AL.

Interestingly, regions demonstrating greater accessibility at P30 were also enriched with binding sites for MEF2B and MEF2D. MEF2B expression has been documented in mouse cortex, hippocampus, dentate gyrus, and olfactory bulb, but its function within the brain is less clear than that of other MEF2 isoforms (Assali et al. 2019; Lisek et al. 2023). MEF2D possesses a similar expression profile to MEF2A in mouse brain, and the two isoforms are thought to similarly influence neuronal maturations and synaptic function (Pfeiffer et al. 2010; Akhtar et al. 2012; Cole et al. 2012; Assali et al. 2019). Thus, observing MEF2D binding site enrichment in regions of greater accessibility at P30 and MEF2A binding site enrichment in regions of greater accessibility at P23 could suggest that the two isoforms are playing subtly distinct roles in developing AL at P23 and P30, potentially through the regulation of different gene sets, or that MEF2 TFs are playing regulatory roles both prior to, and during, the CP for TSM.

Genes ascribed to regions of greater accessibility at P30 were enriched for cellular component GO terms highlighting synaptic transmission (e.g., Synapse, Neurotransmitter receptor complex, Endocytic vesicle membrane) and biological process and molecular function GO terms highlighting glutamatergic synapse function (Synaptic transmission, glutamatergic, Reg. of synaptic transmission, glutamatergic, and Glutamate receptor activity). The genes that gave rise to these terms were CACNG3, SYT1, GRM4, GRIA3, CAMK2N1, and KCTD16. CACNG3 (Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 3) is a transmembrane AMPA

receptor regulatory protein (TARP) that modulates the AMPA receptor trafficking and function (Burgess et al. 1999; Payne 2008). SYT1 (Synaptotagmin 1) is expressed on synaptic vesicles and contributes to the triggering of neurotransmitter release in response to calcium binding (Wu et al. 2014; Bornschein & Schmidt 2019). GRM4 (Glutamate Metabotropic Receptor 4) is a G-protein coupled glutamate receptor implicated in regulating synaptic plasticity supporting spatial and motor learning (Pekhletski et al. 1996; Gerlai et al. 1998). GRIA3 (Glutamate Ionotropic Receptor AMPA Type Subunit 3) is an ionotropic glutamate receptor, which, when mutated, causes neurodevelopmental disorders including cognitive impairment and epilepsy (Wu et al. 2007; Trivisano et al. 2020). CAMK2N1 (Calcium/Calmodulin Dependent Protein Kinase II Inhibitor 1), an endogenous inhibitor of CAMK2D, a calcium/calmodulin dependent protein kinase, is upregulated during the consolidation of fear memory and has been implicated in the maintenance of established long-term memories (Chang et al. 1998; Lepicard et al. 2006; Wang 2008; Vigil et al. 2017). KCTD16 (Potassium Channel Tetramerization Domain Containing 16) is an auxiliary subunit of GABAB receptors that contributes to receptor kinetics (Zuo et al. 2019). Collectively, these genes code for receptor units and enzymes that modify synaptic transmission between neurons. Alterations to the regulation of transcription of these genes at the onset of TSM could alter synaptic efficacy, thereby increasing neuronal responsivity among cell populations within AL. Although not contributing to the identification of any enriched GO terms, genes implicated in the regulation of a breadth of other neurodevelopmental processes, including neurite outgrowth and spinogenesis (NEGR1, NCKIPD, NR4A3), neuron migration (NEGR1, MIR139, NR4A3), cellular adhesion (NEGR1, TMEM132B, JAM3, FBLN2), and neuronal signaling (PDZD8, BHLHE40, NEDD8), were also ascribed to regions of greater accessibility at P30.

Female Onset: Differences in regulatory region accessibility between P23 and P30 in females

Females do not sing because they do not develop the brain circuitry required for song production (Nottebohm & Arnold 1976; Nixdorf-Bergweiler 1996). However, it is known that females perform sensory song learning during the CP for TSM and that song heard during this period of development influences song preferences in adulthood (Miller 1979b, 1979a; Clayton 1988; Riebel 2000; Riebel et al. 2002; Riebel 2003b, 2003a; Lauay et al. 2004; Terpstra et al. 2006; Svec & Wade 2009; Holveck & Riebel 2014; Chen et al. 2017; Diez et al. 2019; Wei et al. 2022). Still, it is not clear whether or not TSM in males and sensory song learning in females are distinct types of learning mediated by different molecular and cellular processes, nor have the effective ages for female learning been established with the same degree of certainty that they have been in males. Although, current evidence supports onset for female sensory song learning occurring at approximately P30 as well (Clayton 1988; Braaten 2010). Thus, to determine if female regulatory region accessibility profiles change across the onset for TSM I compared the regulatory region accessibility profiles of cells composing the AL in P30 females with those of P23 females. Additionally, I compared the changes in regulatory region accessibility observed in females to those observed in males to identify potential similarities and differences in their regulatory region accessibility development.

Cis-regulatory elements shifting in accessibility between P23 and P30 in males and females differ in number, genomic location, and putative regulatory function. If these changes do indeed regulate transitions in sensory song learning capability, then these findings suggest that the onset of sensory song learning in each sex may be partly under the regulation of different biological processes. P23 and P30 females did not significantly differ in regulatory region number and differential analysis identified 10-fold fewer regions changing in accessibility between P23 and

P30 in females than in males, suggesting that P23 and P30 males differ from one another to a greater extent than P23 and P30 females. Regions changing in accessibility in females were predominately located at different genomic locations and were enriched with binding sites for different transcription factors, supporting the notion that the onset of sensory song learning in each sex may be influenced by the regulation of different molecular mechanisms. Considering males and females demonstrate differing patterns of molecular induction in AL during development, it is sensible to hypothesize that cell populations of different identities are supporting song induced molecular responsivity in males and females during development (Bailey & Wade 2003; Ahmadiantehrani & London 2017a). The development of cell populations differing in identity would likely be under the regulation of different TFs and patterns of genomic accessibility, as demonstrated by my results.

A majority of the regions differentially accessible between P23 and P30 females were more accessible at P30. These regions were enriched with binding sites for transcription factors that highlight themes of cellular responsivity (e.g. Response to oxygen-containing compound, Response to lipid, Response to ketone, Response to cAMP) (HOXD13, JUND, HOXB13, FOSL1, JUN, RORB, ZNF148), epithelium development (FOSL2, GRHL2, ETV2, HOXB5, HOXD13, HOXB13), and circulatory system development (GRHL2, ETV2, HOXB13, FOSL1, JUN). While these themes are certainly important for supporting brain function, their implications in supporting a brain state capable of sensory song learning is less apparent than the themes associated with TFs shifting in regulatory potential in males.

TFBSs enriched in regions with greater accessibility at P30 than P23, were for transcription factors belonging to the AP-1/ATF transcription factor family (FOSL1::JUND, BATF, BATF3, FOSL2::JUN, BATF::JUN). The AP-1/ATF transcription factor family has been implicated in

virtually all cellular and physiological functions and is vital in supporting cellular gene expression in response to internal and external cues, including those supporting learning and memory formation (Herdegen & Waetzig 2001; Alberini 2009; Bejjani et al. 2019). Interestingly, FOS, a TF belonging to the AP-1/ATF family, for which I did not observe binding sites enriched in P30 female regions, is induced by song experience within the AL of P30 females, but not males (Bailey & Wade 2003). The AP-1/ATF family is composed of at least 24 different TFs that dimerize together to perform overlapping regulatory functions in response to external stimuli (Herdegen & Waetzig 2001; Alberini 2009; Bejjani et al. 2019). Thus, there is the potential that other AP-1/ATF TF family members, including those for which binding site enrichment was identified, are also induced by song within female AL at this stage of development and that these TFs regulating the transcriptomic response to song at the identified putative regulatory regions. However, the functional role of FOS induction in females following song exposure has yet to be determined, nor has it been established that there is a developmental shift in female FOS responsivity within the AL. Further investigations focused on FOS, and potentially other AP-1/ATF TF family members, could clarify their roles in supporting sensory song learning in females and shed further light on differences in developmental song responsivity between males and females.

Although themes of brain development were not identified by GO term enrichment analysis performed on TFs with binding sites enriched in P30 female regions, some of the TFs identified, including ZNF148, RFX7, and RORB have been implicated in coordinating brain development that could be relevant to supporting the onset of sensory song learning. ZNF148 is highly expressed throughout the brain (Passantino et al. 1998), functionally suppresses muscle cell differentiation (Bakke et al. 2017), and its disruption has been implicated in the occurrence of neurological disorders including ASD, ADHD, and intellectual disability (Stevens et al. 2016; Miao et al. 2023).

Similarly, RFX7 is broadly expressed throughout the brain (Aftab et al. 2008; Sugiaman-Trapman et al. 2018) and its disruption has been implicated in the occurrence of neurological disorders including ASD, ADHD, intellectual disability, and global developmental delay (Rhie et al. 2021; Ledger et al. 2023). As potential regulatory roles in brain development for these TFs have only recently been identified, it will be exciting to incorporate finding from future, likely more targeted experiments, aimed at elucidating their functions into our understanding of mechanisms potentially regulating the onset of female sensory song learning. RORB (retinoid-related orphan receptor β), a TF that functions as a nuclear receptor for retinoic acid and other retinoids, is broadly implicated in the transcriptional control of neuronal differentiation in neural substrates spanning from the retina (Jia et al. 2009; Liu et al. 2013) to cortical sensory areas (Jabaudon et al. 2012; Oishi et al. 2016). RORB expression occurs in maturing neurons suggesting a role in regulating cell fate outcome (Nakagawa & O'Leary 2003; Liu et al. 2013; Fu et al. 2014) and has been used as a selective marker for barrel cortex in mice, suggesting that RORB may play a particularly important role in specifying neurons involved in the processing of sensory neurons (Nakagawa & O'Leary 2003; Jabaudon et al. 2012). Finally, similar to ZNF148 and RFX7, RORB disruption in humans has been associated with cases of epilepsy and intellectual disability (Boudry-Labis et al. 2013; Rudolf et al. 2016). Thus, these TFs may be important for brain development in ways that support the onset of sensory song learning in females.

Genes ascribed to regions of greater accessibility at P30 included JAM3, DRD5, and BTF3 (all other genes were uncharacterized). Although the differential region associated with JAM3 at P30 in females was located at a different genomic location than the differential region associated with JAM3 at P30 in males, the overlapping identification of this gene as a potential regulatory target at P30 supports there being a potential relationship between JAM3 regulation and sensory

song learning in both sexes. DRD5 (Dopamine receptor D5) is a G-protein coupled dopamine receptor that promotes the persistence in long-term plasticity within the hippocampus and forebrain (Hansen & Manahan-Vaughan 2014; Shivarama Shetty et al. 2016) and has also been associated with ADHD (Daly et al. 1999; Kustanovich et al. 2004; Lowe et al. 2004). BTF3 (Basic Transcription Factor 3) is one of the proteins that forms a stable complex with RNA polymerase II to initiate transcription, suggesting that differences in general transcriptional machinery may also be of relevance for female AL development (Zheng et al. 1987).

Collectively, these results suggest that males and females undergo different developmental shifts in regulatory region accessibility between P23 and P30 and that TFs with increased regulatory potential at these sites, as well as the genes associated with the identified regulatory regions, are regulating distinct biological, cellular, and molecular processes. The time points assayed in this project were selected based on the established timeline for the CP for male TSM. While there is evidence to support an onset for sensory song learning in females occurring between P23 and P30 the exact age of initiation has not been established with the same degree of certainty as it has been in males (Clayton 1988; Braaten 2010). There is the potential that this experimental design does not encapsulate the robust age of onset for sensory song learning in females, if one does in fact exist, and that comparisons of different developmental timepoint would be more informative in understanding the regulation of female sensory song learning. Additionally, beyond limited behavioral evidence, there is little evidence suggesting that the onset for female sensory song learning mechanistically parallels the onset of TSM in males. Females are capable of sensory song learning at P30, but they do not demonstrate the induction of molecular cascades necessary for male TSM at this age (Bailey & Wade 2003; Ahmadiantehrani & London 2017a). Instead, P30 females demonstrate an induction of FOS protein following exposure to conspecific song (Bailey

& Wade 2003). However, the experiments necessary to demonstrate the importance of FOS in regulating female sensory song learning have not been performed, nor has it been shown that there is a shift in FOS inducibility between females capable and incapable of sensory song learning, as has been demonstrated from male markers of song responsivity across the onset of the CP for TSM (Jin & Clayton 1997; Stripling et al. 2001; Cheng & Clayton 2004; Ahmadiantehrani & London 2017a). With these considerations in mind, a potential interpretation of this result is that the onset of TSM in males and sensory song learning in females rely on the functions of different TFs at different regulatory regions, which are likely working in conjunction with commonly functioning TFs at regulatory regions unchanging in accessibility between P23 and P30 and regions that differ in accessibility between sexes. This interpretation could support the hypothesis that TSM is a male-specific form of learning; providing a potential explanation for why the differences in female regulatory region accessibility pale in comparison to those observed in males. Alternatively, this could support the hypothesis that males and females achieve different states of brain maturation at P30, each of which is sufficient to support sensory song learning and that are differences between male and female sensory song learning.

Sex Differences: Differences in regulatory region accessibility between P23 and P30 males and females

To determine if regulatory region accessibility profiles vary across sex in the same ways they vary across maturation in males, I compared the regulatory region accessibility profiles of P30 males with those of P30 females. The results suggest that P30 males do not differ from P30 females in the same way that they differ from P23 males. Sexually dimorphic regions at P30 were 26X time greater in number that regions differing in accessibility across development in males, were located at different genomic locations (excluding a single differentially accessible region

ascribed to NEDD8), and were enriched with binding sites for a far greater number of TFs. Comparison of TFBS enrichment profiles and putative target genes found that binding sites for MEF2B, MEF2D, NFIC, PRDM9, TFAP2B, and ZNF263 were enriched in both P30M-over-P30F and P30M-over-P23M regions and that NYX, CACNG3, and NEDD8 were among the putative gene targets for each set of regions. As such, approximately half of the TFBS enriched in P30M-over-P23M regions were also present in P30M-over-P30F regions, suggesting some overlap in the transcriptional regulatory profiles distinguishing P30 males from P30 females and P23 males. However, the overlapping TFBSs compose only about 5% of those enriched in regions more accessible in males than females at P30, suggesting that the shifts in regulatory region accessibility characterizing the onset of TSM in males predominately differ from those potentially regulating differences in sensory song learning between sexes.

To assess if and to what extent sexually dimorphic regulatory region profiles shift across development, I compared the regulatory region accessibility profiles of P23 males with those of P23 females. Sexually dimorphic regions at P23 outnumbered those at P30, as did the number of TFs enriched within them, suggesting that male and females regulatory region accessibility profiles become more similar between P23 and 30. There is the potential that increasing similarity in regulatory region accessibility profiles between P23 and P30 reflects the onset of sensory song learning in both males and females. However, as the developmental differences in regulatory region accessibility between P23 and P30 in males and females differ number, genomic location, and putative regulatory function, this may not be the case. The decrease in sexually dimorphic regions between P23 and P30 resulted from a reduction both in regions with greater accessibility in males and regions with greater accessibility in females, but the proportion of reduction was greater in females. The same trend was present for the number and identities of enriched TFBSs.

Thus, the observed increase in similarity between males and females across development is primarily at the expense of decreasing female regulatory region accessibility.

This difference in decrement between sexes was further accentuated by the high-level GO term analysis illustrating that males and females appear more similar in terms of the regulatory functions of TFs possessing enriched binding sites at P23 than P30. At P30, a greater number of TFs were associated with all high-level GO terms for males than females. Similarly, GO term enrichment analysis showed that commonly enriched GO terms demonstrate a greater degree of enrichment in males than females at P30, but it also identified GO terms uniquely enriched in both sexes. Terms commonly enriched in males and females highlighted themes of cellular development (e.g., Regulation of cell population proliferation, Regulation of cell differentiation), pri-mRNA transcription (e.g., Pri-mRNA transcription by RNA polymerase II) and responsivity (e.g., Response to endogenous stimulus, Response to oxygen-containing compound, Response to nitrogen containing compound). Terms unique to males at this age further highlighted the regulation of cell proliferation and survival (e.g., Somatic stem cell population maintenance, Positive regulation of cell population proliferation, Regulation of apoptotic process, Regulation of cell death) and themes of brain development (e.g., Diencephalon development, Neuron differentiation).

Genes ascribed to regions of greater accessibility in males than females at P30 were enriched for GO terms, including Ciliary neurotrophic factor-mediated signaling pathway, Regulation of Rho protein signal transduction, and Regulation of Ras protein signal transduction. Ciliary neurotrophic factor (CNTF), a polypeptide hormone that functions as a survival factor for nervous system cells, functions within sensory neurons to influence the developmental sex-specific behaviors during postnatal brain maturation (Morris et al. 2004; Koemeter-Cox et al. 2014; Jia et

al. 2019). Ras and Rho are highly homologous interconnected GTPase-dependent molecular cascades that are critically involved in almost all aspects of neurodevelopment, including neurogenesis, neuron differentiation, vesicular trafficking, and synaptic plasticity (Chang et al. 2003a; Chang et al. 2003b; Govek et al. 2005; Ye & Carew 2010; Liang et al. 2019; Soriano et al. 2021). The Ras and Rho signaling cascades link cellular signals transduced from cell surface receptors, including neurotrophic factor receptors like CNTFR, to transcription factors, which then regulate gene expression (Chang et al. 2003a; Chang et al. 2003b; Soriano et al. 2021). Two major downstream effector pathways of Ras and Rho are the MEK/ERK cascade and the PI3K/AKT/mTOR cascade, each of which has been implicated in the regulation of sensory song learning and is induced by song exposure in P30 males, but not P30 females (Bailey & Wade 2003; Cheng & Clayton 2004; London & Clayton 2008; Ahmadiantehrani & London 2017a). Transcriptional regulation of genes involved in neurotrophic factor response and their downstream effector pathways provide a compelling explanation for the documented differences in molecular responsivity to song observed between males and females at P30. However, at P23, the same terms were enriched in genes ascribed to regions of greater accessibility in males, but not females. This suggests that these regulatory processes are insufficient to entirely explain the patterns of molecular responsivity observed in P30 males, as males younger than P30 do not demonstrate a molecular induction of the MEK/ERK or PI3K/AKT/mTOR cascades following song exposure, but does not preclude the possibility that these regulatory processes are functioning alongside others to regulate the onset of TSM in males(Jin & Clayton 1997; Stripling et al. 2001; Ahmadiantehrani & London 2017a).

A large proportion of sexually dimorphic regions were located on sex chromosomes at P23 (80.1%) and at P30 (94.2%), suggesting that the regulation of genes located on sex chromosomes

may be highly influential in establishing differences in AL development and responsivity. Understanding that male zebra finches are homogametic (ZZ) and lack chromosome-wide mechanisms of dosage compensation, this finding aligns with the expectations that a large proportion of sexually dimorphic regions with greater accessibility in males would be located on the Z chromosome (Itoh et al. 2007; Itoh et al. 2010). Studies have suggested that the sexually dimorphic expression of Z-linked genes drives the developmental divergence of telencephalic brain regions that support sensory song learning and production (Chen et al. 2005; Duncan & Carruth 2007; Tang et al. 2007; Tomaszycki et al. 2009) and more recently, large-scale genomic investigations have also pointed towards Z-linked gene regulation as a key governor of neuroanatomical specification and sensory song learning (Davenport et al. 2021; Diddens et al. 2021). These investigations examined song nuclei that demonstrate robust anatomical differences between males and females, but, as AL volume is sexually monomorphic, there has been limited evidence to suggest that Z-linked genes play a similar regulatory role within AL. The results of my experiment suggest that Z-linked gene regulation may be just as critical in defining sexually dimorphic transcriptional landscapes in the AL. Although such regulation does not yield notable differences in AL volume, it may be integral for refining molecular and cellular properties that differentially influence sensory song learning in males and females.

Comparison across TFBSs enriched in sexually dimorphic regions identified a subset of TFBSs enriched in sexually dimorphic regions independent of age and sex (‘core’) and subsets specific to each sex (‘sex-specific’). Core TFBSs were for IEGs prominently involved in learning and memory formation, including CREB1 and AP-1 TF complex members, JUN, JUNB, JUND, FOS, FOSL1, and FOSL2. This subset also included binding sites for TFs that regulate brain development, including SP4, ZNF148, ZNF281, and RFX family members 1-6. SP4 regulates

dendrite patterning in cerebellar neurons during development (Ramos et al. 2007), and SP4 mutant mice display reduced levels of NMDAR subunit 1 protein and memory deficits (Zhou et al. 2010). ZNF281 inhibits neuronal differentiation of murine cortical neurons (Pieraccioli et al. 2018) and has been reported to play a functionally redundant role to ZNF148 during erythroid development (Woo et al. 2019). Because these TFBSs were derived from sexually dimorphic regions, they are, by definition, located at different genomic locations in males and females. Therefore, likely regulate the expression of different gene sets. Such data emphasize the importance of carefully examining downstream regulatory targets for molecular cascades induced by song experience, as these results support the notion that comparable molecular responses in males and females result in the regulation of different target genes.

Male-specific and female-specific binding sites included binding sites for TFs implicated in regulating various aspects of brain development and function. For example, binding sites for EGR1, ATF2, POU3F2, and SOX2 were among male-specific TFBSs, while DMRTA2, ONECUT1, and ZNF382 were among the female-specific TFBSs. Enrichment of EGR1 binding sites in regulatory regions of greater accessibility in males, but not females, is particularly interesting given that EGR1 transitions from a state of high constitutive expression to an inducible state between P20 and P30 (Jin & Clayton 1997; Stripling et al. 2001). As EGR1 binding sites were not enriched in regions differentially accessible between P23 and P30 males, it is reasonable to speculate that sexually dimorphic EGR1 binding site enrichment (as well all other sex-specific TFBS demonstrating a comparable pattern of enrichment) arose from differences in accessibility at the same genomic locations at P23 and P30. If this were to be true, it suggests that EGR1 may be regulating the same sets of genes at both P23 and P30 in a sex-specific manner. ATF2 limits the formation of new synaptic connections and prevents long-term synaptic facilitation when over-

expressed in aplysia (Bartsch et al. 1995; Abel et al. 1998). POU3F2 and SOX2 are highly expressed in neural progenitor cells (NPCs) and demonstrate high TFBS co-enrichment at ChIP-Seq peaks (Kondoh & Kamachi 2016). Additionally, POU3F2 regulates a gene network associated with psychiatric disorders schizophrenia and bipolar (Chen et al. 2018). DMRTA2 knockout mice display a reduced brain size, loss of hippocampal structure, and midline defects compared to wild-type mice (Konno et al. 2012; Saulnier et al. 2013). Additionally, loss of function DMRTA2 mutations in zebrafish and Xenopus cause neurogenesis defects in the telencephalon and olfactory placode, respectively (Yoshizawa et al. 2011; Parlier et al. 2013). ONECUT1 expression induces neuronal characteristics in fibroblasts, potentially through the rapid remodeling of chromatin structure, allowing for organized changes in gene expression (van der Raadt et al. 2019). ZNF382 influences cell proliferation, survival, and apoptosis by repressing AP-1 signaling (Cheng et al. 2010). Together, these TFs may function across development to regulate AL gene expression in a sex-specific manner and may work in conjunction with TFs shifting across development in males and females to regulate sensory song learning.

Chapter III: Developmental shifts in regulatory region accessibility spanning the closure of the CP for TSM

Introduction

Based on existing molecular and behavioral data, there were three major questions regarding epigenetic regulation that I sought to address: [1] Are regulatory region accessibility profiles changing in males as they transition out of their CP for TSM, and if so, how? [2] Are female regulatory region accessibility profiles changing similarly, or do females demonstrate different epigenetic profiles for learning at this stage of development? [3] How does experience, independent of maturation, influence regulatory region accessibility in males? Utilizing ChIP-Seq for H3K27ac, I identified putative accessible regulatory regions spanning the genome in birds differing in age, sex, and experience. I compared experimental groups to identify differentially accessible regions, identified TFBSs enriched with those regions, and assigned genes to each differentially accessible regulatory region by nearest TSS. Finally, I utilized GO ontology analysis and an in-depth literature search to examine how TFs with enriched binding sites and putatively regulated genes may influence AL function in the context of development and sex.

Methods

The methods utilized to produce the data presented and discussed in this chapter (Chapter 3) are described in detail in the methods section of Chapter 2. As the two chapters present and discuss separate findings obtained from the same experiment, I did not think it sensible to include identical information for a second time here.

Results

P60 and P67 ChIP-Seq data meet quality control measures

Sample quality and reproducibility measures indicate that the data are suitable for further downstream analysis. An average of 33,723,906 reads (75 nts in length) were obtained per ChIP, with at least 26,844,161 reads obtained for any individual sample (Figure 15A). Following read trimming, samples maintained an average of 32,477,139 reads, with at least 25,681,635 reads maintained for any individual sample (Figure 15C, Table 1). A one-way ANOVA revealed no main effect ($F(5,12)= 0.818$, $p = 0.56$) of condition on read filtering (Figure 15B), suggesting that there was no overall difference in the quality of reads obtained across samples. At least 94.6% of reads from each sample aligned to the genome, and a one-way ANOVA revealed no main effect of condition on the proportion of reads mapped to the genome ($F(5,12)= 1.834$, $p = 0.18$) (Figure 15D). An average of 52,669, but no fewer than 43,148, peaks were called for each sample (Figure 15E). One-way ANOVAs revealed no main effect of condition on average peak length ($F(5,12)= 0.496$, $p = 0.70$) or average number of reads per peak ($F(5,12)= 1.083$, $p = 0.059$) (Figure 15F, 15G). However, a one-way ANOVA revealed a significant main effect ($F(5,12)= 5.985$, $p = 0.005$) of condition on average peak signal value, and post hoc Tukey tests showed that P67 female peaks had, on average, greater peak signal value than P67 males (p adj = 0.007) and P60 females (p adj = 0.007) (Figure 15H).

Assessment of peak sets showed that metrics of quality control were similar across samples (Table 1) and consistent with published profiles, peaks were predominately located in promoter, intronic, and distal intergenic regions for all samples (Figure 16A). Correlation values between individual replicates were all relatively high ($R2 > .0.75$) and did not highlight a clear divergence of samples based on sex, age, or learning potential (Figure 16B). However, principal component

analysis revealed that male and female samples separated along PC2. Independent of age and tutor song experience, most male samples closely clustered along PC1, while female samples were more distributed across PC1 (Figure 16C).

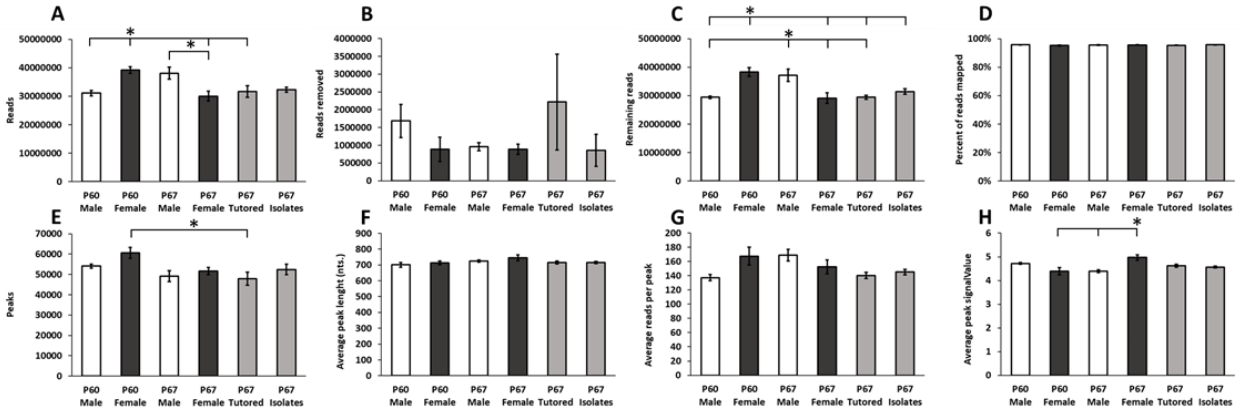

Figure 15. Comparison of read processing and peak characteristics across P60, P67, Tutored, and Isolate experimental groups. Bars and asterisks indicate groups significantly differing from each other with a p-value < 0.05. Asterisks have been placed above the condition significantly differing from the others for significance indicators involving more than two groups. (A) The number of reads obtained from ChIP-Seq. (B) The number of reads removed following QC. (C) Number of reads remaining following read removal. (D) The percentage of remaining reads mapped to the genome. (E) The number of called peaks. (F) Average peak length in nucleotides (nts.). (G) Average number of reads per peak. (H) Average peak signalValue. SignalValue is a measurement of the average enrichment of a genomic region.

Sample ID	Reads	Map%	Filt%	Dup%	ReadL	FragL	RelCC	SSD	RiP%
P60-Male-N1	30239232	100	7.74	0	74	237	3.25	0.788	26.1
P60-Male-N2	29397623	100	7.86	0	73	232	2.69	0.791	24.8
P60-Male-N3	28605716	100	8.05	0	74	204	2.65	0.799	24.8
P60-Female-N1	38117870	100	7.86	0	74	204	2.96	0.901	28.1
P60-Female-N2	35785647	100	8.81	0	74	232	2.2	0.834	22.4
P60-Female-N3	41057160	100	8.97	0	74	233	2.32	0.956	29.1
P67-Male-N1	37121766	100	7.66	0	74	233	3.05	0.88	24.1
P67-Male-N2	33337556	100	8.63	0	74	239	2.48	0.827	20.6
P67-Male-N3	40889470	100	8.29	0	74	236	2.67	0.894	22.4
P67-Female-N1	32169416	100	7.16	0	73	239	3.71	0.843	28.6
P67-Female-N2	29584426	100	7.85	0	74	243	3.46	0.85	25.8
P67-Female-N3	25681635	100	7.2	0	74	242	3.99	0.75	26.7
P67-Tutored-N1	29094264	100	8.48	0	74	242	2.63	0.811	22.3
P67-Tutored-N2	28324023	100	8.92	0	74	237	2.22	0.808	20.3
P67-Tutored-N3	30847364	100	8.01	0	74	238	2.88	0.865	26
P67-Isolate-N1	30127955	100	7.87	0	74	236	2.53	0.807	26.4
P67-Isolate-N2	33299542	100	8.09	0	74	235	2.98	0.822	21.8
P67-Isolate-N3	30907841	100	7.86	0	74	238	2.81	0.813	24.5

Table 2. Summary of ChIP-Seq quality metrics for P60, P67, Tutored, and Isolate experimental replicates. Each row pertains to a single replicate. Sample ID indicates the age and sex of the birds from which the sample was derived and the replicate number. Reads indicate the number of reads remaining following read trimming and removal. Map% is the percentage of reads that aligned to the genome. Filt% is the percentage of reads removed from downstream analysis for not meeting the q-value cutoff threshold of 15. Dup% is the percentage of reads for which there was an identical read aligned to the same genomic location. ReadL is the average length of reads. FragL is the estimated mean fragment length determined by systematically shifting the reads on each strand toward each other until the minimum genome coverage is achieved. RelCC is obtained by comparing the maximum cross-coverage peak to the cross-coverage at a shift size corresponding to the read length. Higher scores (generally 1 or greater) indicate good enrichment. SSD (standardized standard deviation) is computed by looking at the standard deviation of signal pile-up along the genome normalized to the total number of reads. RiP% indicates the percentage of reads located in peaks.

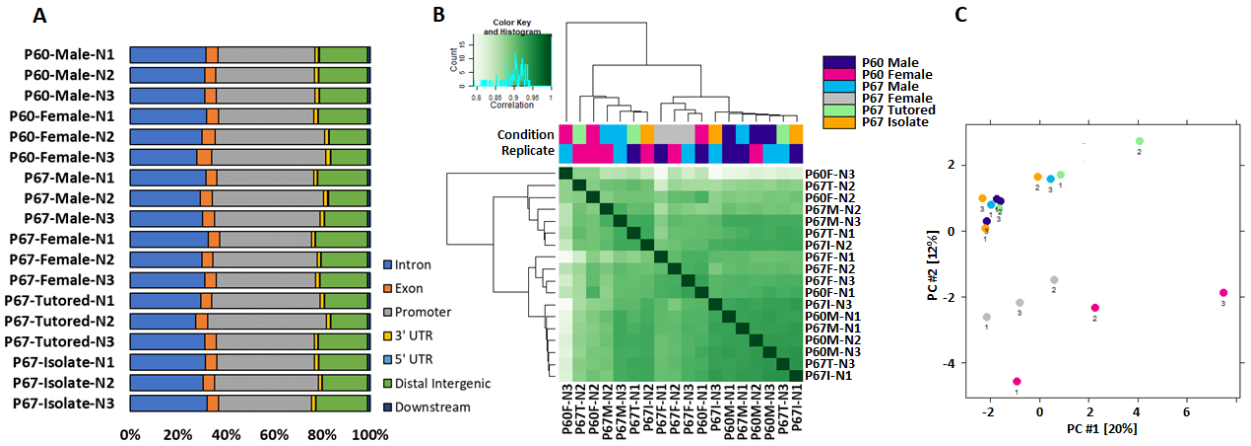

Figure 16. Comparison of replicate peak profiles of P60, P67, Tutored, and Isolate experimental groups. (A) Distribution of peaks across genomic features for biological replicates from each experimental condition. (B) Heatmap of correlation values between experimental replicate peak profiles resulting from unsupervised hierarchical clustering. All samples are listed along the bottom and right side of the figure. Each shaded square indicates the correlation coefficient between two samples. A histogram in the upper left displays the frequency of correlation coefficients and the color of green shading associated with each coefficient. Correlation coefficients ranged from 0.75 to 1. Correlation coefficients of 1 were only obtained when comparing samples to themselves. Identical dendrograms are shown at the top and along the left side of the figure. Solid colored rectangles along the top figure correspond to condition (dark blue = P60 male, pink = P60 female, light blue = P67 male, gray = P67 female, light green = P67 Tutored, orange = P67 Isolate) and replicate number (dark blue = replicate 1, pink = replicate 2, light blue = replicate 3). (C) PCA of all replicates colored according to experimental group (dark blue = P60 male, pink = P60 female, light blue = P67 male, gray = P67 female, light green = P67 Tutored, orange = P67 Isolate). Principal component 1 is represented along the x-axis and accounts for 20% of the variance in the data. Principal component 2 is represented along the y-axis and accounts for 12% of the variance in the data.

Males and females possessed similar numbers of accessible regulatory regions at P60 and P67

I first sought to determine how the number of accessible regulatory regions changes between P60 and P67 in males and females. A one-way ANOVA revealed a significant main effect ($F(5,12)= 3.364$, $p = 0.04$) of condition on peak number. Post hoc Tukey tests showed that P60 females had a significantly greater number of peaks than P67 Tutored birds (p adj = 0.03) and that

no other groups significantly differed from one another (Figure 15E). Thus, neither males nor females appear to undergo a significant change in regulatory region number between P60 and P67.

Differential analysis revealed regulatory regions with greater accessibility in P60 and P67 males

To investigate the effects of age on male regulatory region accessibility, I compared P60 and P67 male peak sets to identify differentially accessible regions. I identified 103 differentially accessible regions between P60 and P67 males, 23 of the regions demonstrated greater accessibility in P60 males ('P60M-over-P67M'), and 80 of the regions demonstrated greater accessibility in P67 males ('P67M-over-P60M') (Figure 17B).

Differentially accessible regions in males are associated with distinct biological processes

To understand how regions differing in accessibility between P60 and P67 males might influence the neural properties that regulate TSM, I determined what TFs may contribute to differences in learning potential between experimental groups by identifying TFBSs enriched in the regions differing in accessibility between P60 and P67 males. P60M-over-P67M regions were enriched with TFBSs for two TF, PAX4 and ZNF707. P67M-over-P60M regions were enriched with TFBSs for 11 TFs, including RFX2, REST, and MEF2A (Figure 17C, 17D). I performed GO term enrichment analysis on TFs with binding sites enriched in P67M-over-P60M regions to determine the biological processes that might be coming online to limit TSM at P67 in males. GO term enrichment analysis of P67M-over-P60M TFs highlighted themes of cellular responsivity to various endogenous stimuli, including hormones and lipids, as well as themes relevant to brain function, such as cell projection assembly, intracellular receptor signaling, and regulation of neuron death (Figure 17E).

To further investigate how regions differing in accessibility between P60 and P67 males might influence the neural properties that regulate TSM, I ascribed the gene with the nearest TSS to each differentially accessible region and performed GO analysis for biological process (BP), cellular component (CC), and molecular function (MF). There were no terms enriched for the genes associated with P60M-over-P67M regions, while genes associated with P67M-over-P60M regions highlighted themes of neuronal maturation, synaptic function, and transporter activity (Figure 18).

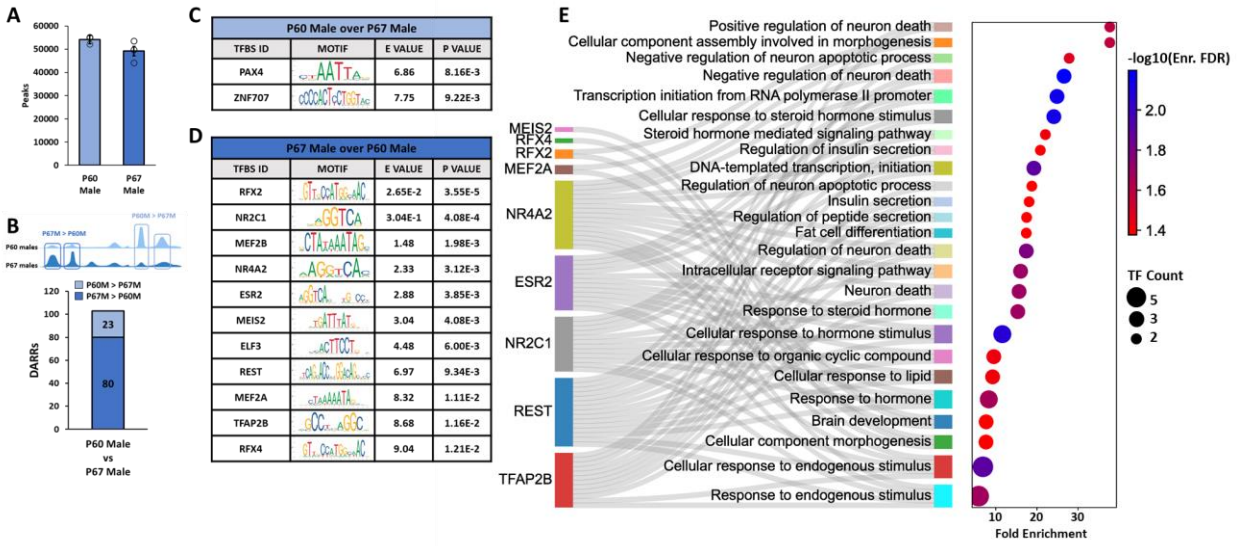

Figure 17. Comparison of P60 and P67 male regulatory region accessibility profiles. (A) The number of called peaks for P60 and P67 males. (B) The number of differentially accessible regions for P60 and P67 males. (C) TFBSs enriched in P60M-over-P67M regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (D) TFBSs enriched in P67M-over-P60M regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (E) Sankey dot plot displaying results from biological process GO term enrichment analysis performed on P67M-over-P60M TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results files. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

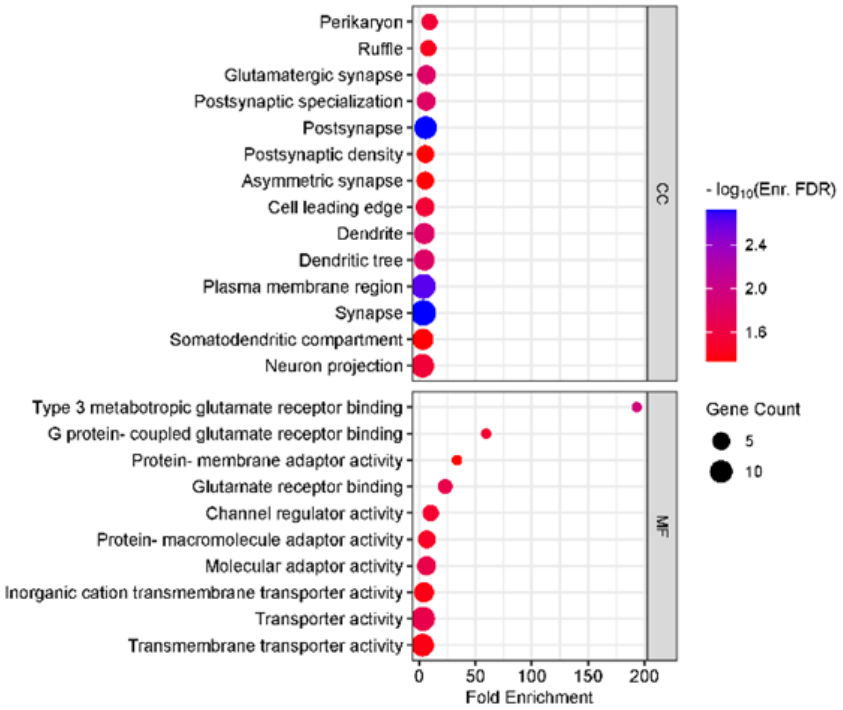

Figure 18. GO term enrichment for P67M-over-P60M genes. Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P67M-over-P60M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Far more regulatory regions were differentially accessible between P60 and P67 in females than in males

Although there is no established closure to female sensory song learning, I also sought to investigate the effects of age on female regulatory region accessibility to determine if the changes in regulatory region accessibility observed in males are occurring concurrently in females. To this end, I compared P60 and P67 female peak sets to identify differentially accessible regions. Unexpectedly, there were seventeen-fold greater differentially accessible regions between P60 and P67 females than between P60 and P67 males. I identified 1748 differentially accessible regions

between P60 and P67 females, 1400 of the regions demonstrated greater accessibility in P60 females ('P60F-over-P67F'), and 348 of the regions demonstrated greater accessibility in P67 females ('P67F-over-P60F') (Figure 19B). Notably, there were 15 regions differing in accessibility between P60 and P67 females the overlapped regions differing in accessibility between P60 and P67 males, suggesting that there is, albeit minimal, overlap in the regulatory regions shifting in accessibility between P60 and P67 in males and females.

Differentially accessible regions in females associated with distinct biological processes

To understand how regions differing in accessibility between P60 and P67 females might influence the neural properties that regulate sensory song learning, I determined what TFs may contribute to differences in learning potential between experimental groups by identifying TFBSs enriched in the regions differing in accessibility between P60 and P67 females. P60F-over-P67F regions were enriched with 307 TFBSs, and P67F-over-P60F regions were enriched with 101 TFBSs. There were no TFBSs commonly enriched in P60F-over-P67F regions and P60M-over-P67M regions, but binding sites for MEF2A, MEF2B, RFX2, and RFX4, were commonly enriched in P67F-over-P60F regions and P67M-over-P60M regions, suggesting that there is some overlap in the TFs that increase in their potential to influence transcription at P67 in males and females.

GO term enrichment analysis of P60F-over-P67F TFs highlighted themes of general cellular development, including regulation of cell population proliferation and cell fate commitment, but also emphasized themes crucial for brain development and organization, including neurogenesis, neuron differentiation, and forebrain development (Figure 19C). GO term enrichment analysis of P67F-over-P60F TFs also highlighted themes of cell population and cellular differentiation but not themes associated with brain development and organization.

Instead, terms with the highest enrichment highlight the regulation of transcription by RNA polymerase II and themes of responsivity to various endogenous stimuli (Figure 19D).

To further investigate how regions differing in accessibility between P60 and P67 females might influence the neural properties that regulate sensory song learning, I ascribed the gene with the nearest TSS to each differentially accessible region and performed GO analysis to derive biological function. The genes ascribed to P60F-over-P67F regions, and the genes ascribed to P67F-over-P60F regions, were highly enriched with terms associated with neuron development and organization, suggesting that different sets of genes, important for regulating overlapping biological processes, are increasing, and decreasing in their potential for regulation between P60 and P67 in females (Figure 20A, 20B).

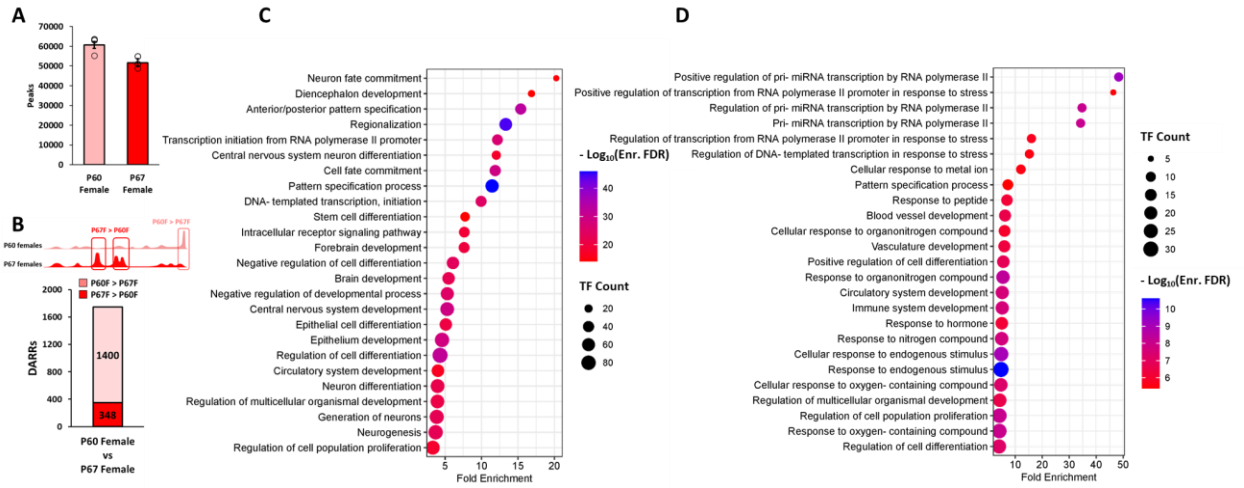

Figure 19. Comparison of P60 and P67 female regulatory region accessibility profiles. (A) The number of called peaks for P60 and P67 females. (B) The number of differentially accessible regions for P60 and P67 females. (C) Dot plot displaying the results from biological process GO term enrichment analysis performed on P60F-over-P67F TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term in reference to all protein-coding genes in the genome. GO terms completely unrelated to brain development and function were removed for the generation of this figure . GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed. (D) Dot plot displaying the results from biological process GO term enrichment analysis performed on P60F-over-P67F TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term in reference to all protein-coding genes in the genome. GO terms completely unrelated to brain development and function were removed for the generation of this figure but are but are maintained in the results files. GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed.

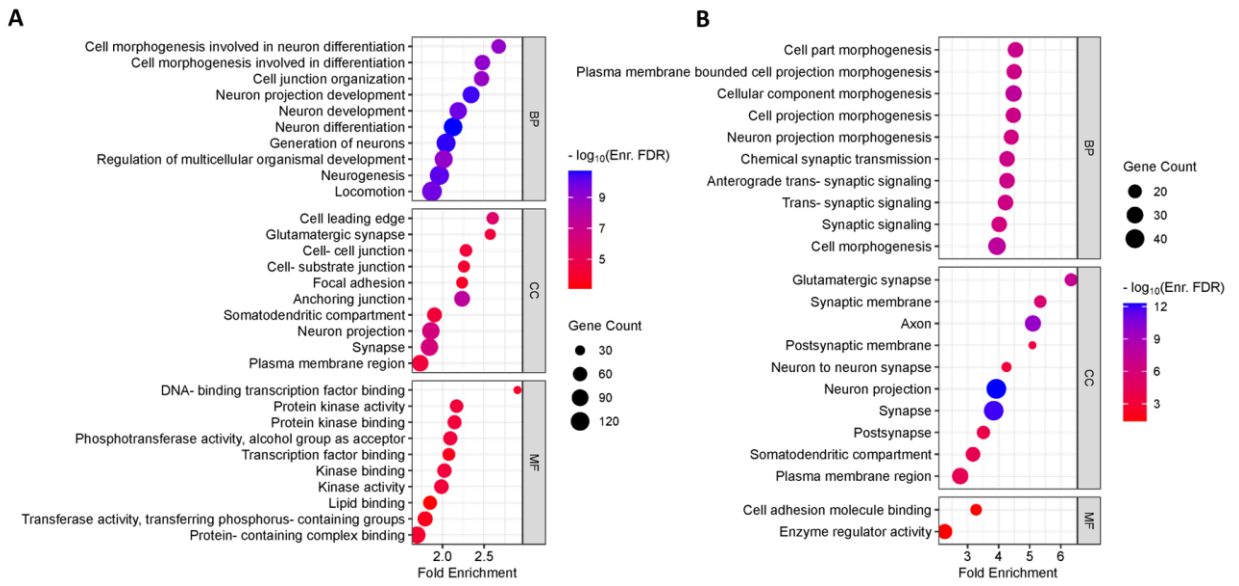

Figure 20. GO term enrichment for P60F-over-P67F and P67F-over-P60M genes. (A) Dot plot displaying the results from GO term enrichment analysis performed on P60F-over-P67F genes. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term in reference to all protein-coding genes in the genome. GO terms completely unrelated to brain development and function were removed for the generation of this figure but are maintained in the results file. GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed. (D) Dot plot displaying the results from GO term enrichment analysis performed on P67F-over-P60F genes. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term in reference to all protein-coding genes in the genome. GO terms completely unrelated to brain development and function were removed for the generation of this figure but are maintained in the results file. GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed.

Males and female regulatory region accessibility profiles are more similar at P67 than at P60

To investigate the influence of sex on regulatory region accessibility and explore potential differences in mechanisms of sensory song learning, I compared P60 male and female peak sets to identify regulatory regions differing in accessibility between them. Additionally, to determine how sexually dimorphic regions change across development, I identified regulatory regions differing

in accessibility between P67 males and females. The results suggest that regulatory region accessibility differs to a greater extent at P60 than at P67. At P60, a greater number of differentially accessible regions were more accessible in females than in males, while at P67 the opposite was observed (Figure 21A). Comparison of age-matched males and females identified 4659 differentially accessible regions at P60 and 1852 differentially accessible regions at P67. At P60, 2057 regions were more accessible in males ('P60M-over-P60F'), and 557 regions were more accessible in females ('P60F-over-P60M'). At P67, 1294 regions were more accessible in males ('P67M-over-P67F'), and 302 regions were more accessible in females (Figure 21A) ('P67F-over-P67M').

Sexually dimorphic regulatory regions were enriched with fewer TFBS at P67 than at P60

To determine how sexually dimorphic regulatory regions might influence neural properties that support sensory song learning, I identified TFBSs enriched in sexually dimorphic regions at P60 and P67 and compared across sets of TFs potentially functioning within differentially accessible regions to investigate how themes of biological regulation may differ between males and females at P60 and P67. P60M-over-P60F regions were enriched with 188 TFBSs, P60F-over-P60M regions with 340, P67M-over-P67F regions with 173, and P67F-over-P67M regions with 98 (Figure 21B).

Biological themes associated with sexually dimorphic TF profiles suggest males and females differ more at P60 than at P67

To understand how TFBSs enriched in sexually dimorphic regions might influence biological function, I compared biological themes associated with the TFs with enriched binding sites in P60M-over-P60F, P60M-over-P60F, P67M-over-P67F, or P67F-over-P67M regions. Intending to identify broad themes of differential regulation, I grouped individual TFs by

functional categories defined by high-level GO terms. This approach identified 102 biological themes regulated by at least one TF with enriched TFBS in P60M-over-P60F, P60F-over-P60M, P67M-over-P67F, or P67F-over-P67M regions. At P60, 56 biological themes differed in the number of contributing TFs by 5 or more. At P67, 34 biological themes differed in the number of contributing TFs by 5 or more. The results suggest that in comparison to other sexually dimorphic regions at P60 and P67, P60F-over-P60M regions have a greater number of binding sites for TFs regulating all identified high-level GO terms. This notably greater number of TFs associated with each functional theme is certainly driven by the comparably higher number of TFs with enriched binding sites in P60F-over-P60M regions than the other comparisons (Figure 21D). Thus, to further investigate how TFBSs, enriched in regions sexually dimorphic at P60 and P67, might influence differences in sensory song learning at these ages, I performed GO term enrichment analysis for biological process on the TFs with binding sites enriched in regions sexually dimorphic at each age (Figure 22, 24) and GO term enrichment analysis for all GO groups on the genes associated with those regions (Figure 23B, 23C, 25B, 25C).

At P60, biological process terms related to brain development and organization (neurogenesis, neuron differentiation, central nervous system development, regionalization, and pattern specification) were highly enriched in both P60M-over-P60F and P60F-over-P60M TFs, but associated with greater numbers of TFs and possessed lower enrichment FDRs for P60F-over-P60M TFs (Figure 22). Terms enriched in P60M-over-P60F TFs and absent in P60F-over-P60M highlighted themes of epithelium development, core promoter binging, and response to calcium ion, while terms enriched in P60F-over-P60M TFs and absent in P60M-over-P60F highlighted the regulation of neurogenesis, regulation of gliogeneisis, and response to steroid hormone (Figure 22). At P67, biological process terms related to the regulation pri-miRNA transcription by RNA-

polymerase II, the regulation of cell differentiation, and cellular responsivity were commonly enriched in both P67M-over-P67F and P67F-over-P67M TFs (Figure 24). In contrast to P60, themes of brain development and organization (neurogenesis, neuron differentiation, central nervous system development, regionalization, and pattern specification) were enriched in P67M-over-P67F TFs, but not P67F-over-P67M TFs (Figure 24).

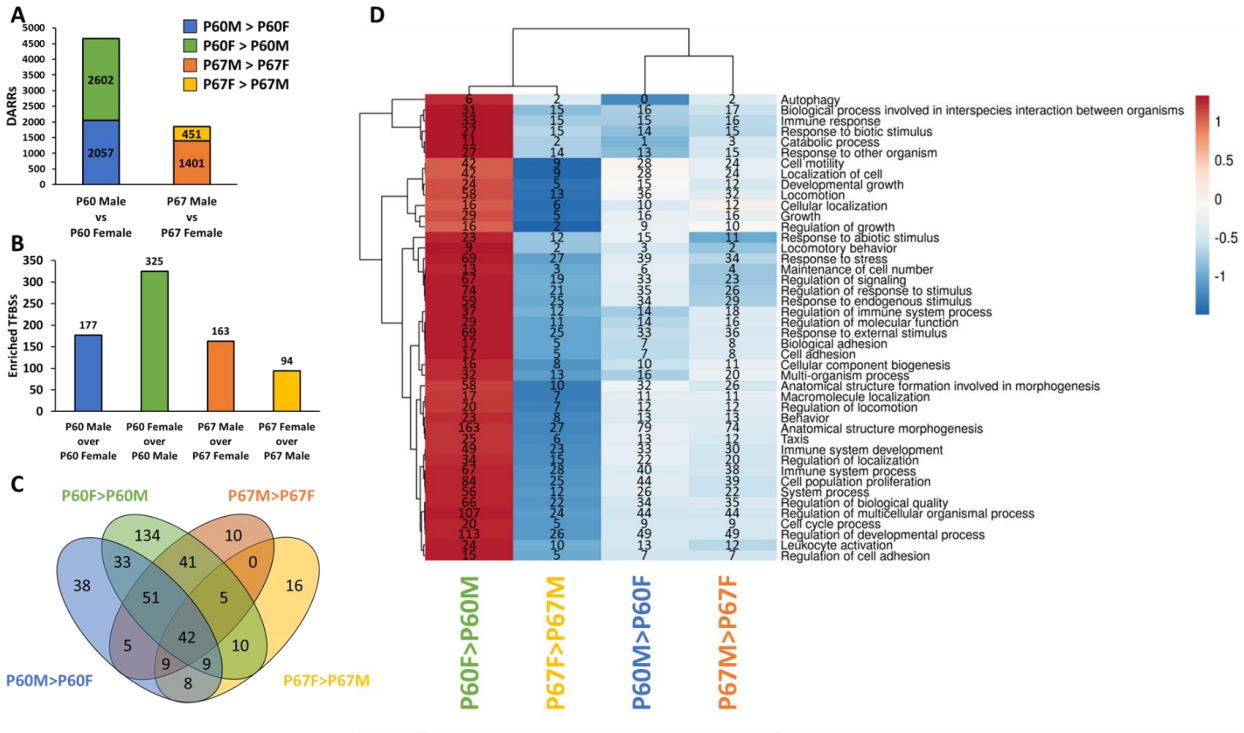

Figure 21. Comparison of sexually dimorphic regulatory region accessibility profiles. (A) The number of sexually dimorphic differentially accessible regions at P60 and P67. The color of the bar indicated the directionality of greater accessibility. (B) The number of unique TFBSs enriched in sexually dimorphic regions at P60 and P67. (C) Venn diagram examining the overlap between TFBSs enriched in sexually dimorphic differentially accessible regions at P60 and P67. (D) Heatmap of high-level GO terms differing by 5 or more contributing TFs at P60 or P67. GO term values were centered, and unit variance was applied to each set of GO term counts. Rows and columns were clustered using correlation distance and average linkage. Color indicates the relationship between the number of contributing TFs and the center GO term value. The number TFs associated with each GO term is displayed in each cell.

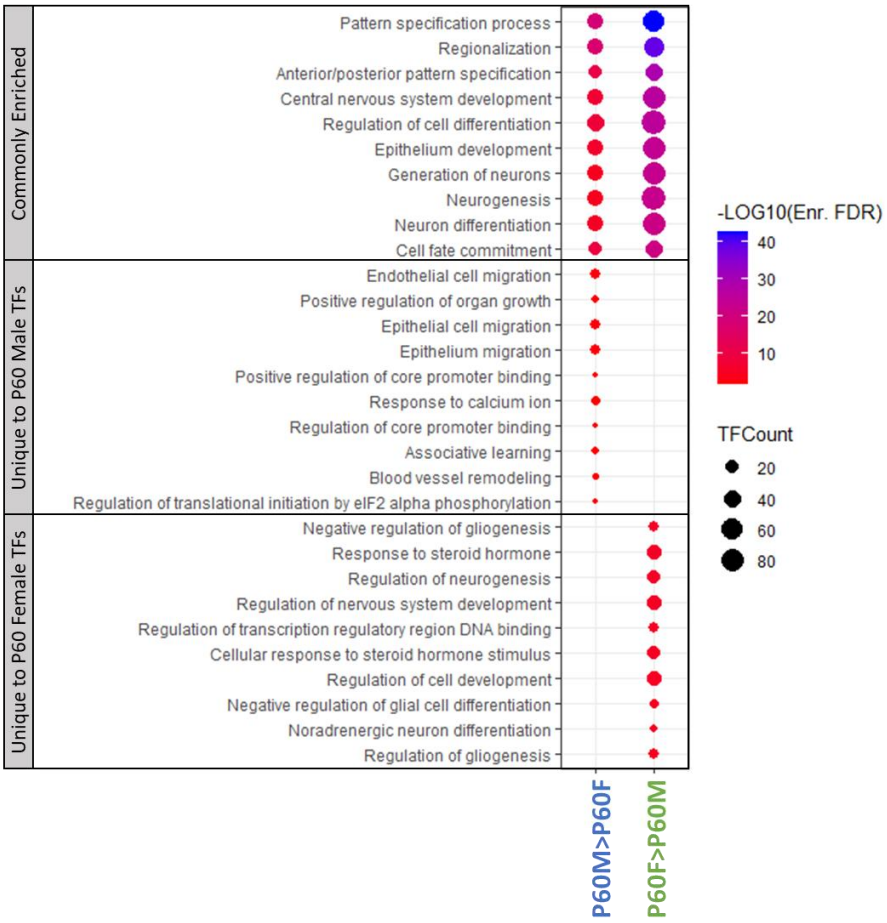

Figure 22. Comparison of GO term enrichment results for P60M-over-P60F and P60F-over-P60M TFs. Dot plot comparing the results from biological process GO term enrichment analysis performed on P60M-over-P60F TFs and P60F-over-P60 TFs. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the comparison(s) from which they were derived. 'Commonly enriched terms' denotes terms with the lowest enrichment FDRs commonly enriched in both P60M-over-P60F TFs and P60F-over-P60M TFs, 'Unique to P60 Male TFs' denotes terms with the lowest enrichment FDRs enriched in P60M-over-P60F TFs, but not P60F-over-P60M TFs, and 'Unique to P60 Female TFs' denotes terms with the lowest enrichment FDRs enriched in P60F-over-P60M TFs, but not P60M-over-P60F TFs. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the comparison from which the enrichment analysis was derived.

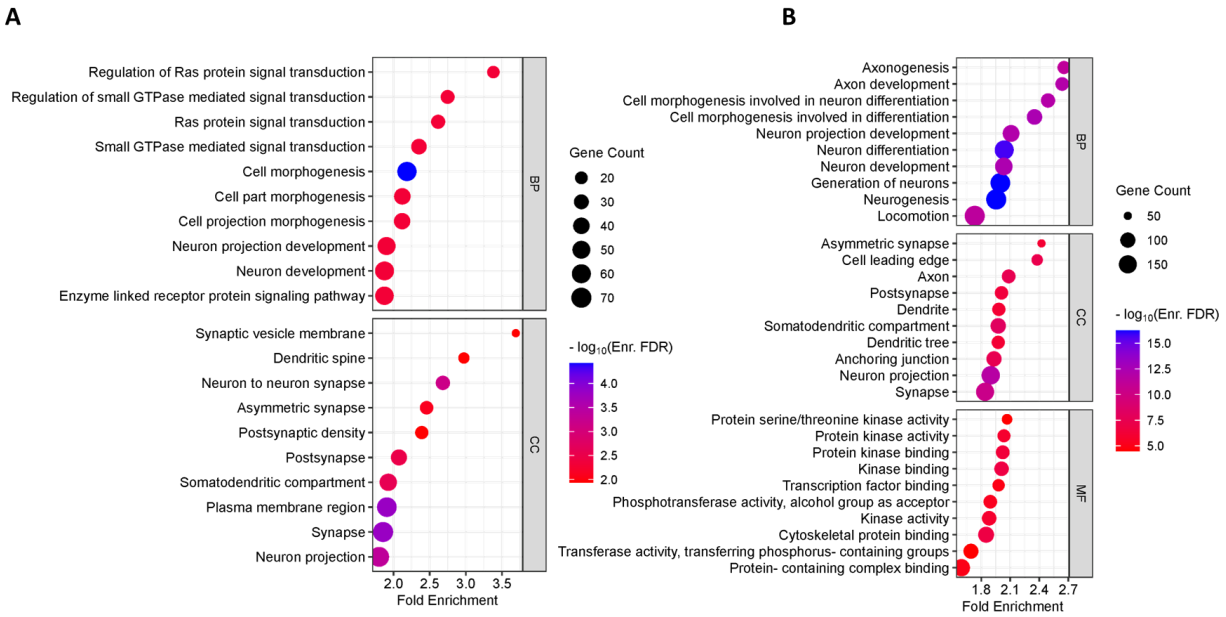

Figure 23. Comparison of GO term enrichment results for P60M-over-P60F and P60F-over-P60M genes. (A) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P60M-over-P60F genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. (B) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P60F-over-P60M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

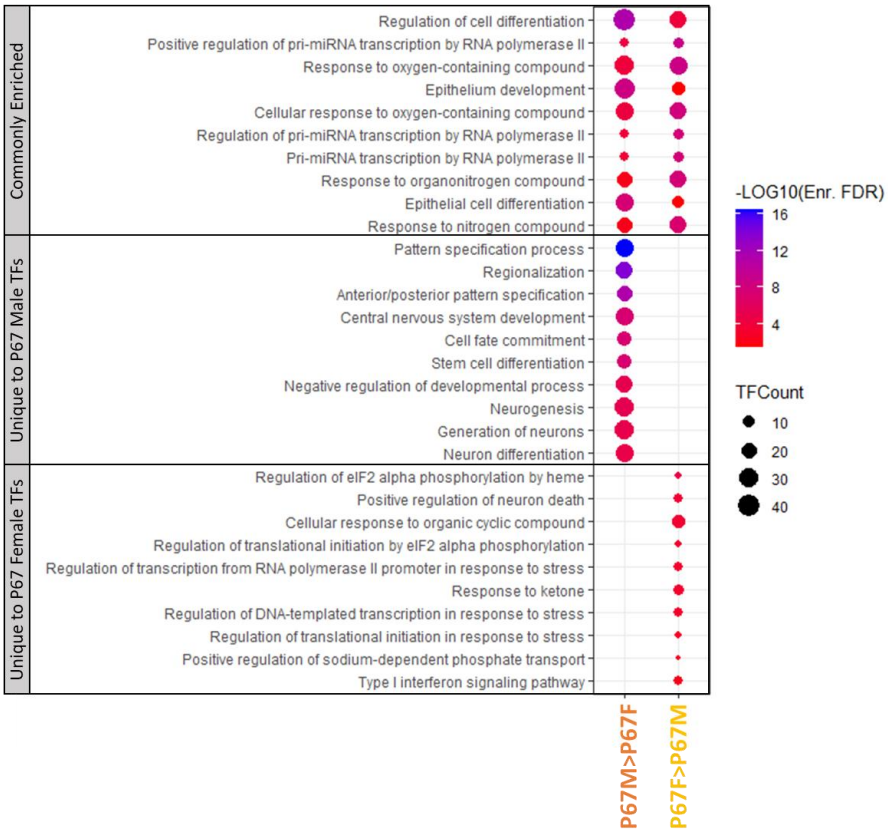

Figure 24. Comparison of GO term enrichment results for P67M-over-P67F and P67F-over-P67M TFs. Dot plot comparing the results from biological process GO term enrichment analysis performed on P67M-over-P67F TFs and P67F-over-P67M TFs. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the comparison(s) from which they were derived. 'Commonly enriched terms' denotes terms with the lowest enrichment FDRs commonly enriched in both P67M-over-P67F TFs and P67F-over-P67M TFs, 'Unique to P67 Male TFs' denotes terms with the lowest enrichment FDRs enriched in P67M-over-P67F TFs, but not P67F-over-P67M TFs, and 'Unique to P60 Female TFs' denotes terms with the lowest enrichment FDRs enriched in P60F-over-P60M TFs, but not P67M-over-P67F TFs. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the comparison from which the enrichment analysis was derived.

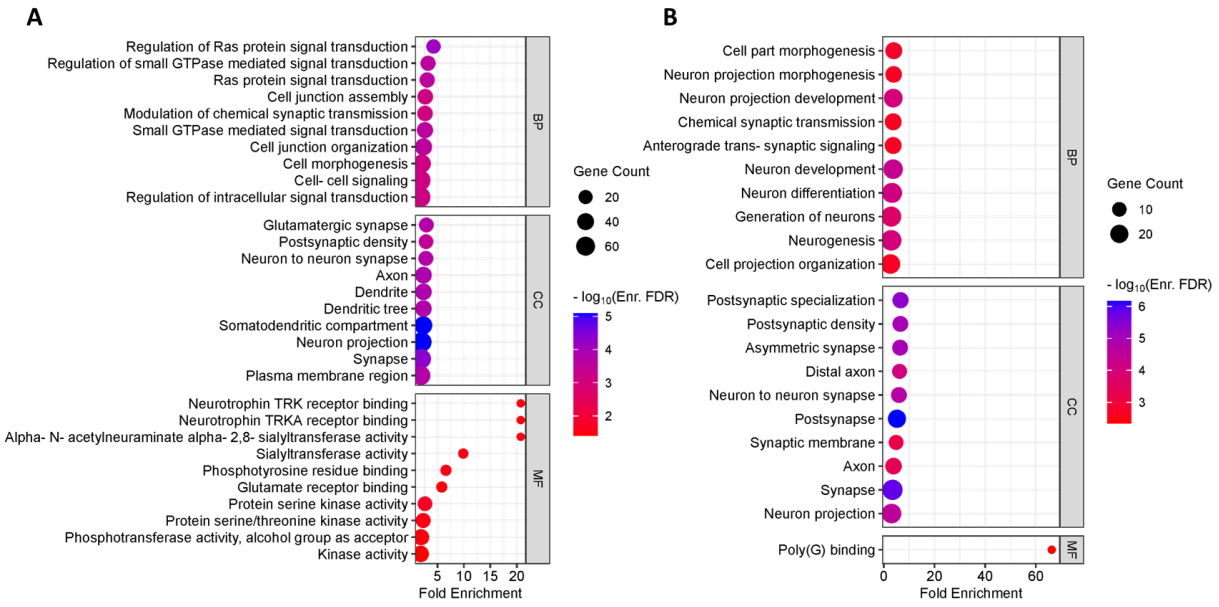

Figure 25. Comparison of GO term enrichment results for P67M-over-P67F and P67F-over-P67M genes. (A) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P67M-over-P67F genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. (B) Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P67F-over-P67M genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Comparison of sexually dimorphic regions identified 'Core ' and 'Sex-specific' TFBS

I compared the identities of TFBSs enriched in sexually dimorphic regions to identify similarities and differences in the TFBSs enriched in sexually dimorphic regions at both P60 and P67. There were 388 unique TFBSs enriched in sexually dimorphic regions: 175 (45.1%) TFBSs were unique to a single comparison, 97 (25%) TFBSs were enriched in two comparisons, 74 (19.1%) TFBSs were enriched in 3 comparisons, and 20 (10.8%) TFBSs were enriched in all four

comparisons (Figure 21C). A large majority (134) of the TFBSs uniquely enriched in a single comparison were derived from the P60F-over-P60M regions, while each other set of sexually dimorphic regions was enriched with comparably few unique TFBSs (Figure 21C).

At P60, 367 TFBS were enriched in sexually dimorphic regions, of which, 135 (36.8%) were enriched in both sets of regions, 42 (11.4%) were only enriched in regions demonstrating greater accessibility in males, and 62 (51.8%) were only enriched in regions demonstrating greater accessibility in females. At P67, 201 TFBS were enriched in sexually dimorphic regions, of which, 56 (27.9%) were enriched in both sets of regions, 107 (53.2%) were only enriched in regions demonstrating greater accessibility in males, and 38 (18.9%) were only enriched in regions demonstrating greater accessibility in females. As such, a greater proportion (36.8% > 27.9%) of TFBSs were commonly enriched in sexually dimorphic regions independent of the sex in which the regions were more accessible at P67 than P60. Furthermore, between P60 and P67, there was an increase in the proportion of TFBSs solely enriched in sexually dimorphic regions with greater accessibility in males (11.4% to 53.2%) and a decrease in the proportion of TFBSs solely enriched in regions with greater accessibility in females (51.8% to 18.9%).

Lastly, to determine how TFBSs enriched in sexually dimorphic regions change across development, I compared TFBSs enriched in sexually dimorphic regions with greater accessibility in males at P60 and P67 and TFBSs enriched in sexually dimorphic regions with greater accessibility in females at P60 and P67. P60M-over-P60F regions and P67M-over-P67F regions were commonly enriched with 107 (50.6%) TFBSs. In contrast, P60F-over-P60M and P67F-over-P67M regions were commonly enriched with 66 (18.7%) TFBSs, suggesting that there is greater similarity between the TFBSs enriched in sexually dimorphic regions across development in males than in females.

Comparison of TFBSs enriched in sexually dimorphic regions revealed a subset of TFBSs enriched in sexually dimorphic regions independent of age and sex – termed ‘core’ TFBSs above (Figure 26A). This analysis also revealed TFBSs exclusively enriched in sexually dimorphic regions with greater accessibility in either males or females P60 and P67 – termed ‘sex-specific’ TFBSs above. 19 TFBSs were exclusively enriched in P60M-over-P60F and P67M-over-P67F regions, and 7 TFBSs were exclusively enriched in P60F-over-P60M and P67F-over-P67M. To understand how ‘core’ and ‘sex-specific’ TFBSs might influence differences in sensory song learning, I performed GO term enrichment analysis for biological process on the TFs with binding sites enriched in ‘core’ and ‘sex-specific’ TFBSs (Figure 26B, 27C).

A

TFBS ID	MOTIF	P60 Male		P60 Female		P67 Male		P67 Female	
		E VALUE	P VALUE	E VALUE	P VALUE	E VALUE	P VALUE	E VALUE	P VALUE
RFX2		1.80E-26	2.14E-29	8.06E-7	9.58E-10	1.82E-7	2.17E-10	6.72E-10	7.99E-13
RFX3		4.21E-24	5.00E-27	1.52E-12	1.81E-15	5.09E-8	6.05E-11	2.28E-10	2.71E-13
RFX1		1.98E-21	2.36E-24	6.37E-9	7.58E-12	6.34E-9	7.54E-12	9.34E-09	1.11E-11
PRDM9		9.23E-17	1.10E-19	9.70E-25	1.15E-27	2.18E-10	2.59E-13	1.80E-4	2.14E-7
RREB1		5.14E-16	6.12E-19	5.70E-11	6.78E-14	3.39E-10	4.03E-13	8.83E-4	1.05E-6
ZKSCAN5		3.67E-14	4.37E-17	2.29E-14	2.73E-17	9.02E-4	1.07E-6	3.45E-2	4.11E-5
RFX5		1.19E-11	1.41E-14	1.16E-8	1.38E-11	4.46E-13	5.30E-16	3.80E-8	4.52E-11
FOSL2::JUN		2.85E-2	3.38E-5	3.26E-7	3.88E-10	1.44	1.71E-3	8.19E-2	9.74E-5
FOSL2::JUND		6.98E-1	8.30E-4	3.54E-8	4.21E-11	1.78	2.12E-3	1.77E-1	2.10E-4
FOSL2::JUNB		3.23E-1	3.84E-4	5.78E-6	6.87E-09	2.51E	2.99E-3	3.24E-1	3.85E-4
FOS		1.94E-10	2.31E-13	2.26E-1	2.68E-4	3.50E-5	4.16E-8	1.65	1.97E-3
FOSL1::JUN		6.80E-6	8.08E-9	1.02E-2	1.21E-5	2.63	3.13E-3	1.93	2.29E-3
FOSB::JUNB		8.66	1.03E-2	7.59E-1	9.03E-4	6.66	7.92E-3	4.85E-2	5.77E-5
JUNB		4.03E-9	4.79E-12	4.06E-1	4.83E-4	8.49E-5	1.01E-7	1.41	1.68E-3
JUND		1.44E-1	1.71E-4	2.83E-1	3.37E-4	2.52	3.00E-3	9.99	1.19E-2
SP2		1.91E-8	2.27E-11	2.40E-6	2.85E-9	5.61E-3	6.67E-6	4.22E-3	5.02E-6
ATF3		5.98E-4	7.12E-7	8.67E-4	1.03E-6	1.32	1.57E-3	2.00	2.38E-3
SP1		3.48E-8	4.14E-11	9.33E-7	1.11E-9	1.86E-4	2.22E-7	9.24E-4	1.10E-6
JDP2		8.84E-8	1.05E-10	6.96	8.27E-3	7.05E-3	8.38E-6	4.91	5.84E-3
MEF2D		3.55E-7	4.22E-10	1.37E-17	1.63E-20	1.55E-14	1.85E-17	1.05E-1	1.25E-4
BNC2		1.56E-6	1.85E-9	2.31E-1	2.74E-4	2.00E-3	2.38E-6	5.19	6.17E-3
SP4		2.16E-6	2.57E-9	1.08E-6	1.28E-9	3.62E-4	4.31E-7	7.94E-1	9.44E-4
NFIC		6.80E-6	8.08E-9	1.02E-2	1.21E-5	2.63	3.13E-3	1.93	2.29E-3
PATZ1		1.56E-5	1.86E-8	3.02E-4	3.59E-7	7.49E-5	8.90E-8	1.22E-5	1.45E-8
JUN		1.24	1.47E-3	8.39E-3	9.98E-6	7.59	9.02E-3	9.91E-1	1.18E-3
SPI1		5.51E-5	6.55E-8	6.01E-16	7.14E-19	3.91E-4	4.65E-7	1.25	1.49E-3
KLF10		8.41E-5	1.00E-7	2.12E-7	2.52E-10	1.98E-1	2.35E-4	8.44E-1	1.00E-3
KLF7		3.24E-4	3.85E-7	2.96E-6	3.52E-9	8.69E-3	1.03E-5	3.43	4.08E-3
CREB5		1.31E-3	1.56E-6	7.20E-2	8.56E-5	3.75E-1	4.46E-4	7.76	9.23E-3
ZNF530		1.56E-3	1.86E-6	7.57E-10	9.00E-13	3.96E-3	4.70E-6	5.75E-3	6.83E-6
KLF15		1.65E-3	1.96E-6	2.42E-1	2.87E-4	7.29E-3	8.67E-6	1.08	1.29E-3
ARGFX		3.68E-3	4.37E-6	2.23E-13	2.65E-16	2.26	2.69E-3	6.28	7.47E-3
CREB1		1.26E-2	1.50E-5	2.84E-4	3.37E-7	5.25E-2	6.24E-5	4.56E-1	5.42E-4
ZNF93		1.84E-2	2.19E-5	2.31E-15	2.75E-18	3.77E-3	4.49E-6	1.65E-4	1.96E-7
FOSB::JUN		3.53E-2	4.20E-5	1.22E-3	1.45E-6	2.88E-1	3.43E-4	5.37E-2	6.38E-5
CREM		4.34E-2	5.16E-5	3.45E-5	4.10E-8	1.62	1.93E-3	3.14E-1	3.73E-4
KLF1		6.88E-2	8.18E-5	2.12E-5	2.52E-8	8.10	9.63E-3	2.07E-1	2.46E-4
ATF2		8.72E-1	1.04E-3	1.44E-8	1.71E-11	2.72	3.23E-3	2.28	2.71E-3
RFX7		1.15	1.36E-3	1.33	1.59E-3	4.14	4.92E-3	2.18E-4	2.59E-7
ELF5		1.25	1.49E-3	7.23E-7	8.60E-10	1.05E-4	1.25E-7	4.10E-4	4.87E-7
HOXA1		2.83	3.36E-3	2.03E-6	2.42E-9	1.90	2.25E-3	8.91	1.06E-2
HOXB4		5.78E	6.87E-3	5.25E-8	6.24E-11	1.28E-1	1.52E-4	7.05	8.39E-3

B

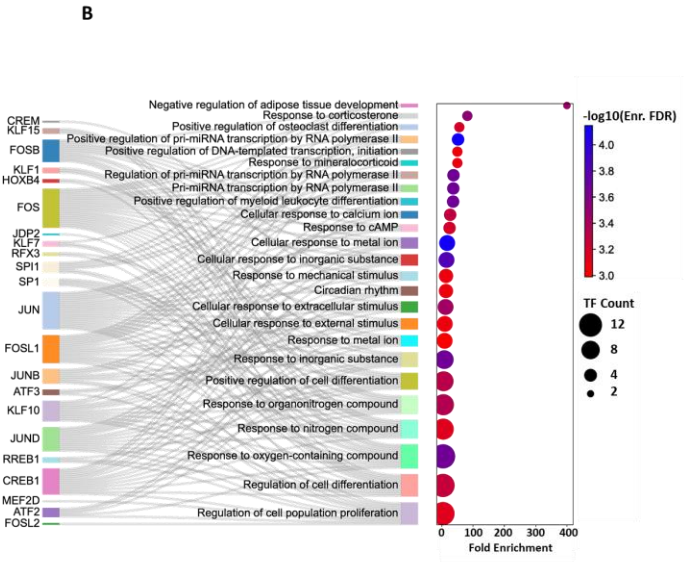

Figure 26. TFBSs enriched in sexually dimorphic regulatory regions in males and females at P60 and P67. (A) TFBSs enriched in P60M-over-P60F, P60F-over-P60M, P67M-over-P67F, and P67F-over-P67M regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (B) Sankey dot plot displaying the results from GO term enrichment analysis performed on core TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the TF colored bars to the GO term colored bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function were removed for the generation of this figure but are maintained in the results files. GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed.

A

		P60 Male		P67 Male	
TFBS ID	MOTIF	E VALUE	P VALUE	E VALUE	P VALUE
NFE2		5.02E-7	5.97E-10	2.16	2.57E-3
FOSL2		9.45E-6	1.12E-8	2.26E-5	2.69E-8
NFIX		8.02E-2	9.54E-5	2.63	3.13E-3
ZNF263		1.36	1.62E-3	7.80E-1	9.27E-4
MAFG::NFE2L1		1.53	1.81E-3	2.66	3.16E-3

B

		P60 Female		P67 Female	
TFBS ID	MOTIF	E VALUE	P VALUE	E VALUE	P VALUE
POU4F2		3.34E-7	3.97E-10	1.83	2.18E-3
IRF1		1.91E-4	2.27E-7	6.95	8.27E-3
ZNF257		6.21E-3	7.38E-6	5.90E-1	7.01E-4
IRF7		2.93E-2	3.48E-5	2.66E-2	3.17E-5
GABPA		6.66E-2	7.92E-5	2.55	3.03E-3
DUX		2.25E-1	2.68E-4	2.84	3.37E-3
NPAS4		8.16E-1	9.70E-4	8.40	9.99E-3
ERG		3.59	4.26E-3	1.73E-1	2.05E-4
IRF5		4.32	5.14E-3	1.93	2.29E-3
ATF6		6.14	7.30E-3	1.47E-2	1.75E-5

C

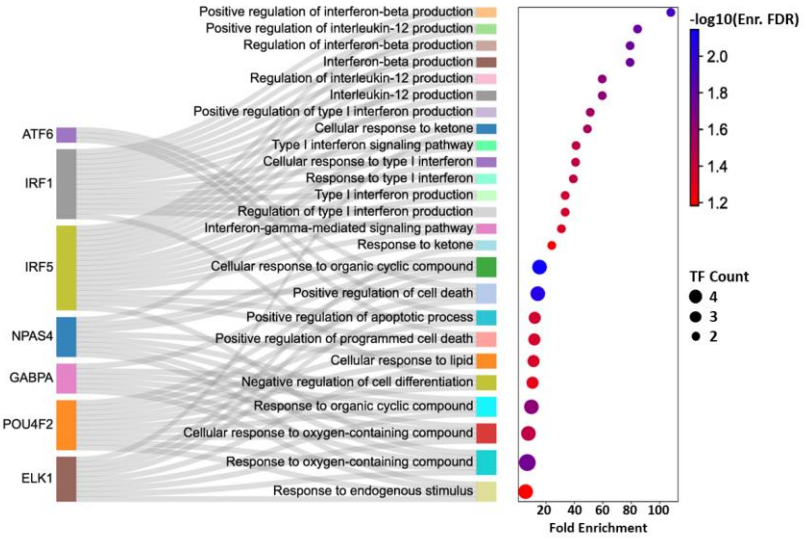

Figure 27. Male-specific and female-specific TFBS enriched in sexually dimorphic differentially accessible regions at P60 and P67. (A) 'Male-specific TFBSs" – TFBSs enriched in P60M-over-P60F and P67M-over-P67F differentially accessible regions and absent in P60F-over-P60M and P67F-over-P67M differentially accessible regions. Corresponding motif logos, enrichment values (E VALUES), and p values and displayed following each TFBS. (B) Sankey dot plot displaying the results from GO term enrichment analysis performed on 'male-specific' TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the TF colored bars to the GO term colored bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function were removed for the generation of this figure but are maintained in the results files. GO terms with the highest number of contributing TFs were then ordered by fold enrichment and displayed. (C) 'Female-specific TFBSs" – TFBSs enriched in P60F-over-P60M and P67F-over-P67M differentially accessible regions and absent in P60M-over-P60F and P67M-over-P67F differentially accessible regions. Corresponding motif logos, enrichment values (E VALUES), and p values and displayed following each TFBS.

Tutored and Isolate males possessed similar numbers of accessible regulatory regions

Previous experiments examining differences in epigenetic regulation between P67 Tutored and Isolate males demonstrate that experience can have a dramatic effect on post-translational histone modifications and resulting gene expression (Kelly et al. 2018). To investigate if, and how, song experience alters regulatory region accessibility profiles in male birds I compared regulatory region accessibility profiles of P67 Tutored and Isolate males. As previously stated, Tutored and Isolate males did not significantly differ in number of accessible regulatory regions (p adj = 0.78) (Figure 15E, 28A).

Differential analysis revealed regulatory regions with greater accessibility in P67 Tutored and Isolate males

To investigate the effects of experience, independent of maturation, on male regulatory region accessibility, I compared P67 Tutored and P67 Isolate male peak sets to identify differentially accessible regions. I identified 51 differentially accessible regions between Tutored and Isolate males, 39 of the regions demonstrated greater accessibility in P67 Tutored males and ('P67T-over-P67I'), and 12 of the regions demonstrated greater accessibility in P67 Isolate males ('P67I-over-P67T') (Figure 28B).

Regions differentially accessible between P67 Tutored and Isolate males were associated with distinct biological processes

To understand how regions differing in accessibility between P67 Tutored and P67 Isolate males might influence the neural properties related to the experience-dependent closure of the CP for TSM, I determined what TFs might contribute to differences in learning potential between experimental groups by identifying TFBSs enriched in the regions differing in accessibility between P67 Tutored and P67 Isolate males. P67T-over-P67I regions were enriched with TFBSs

for 3 TFs, ZNF708, SPIB, and ELF3, and P67I-over-P67T regions were enriched with TFBSs for 13 TFs, including SP1, THAP11, and many AP-1 family members (Figure 28C, 28D). Notably, there was little overlap in the identities of the TFs with binding sites enriched in regions differing in accessibility between P60 males and P67 males and identities of the TFs with binding sites enriched in regions differing in accessibility between P67 Tutored males and P67 Isolate males. Interestingly, a single TF, ELF3, possess binding sites enriched in both P67M-over-P60M regions and P67T-over-P67I regions.

I performed GO term enrichment analysis on TFs with binding sites enriched in P67T-over-P67I regions to determine the biological processes that might be contributing to the experience-dependent closure of the CP for TSM and on TFs with binding sites enriched in P67I-over-P67T regions to determine the biological processes that might be contributing to a maintained state of neural plasticity associated with open CPs. No BP GO terms were enriched in the P67T-over-P67I TFs, and beyond general themes of cellular responsivity, GO term enrichment analysis of P67I-over-P67T TFs did not highlight any themes apparently relevant for maintaining a state of neural receptivity (Figure 28E).To further investigate how regions differing in accessibility between P67 Tutored and P67 Isolate males might influence the neural properties that regulate TSM, I ascribed the gene with the nearest TSS to each differentially accessible region and performed GO analysis for biological process (BP), cellular component (CC), and molecular function (MF). No terms were significantly enriched in the genes associated with P67T-over-P67I regions. Terms significantly enriched in the genes associated with P67I-over-P67T regions highlight processes of chromosomal regulation and cell adhesion molecular binding (Figure 29).

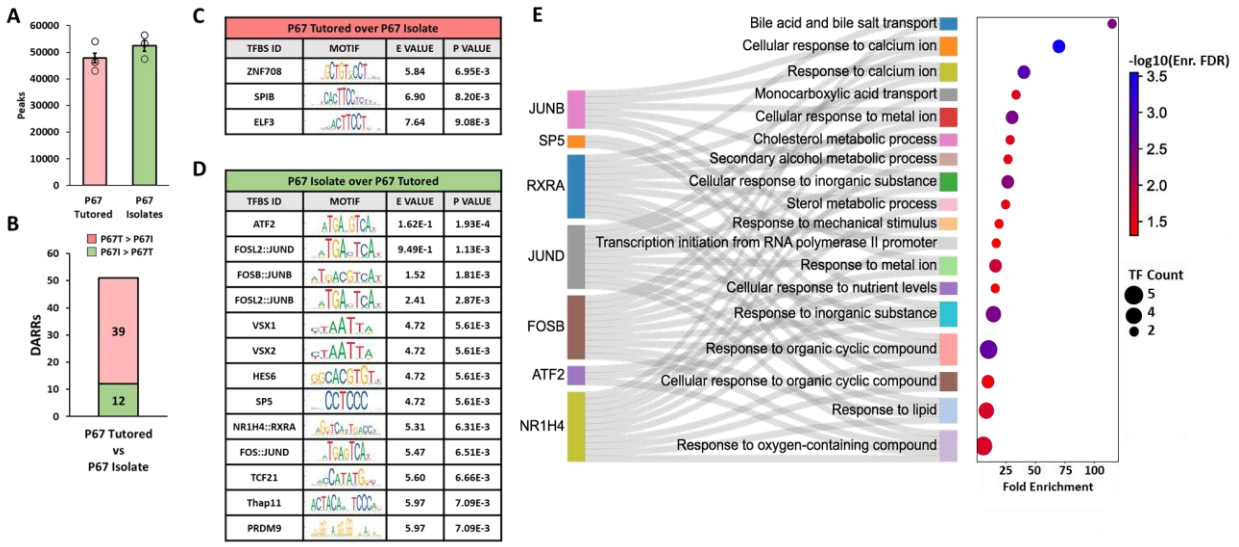

Figure 28. Comparison of P67 Tutored and Isolate male regulatory region accessibility profiles. (A) The number of called peaks for P67 Tutored and Isolate males. (B) The number of differentially accessible regions for P67 Tutored and Isolate males. (C) TFBSs enriched in P67T-over-P67I differentially accessible regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (D) TFBSs enriched in P67I-over-P67T differentially accessible regions and their corresponding motif logos, enrichment values (E VALUES), and p values. (E) Sankey dot plot displaying results from biological process GO term enrichment analysis performed on P67I-over-P67T TFs. Resulting GO terms are listed along the y-axis of the dot plot. The size of the dot, as well as the size of the colored bar preceding the GO term, indicates the number of TFs associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term. The TFs that gave rise to the GO terms are displayed along the left side of the figure. The size of the colored bar preceding the TF indicates the number of terms to which it contributes, and the grey lines running from the colored TF bars to the colored GO term bars indicate the GO terms with which the TF is associated. GO terms entirely unrelated to brain development and function and terms inherently related to all TF function were removed for the generation of this figure but are maintained in the results files. The 25 GO terms with the lowest enrichment FDRs were ordered by fold enrichment and displayed.

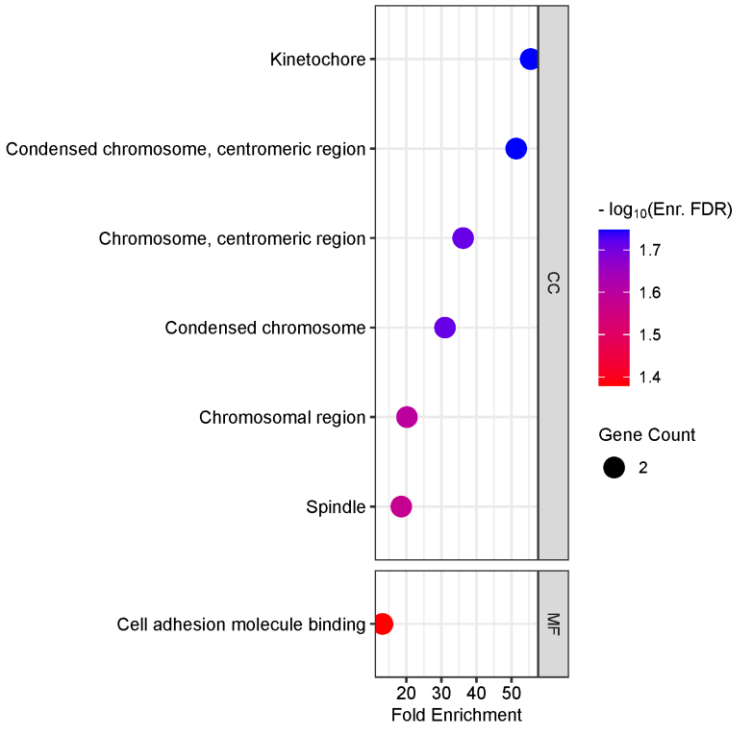

Figure 29. GO term enrichment for P67I-over-P67T genes. Dot plot displaying the results from biological process (BP), cellular component (CC), and molecular function (MF) GO term enrichment analysis performed on P67I-over-P67T genes. Resulting GO terms are listed along the y-axis of the dot plot and segmented based on the GO analysis from which they were derived. The size of the dot indicates the number of genes associated with the GO term. The dot color indicates the –log10 of the enrichment FDR value of the GO term. The dot's location along the x-axis indicates the fold enrichment of the GO term.

Discussion

Male Closure: Differences in regulatory region accessibility between P60 and P67 in males

In normally reared males, song heard after P65 is not incorporated into song produced in adulthood, suggesting that by P65, mechanisms of neural stability predominate over mechanisms of neural plasticity, thereby promoting the persistence of established tutor song memories that are used to inform sensorimotor and motor learning (Böhner 1983; Eales 1985, 1987; Slater et al. 1991; Morrison & Nottebohm 1993). To identify the biological, molecular, and cellular processes

that might facilitate the closure of the CP for TSM and differentiate cell populations capable and incapable of supporting TSM, I compared the regulatory region accessibility profiles of cells composing the ALs of P60 males with those of P67 males.

P60 and P67 males did not significantly differ in regulatory region number, and differential analysis revealed that a greater proportion of differentially accessible regions were more accessible in P67 males than in P60 males. Regions demonstrating greater accessibility at P60 were enriched with binding sites for two TFs, PAX4 and ZNF707. PAX4 has predominately been investigated for its role in pancreatic islet development and the differentiation of insulin-producing beta cells (Sosa-Pineda 2004; Lorenzo et al. 2017). Literature searches identified no studies that have directly examined the role of PAX4 in the brain and relatively few that have tangentially implicated PAX4 if brain function under abnormal or neuropathic conditions (Petschner et al. 2018; Majumder et al. 2021). Thus, it is difficult to infer the potential role of PAX4 may be playing in P60 male AL. Similarly, literature searches identified no studies that have directly examined the role of ZNF707 in any tissue. As such, the identified TFs provide little information regarding the regulatory functions that may be important for maintaining receptivity to tutor song at P60 compared to P67.

Furthermore, genes ascribed to regions of greater accessibility at P60 were not enriched for any GO terms, and literature reviews focusing on individual genes identified few with clear implications for brain development or function that were not equally or more so pertinent to biological function across many other tissues. Some of the notable genes to which regulatory regions were associated include KCNMB2 and NRSN1. KCNMB2 (Potassium Calcium-Activated Channel Subfamily M Regulatory Beta Subunit 2) is an auxiliary regulatory subunit of the calcium-activated potassium (maxiK) channel that can confer rapid and complete inactivation of maxiK channels (Wallner et al. 1999; Bentrop et al. 2001). MaxiK channels play important roles

in action potential firing, neurotransmitter release, learning and memory processes, and alterations in their expression profiles has been connected with the development of cognitive impairment (Jin et al. 2000; Hu et al. 2001; Faber & Sah 2003; Raffaelli et al. 2004; Gu et al. 2007; Matthews et al. 2009; Typlt et al. 2013; Springer et al. 2015; Wang et al. 2015a; Wang et al. 2015b; Griguoli et al. 2016). NRSN1 (Neurensin 1) is a relatively uncharacterized brain-specific protein implicated in memory consolidation (Cho et al. 2015). NRSN1 protein expression in hippocampus is downregulated following fear conditioning and its overexpression impairs long-term memory formation following several learning tasks, but not short-term memory (Cho et al. 2015).

Regions demonstrating greater accessibility at P67 were enriched with binding sites for transcription factors implicated in regulating neuron survival and apoptotic process (TFAP2B, REST, NR4A2), hormone secretion and response to hormone stimulus (REST, NR2C1, ESR2, NR4A2, TFAP2B), and cellular component morphogenesis supporting cell-cell signaling (MEF2A, RFX2, RFX4, NR4A2, TFAP2B, ESR2, REST, NR2C1). TFAP2B expression has been documented throughout the brain in both adult and embryonic mice, suggesting a functional role in both developing and mature neurons (Shimada et al. 1999; Schmidt et al. 2011; Zainolabidin et al. 2017). TFAP2B function is essential for the survival of populations of neural crest cells biased for sympathetic neuron generation but does not affect the induction or maintenance of TH and DBH expression in vivo, and, as discussed previously, has been shown to be important for the specification of GABAergic interneurons (Schmidt et al. 2011; Zainolabidin et al. 2017). REST (also known as Neuron-Restrictive Silencer Factor (NRSF)) (Chong et al. 1995; Schoenherr & Anderson 1995), a proposed master regulator of neurogenesis and neuron specification, regulates suites of neuron-specific genes through mechanisms of epigenetic suppression and transcriptional activation (Ballas et al. 2005; Tang 2009; Baldelli & Meldolesi 2015). REST is depleted in

developing neurons allowing for the expression of neuron-specifying genes. In adult neurons, REST participated in the remodeling of chromatin structure to fine-tune the expression of genes encoding transcription factors, proteins involved in neurotransmitter release, receptors, channels, and signaling proteins (Ballas et al. 2005; Rodenas-Ruano et al. 2012; Baldelli & Meldolesi 2015). NR4A2, mRNA for which is induced within adult male AL upon song exposure (Dong et al. 2009), contributes to the survival of dopaminergic neurons (Saucedo-Cardenas et al. 1998; Le et al. 1999; Sousa et al. 2007; Jo et al. 2009; Kadkhodaei et al. 2009; Decressac et al. 2013) and has been broadly implicated in the regulation of hippocampal synaptic plasticity enhancing long-term memory formation (Hawk et al. 2011; McQuown et al. 2011; McNulty et al. 2012; Aldavert-Vera et al. 2013; Bridi & Abel 2013). Collectively, these TFs may be functioning in conjunction to not only support neuronal survival but also to influence the transcription of genes sets that influence the maintenance of established long-term memories.

Along with the TFs discussed above, NR2C1 and ESR2 contributed to the identification of GO terms pertaining to hormone stimulus response. NR2C1 is a nuclear hormone receptor with no clearly established roles in brain development or function. However, NR2C1 is a critical regulator of cell patterning across the developing retina (Olivares et al. 2017), suggesting the potential for a role in the regulation of cell pattering within other tissues composing the central nervous system, such as the brain. ESR2 is an estrogen receptor that mediates the effects of estrogen on synaptic plasticity to enhance long-term potentiation (Liu et al. 2008). Through ESR2 binding, estrogens promote the sprouting of new dendritic spines and the formation of excitatory synapses (Woolley et al. 1997; Li et al. 2004), increase the establishment of NMDA receptors (NMDAR) (Adams et al. 2004), and enhances NMDAR-mediated synaptic activity and long-term potentiation (Woolley et al. 1997; Smith & McMahon 2006). Estrogen signaling within the AL, and its importance in

regulating juvenile and adult sensory song learning, are topics of active investigation. Estrogen-synthesizing (i.e. aromatase positive) and estrogen-sensitive neurons are prolific in NCM (Jeong et al. 2011). Rapid increases in estradiol levels within AL upon conspecific song exposure enhance auditory-evoked neuronal activity in males and females, and inhibiting estrogen synthesis in the same context decreases auditory-evoked firing and the establishing of song preference, potentially through reducing EGR-1 induction (Remage-Healey et al. 2008; Remage-Healey et al. 2010; Remage-Healey et al. 2012; Krentzel & Remage-Healey 2015). As such, it is interesting to observe increased accessibility for ESR2 in regions of greater accessibility at P67, as the TF certainly plays a role in modulating sensory song responsivity within the AL, and the mechanisms through which it does so are still obscure. Examination of potential ESR2 regulatory targets may be beneficial to further elucidating the mechanisms through which estrogen modulates sensory song responsivity within the AL.

GO terms enriched in P67 male TFs also emphasized themes of neuronal development that support synapse formation and neuronal communication (e.g., Plasma membrane bounded cell projection organization, Cell-cell signaling, and Brain development). The TFs that gave rise to these terms include MEF2A, RFX2, RFX4, NR4A2, TFAP2B, ESR2, REST, and NR2C1. Investigations focusing on RFX TF function within the brain are limited, and the precise regulatory roles of each RFX family member have not yet been determined (Sugiaman-Trapman et al. 2018). RFX TFs, including RFX2 and RFX4, are considered critical regulators of ciliogenesis and cell migration (Ashique et al. 2009; Chung et al. 2012). RFX4 mutant mice demonstrate abnormal brain development and possess higher risk for bipolar disorder (Zhang et al. 2007). Various RFX TF family members demonstrate binding sites enriched in regions changing in accessibility across development in males and females and in sexually dimorphic regions spanning development,

thereby increasing the likelihood that RFX TFs are playing some regulatory role within developing AL. There being limited investigations aimed at understanding the role of these TFs in the brain leads to difficulty in extrapolating their potential importance. This may be the result of little support for such investigations or because the implication of these TFs in brain development is fairly recent. Regardless, sensory song learning in zebra finches would be a well-suited paradigm for furthering such lines of inquiry. The other TFs contributing to the identification of these terms have been introduced above or at an earlier point in this thesis and, as such, are not discussed in further detail here.

Genes ascribed to regions of greater accessibility at P67 were enriched for cellular component GO terms highlighting synaptic transmission (e.g., Synapse, Postsynapse, Dendrite, Dendritic tree, Postsynaptic specialization), as well as specific neuronal cell structures (e.g., Perikaryon, Ruffle). Molecular function GO terms highlighted transporter activity and glutamate receptor binding (e.g., Glutamate receptor binding, Type 3 metabotropic glutamate receptor binding, G protein-coupled glutamate receptor binding). The genes that gave rise to these terms highlighting synaptic signaling between neurons include CACNG3, AP2B1, SDK2, DLGAP4, MAPK1, DRP2, AAK1, PSD2, WASF2, GRM2, DDN, AGRN, SLC6A9, DYNLL2, SLC9A3R2, SLC12A3, FSCN1, SLC9A3R1, KCNE3, OSBP2. CACNG3 (Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 3) is a transmembrane AMPA receptor regulatory protein (TARP) that modulates the AMPA receptor trafficking and function (Burgess et al. 1999; Payne 2008). AP2B1 (Adaptor Related Protein Complex 2 Subunit Beta 1) and AAK1 (AP2 Associated Kinase 1) are kinases that phosphorylate subunits of the AP-2 complex, which interacts with membrane-bound receptors to promote subsequence endocytosis and has been implicated in the regulation and maintenance of hippocampal LTP in murine models (Marcello et al. 2013). SDK2 (Sidekick Cell

Adhesion Molecule 2) is a neural adhesion molecule that promotes the formation of synapses between retinal neurons necessary for the detection of motion (Yamagata et al. 2002; Krishnaswamy et al. 2015). DLGAP4 (DLG Associated Protein 4) is a kinase that supports synaptic organization and signaling at the postsynaptic density (PSD) of neuronal cells through interactions with potassium channels and receptors, as well as other signaling molecules (Takeuchi et al. 1997; Rao et al. 1998). More recently, DLGAP4 expression has also been shown to be critical for cerebral cortex development, as it is required for proper radial glial cell organization and neuronal migration (Romero et al. 2022). MAPK1 (Mitogen-Activated Protein Kinase 1; also commonly known as Extracellular Signal-Regulated Kinase 2 (ERK2)) is a kinase that mediates a diversity of biological functions, including cell growth, survival, and differentiation. MAPK1 activation is required for EGR-1 transcription and to support TSM (Cheng & Clayton 2004; London & Clayton 2008). DRP2 (Dystrophin-Related Protein 2) is a protein enriched at the PSD with particularly high concentrations in brain regions involved in cholinergic transmission, suggesting a role for DRP2 in the organization of cholinergic synapses (Roberts & Sheng 2000). PSD2 (Pleckstrin And Sec7 Domain Containing 2) is a nucleotide exchange factor localized to the PSD that is preferentially expressed in adult Purkinje cells of the cerebellar cortex (Matsuya et al. 2005). WASF2 (WASP Family Member 2) contributes to the formation of a multiprotein complex that links receptor kinases, involved in the transduction of signals changing cell shape, motility, and function, to actin (Xie et al. 2013). GRM2 (Glutamate Metabotropic Receptor 2) is a g-protein coupled glutamate receptor implicated in the regulation of hippocampal synaptic plasticity (Yokoi et al. 1996; Nicholls et al. 2006). Furthermore, GRM2 has recently been shown to regulate the integration of adult born hippocampal neurons through modulating the MEK/ERK1/2 cascade (Ma et al. 2023). DDN (Dendrin) contributes to PSD stabilizing in the forebrain and hippocampus

through cytoskeletal interactions (Herb et al. 1997; Kawata et al. 2006; Kremerskothen et al. 2006). AGRN (Agrin) expression is highest during periods of increased synaptogenesis and remains high brain regions that demonstrate increased synaptic plasticity, including hippocampus and cortex (Bowe et al. 1994; O'Connor et al. 1994; Stone & Nikolics 1995; Li et al. 1997). AGRN suppression in cultured hippocampal neurons significantly reduces synapse formation (Ferreira 1999; Böse et al. 2000; McCroskery et al. 2009). SLC6A9 (Solute Carrier Family 6 Member 9) and SLC12A3 (Solute Carrier Family 12 Member 3) are transporter proteins belonging to the family that function to remove glycine from the synaptic cleft following neurotransmission and maintain cellular sodium and chloride homeostasis, respectively (Harsing et al. 2012; Harvey & Yee 2013). SLC9A3R1 (SLC9A3 Regulator 1; also commonly known as NHERF1) and SLC9A3R2 (SLC9A3 Regulator 2; also commonly known as NHERF2) are scaffolding proteins that associate with metabotropic glutamate receptors (mGluR2 and mGluR3) to regulate the cellular distributions mGluR2/3 (Sato et al. 2013; Ritter-Makinson et al. 2017). DYNLL2 (Dynein Light Chain LC8-Type 2) is a component of the dynein 1 complex, which promotes retrograde movement of vesicles and organelles along axonal microtubules (Vallee et al. 1989; Susalka & Pfister 2000). FSCN1 (Fascin Actin-Bundling Protein 1) is an actin-binding protein capable of inducing membrane protrusions, such as filopodia, that influence cell motility, migration, and axonal growth-cone collapse (Deinhardt et al. 2011; Jansen et al. 2011). KCNE3 (Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 3) is a beta subunit of a voltage-gated potassium channel that modulates gating kinetics by reducing reliance on membrane depolarization for gate opening (Schroeder et al. 2000; McCrossan et al. 2003; Abbott 2016). OSBP2 (Oxysterol Binding Protein 2) is highly expressed in the hippocampus and has been implicated in the regulation of neuronal cholesterol biosynthesis (Martin 2018). Genes regulating

transported activity, but not specifically implicated in synaptic function included other solute carrier family members, SLC50A1 (Solute Carrier Family 50 Member 1) and SLC39A3 (Solute Carrier Family 39 Member 3), as well as genes contributing to ATP production through participation in the mitochondrial respiratory chain, NDUFA8 (NADH:Ubiquinone Oxidoreductase Subunit A8), UQCR11 (Ubiquinol-Cytochrome C Reductase, Complex III Subunit XI), and ATP5MG (ATP Synthase Membrane Subunit G). Collectively, the association of these genes with regulatory regions demonstrating greater accessibility at P67 suggest that alterations in the expression of proteins participating in synaptic transmission may underlie the decrease in neural responsivity characterizing the closure of the CP for TSM.

It is unknown as to whether or not the same biological mechanisms gating the onset of TSM in males also regulate TSM closure. If this were to be the case, one might hypothesize that regulatory region differing in accessibility between P23 and P30 males would be located at the same genomic locations as those differing in accessibility between P60 and P67 males, and that the directionality of differences in accessibility would correspond with differences in learning potential (i.e., regions increasing in accessibility across the onset for the CP for TSM would decrease in accessibility across the closure of the CP for TSM and regions decreasing in accessibility across the onset for the CP for TSM would increase in accessibility across the closure of the CP for TSM). Alternatively, it the same biological mechanisms regulate the onset of TSM and its closure, one might hypothesize that regions differing in accessibility between P23 and P30 males would be enriched with binding sites for the same TFs, or at least TFs with identical regulatory functions, as those differing in accessibility between P60 and P67 males, and that the directionality of differences in TFBS enrichment would correspond with differences in learning potential. The results from my experiment do not support either of these potential hypotheses.

Comparison of differentially accessible regulatory region profiles associated with the onset of TSM and its closure, suggest that the two transitions in learning potential are regulated by overlapping, yet predominately distinct, sets of TFs and genes that have each been implicated in the regulation of overlapping neural processes. TFBSs for MEF2A, MEF2B, and TFAP2B were enriched in regions with greater accessibility at P67 than P60. TFBSs for MEF2A were also enriched in regions with greater accessibility at P23 than P30, while TFBSs for MEF2B and TFAP2B were also enriched in regions with greater accessibility at P30 than P23. Thus, even the TFs changing in regulatory potential at both the onset and closure of the CP for TSM do not perfectly agree with what is known regarding the capacity for learning in males across development. Genes ascribed to differentially accessible regions demonstrate a similar pattern; CACNG3 and NYX were associated with regions changing in accessibility across the onset and the closure of the CP for TSM. Differentially accessible regions ascribed to each demonstrated greater accessibility at P67 than at P60 and at P30 than P23. Notably, there was no overlap in the genomic locations of the regulatory regions differing in accessibility across the onset and those differing in accessibility across the closure, introducing the possibility that regulatory regions may be differently influencing the transcription of these genes at each developmental time point.

Female Closure: Differences in regulatory region accessibility between P60 and P67 in females

Adult female song preference is influenced by developmental song experience (Miller 1979b, 1979a; Clayton 1988; Riebel 2000; Riebel et al. 2002; Riebel 2003b, 2003a; Lauay et al. 2004; Terpstra et al. 2006; Riebel et al. 2009; Svec & Wade 2009; Holveck & Riebel 2014; Chen et al. 2017; Diez et al. 2019; Wei et al. 2022), and there is strong evidence to suggest that song exposure during early development is equally influential in determining adult song preference in

females as it is in shaping adult song production in males (Riebel 2000). There remain substantial gaps in our understanding of the mechanisms that regulate song preference learning. For example, a point in development at which song experience no longer influences female preference has not been established, potentially because investigations into the matter have predominately focused on the outcome of the learning, rather than the learning process itself. Female song preferences established during development appear decidedly stable, persisting even when further song experience is provided, suggesting the process is terminal (Riebel 2000). If TSM in males and preference song learning in females adhere to the same developmental progression, one could expect to observe marked differences in regulatory region accessibility between females spanning the closure of the male-defined CP for TSM. Furthermore, If TSM in males and preference song learning in females are indistinguishable processes under the influence of the same regulatory mechanisms, one could expect that differences in regulatory region accessibility between females spanning the closure of the CP for TSM would mirror differences in regulatory region accessibility between males spanning the closure of the CP for TSM. Thus, to determine if female regulatory region accessibility profiles change across the closure for the CP for TSM, I compared the regulatory region accessibility profiles of cells composing the AL in P60 females with those of P67 females. Additionally, I compared the changes in regulatory region accessibility observed in females to those observed in males to identify potential similarities and differences in their regulatory region accessibility development.

Unexpectedly, regulatory region accessibility profiles differed between P60 and P67 females to a greater extent than was observed across any of the other comparisons made between birds of the same sex. Although not significantly differing in the number of H3K27ac-defined peaks, differential analysis identified thousands of regions, enriched with binding sites for

hundreds of TFs, differing in accessibility between P60 and P67 females, a large portion (80%) of which demonstrated greater accessibility in P60 females. This outcome was particularly surprising as there is no evidence to suggest that the regulatory region accessibility profiles of P60 and P67 females would differ to this extent. As there have been limited investigations examining differences in female AL at these time points, it is difficult to speculate about the implications of this result. Experiments examining responsivity to song in female AL suggest that electrophysiological responsivity to song appears by P20 and molecular signatures of song response emerge sometime between P30 - P45 (Stripling et al. 2001; Bailey & Wade 2003, 2005; Tomaszycki et al. 2006).

Interestingly, unpublished results from the London laboratory have recently demonstrated that the mTOR signaling cascade, as measured by phosphorylation of pS6 in response to acute song experience, appears to become inducible by song between P60 and P67 (Butler and London, unpublished). There is the potential that these changes in molecular responsivity and regulatory region accessibility underlie a shift in the functional role of song between P60 and P67, such that song heard at earlier stages of development is establishing preference, while song heard during later stages of development and in adulthood is informing mate appraisal (Macdougall-Shackleton 1997; Collins 2004; Tomaszycki & Adkins-Regan 2005; Riebel et al. 2009; Woodgate et al. 2012). Females can still form preferences for the song of their mate or a male with whom they have been housed over an unfamiliar song well after 120 days (Miller 1979a; Clayton 1988). However, the novel experience does not appear to overwrite the developmentally established preferences. Rather, it contributes to establishing an additional preference (Clayton 1988; Riebel 2000; Holveck & Riebel 2014). Determining the manner and extent to which the song preferences established in adulthood reflect the generalization of preferences established during early development is crucial

in understanding if these are distinct learning processes, potentially even as separable from one another as TSM and song production learning in males. Independent of these considerations, the results from my experiment support a relatively extreme shift in regulatory region accessibility in female AL between P60 and P67 (time points spanning the closure of the CP for male TSM), potentially opening the door for new lines of inquiry into female AL development during this window of development.

I compared the changes in regulatory region accessibility observed in females to those observed in males to identify potential similarities and differences in their regulatory region accessibility development beyond those evident from examining count alone. There was relatively little overlap in the TFBSs enriched in regions differentially accessible between P60 and P67 in males and females and there was little overlap in the identities of the putative genes ascribed to those regions, suggesting that males and females undergo different developmental shifts in regulatory region accessibility between P60 and P67. Only TFBSs for RFX2, RFX4, MEF2A, and MEF2B were enriched in regions of greater accessibility at P67 in males and females and there were no TFBSs commonly enriched in regions of greater accessibility at P60 in males and females. RHBDF1 (Rhomboid 5 Homolog 1), a regulator of epidermal growth factor receptor-stimulated cell processes and endoplasmic reticulum-associated protein production (Ji et al. 2022) was the only gene commonly ascribed to regions of greater accessibility in P60 males and females, and RASL10B (RAS Like Family 10 Member B), a relatively uncharacterized GTPase belonging to the Ras superfamily, was the only gene commonly ascribed to regions of greater accessibility in P67 males and females. Interestingly, the greatest degree of overlap in TFBS enrichment and putative target genes was among the regions with greater accessibility in P60 females compared to P67 females and regions with greater accessibility in P67 males than in P60 males. These regions

were enriched with binding sites for NR2C1, MEF2B, ESR2, MEIS2, ELF3, MEF2A, and TFAP2B and shared 32 of the same putative gene targets. In sum, these results suggest that males and females undergo drastically different developmental shifts in regulatory region accessibility between P60 and P67.

Considering the extensive number of TFBSs enriched in regions differing in accessibility between P60 and P67 females, the pleiotropic nature of TF function, and the relatively small amount of knowledge surrounding female AL development and behavior at these timepoints, it is difficult to determine which biological themes are of the highest relevance for distinguishing females of each age. Regions more accessible at P60 were enriched with binding sites for TFs that highlight themes of general cellular development, including regulation of cell population proliferation and cell fate commitment, but also emphasized themes crucial for brain development and organization, including neurogenesis, neuron differentiation, and forebrain development. These TFs have also been implicated in regulating the differentiation of more specific neural cell types (e.g., GABAergic neuron differentiation, Dopaminergic neuron differentiation, Astrocyte differentiation, Oligodendrocyte differentiation), as well as countless other processes crucial for regulating brain development and function necessary to support developmental learning (e.g., Axonogenesis, Cell morphogenesis involved in neuron differentiation, Neuron migration). Such themes (e.g., Neuron fate commitment, Diencephalon development, Regionalization, Central nervous system neuron differentiation) were among those most highly enriched in P60 females, suggesting that the regulation of neuronal development and organization may be under a greater deal of transcriptional regulation in P60 females than P67 females.

In contrast, biological processes most enriched among TFs with binding sites enriched in regions more accessible at P67 highlighted themes of Pri-mRNA transcription, circulatory system

development, and general cellular responsivity. Themes of neuron development and organization were still enriched at P67, but to a far lesser extent than at P60. This may reflect the overlap in TFs with increased potential to function at regions with greater accessibility at both P60 and P67. Interestingly, this was the only comparison between birds of the same sex in which binding sites for the same TFs were enriched in regions demonstrating greater accessibility at each age. As TFs were derived from differentially accessible regions, their binding sites, are, by definition, located at different regions of the genome, suggesting that even the same transcription factors are differentially influencing transcription at each age.

GO terms enriched in genes ascribed to regions of greater accessibility at P60 and P67 displayed results mirroring that of the GO terms enrichment analysis performed on TFs. Genes ascribed to regions of greater accessibility at each age were enriched for largely overlapping sets of biological processes (e.g., Neurogenesis, Neuron differentiation, Neuron development, Axonogenesis, Axon guidance, Cell adhesion, Cell-cell signaling, Synaptic signaling) influencing similar cellular components (e.g., Neuron projection, Synapse, Glutamatergic synapse, Axon, Dendrite, Presynapse, Postsynapse, Dendritic tree, Neuron to neuron synapse). Again, as with the TF analysis, these terms were far more enriched at P60 than P67 suggesting that there may be a greater degree of transcriptional regulation influencing neuron development and organization at P60 than at P67 in females. Interestingly, Protein kinase activity and Protein kinase binding were among the terms most highly enriched in the P60 female gene set and absent in the P67 female gene set. The genes that gave rise to these terms code for kinases, phosphatases, and intracellular receptors that have been implicated in the regulation of signal transduction related to synaptic plasticity (CAMK1, CAMKK1, CAMK2B, TNK2, EPHB3, MARK2, STK11, PRKCB), GPCR-mediated intracellular signaling (PIP5K1B, PIK3C2B, DDR2, PKIG, GRK2, GRK5, GRK6), cell

cycle progression (CDK13, CSNK2A2), energy homeostasis and glucose metabolism (GALK1, PGM2L1, INSR, PRKAG1, AATK), and cytoskeletal organization (FGR, EPHA3, SLK, TJP2, ILK); all processes that are integral to the regulation of learning and memory formation and have been identified as potential contributors to differences in learning potential in juvenile zebra finches. For example, among these genes were multiple calcium/calmodulin-dependent protein kinase family members (e.g., CAMK1, CAMKK1, CAMK2B), which influence the activity of proteins regulating mechanisms of synaptic plasticity in response to changes in cellular calcium concentration (Liu & Murray 2012; Zalcman et al. 2018; Yasuda et al. 2022). Furthermore, many of the genes contributing to the identification of these terms directly participate in the MAPK signaling cascade and its regulation, or are among its downstream targets (MAPK6, MAP2K6, TGFBR3, STK10, PDGFRB, ACVR1, KSR1, FGFR4, WNK2, MELK, PRKCB, SPHK1), providing support for the idea that a signaling cascade necessary for TSM memorization in males is being differentially regulated between P60 and P67 in females (Cheng & Clayton 2004; London & Clayton 2008). Focusing on the genes implicated in the regulation of kinase activity alone, while ignoring other overlapping brain-specific processes, still implicated the overlapping processes enriched in both P60 and P67 females. This outcome illustrates the difficulty of drawing meaningful conclusions from the comparison of GO enrichment analyses highlighting an extensive number of regulatory processes and emphasizing a need for further targeted investigations to disentangle which regulatory processes are biologically relevant for differentiating P60 and P67 females AL. However, as was the case with the comparison of P60 and P67 females, large-scale bioinformatic experiments have a history of motivating new lines of inquiry and implicating previously uninvestigated genes and biological processes. Collectively, the differences between P60 and P67 females identified from this experiment support the prevalence of extreme differences

in transcriptional regulation within the ALs of these birds and implicate countless previously unexplored transcription factors, genes, and themes of biological regulation that could lead to drastic differences in the influence of song experience at each age.

Sex Differences: Differences in regulatory region accessibility between P60 and P67 males and females

To investigate the influence of sex on regulatory region accessibility and explore potential differences in mechanisms of sensory song learning, I compared P60 male and female peak sets to identify regulatory regions differing in accessibility between them. Additionally, to assess if and to what extent sexually dimorphic region profiles shift across development, I compared the regulatory region accessibility profiles of P67 males with those of P67 females. Current evidence suggests that measures of molecular and electrophysiological responsivity to song become more similar in males and females across development (Chew et al. 1996; Stripling et al. 2001; Bailey & Wade 2003, 2005; Tomaszycki et al. 2006; Ahmadiantehrani & London 2017a; Scully et al. 2017; Ahmadiantehrani et al. 2018). For example, at P30, song experience results in the induction of EGR-1 protein following exposure to conspecific song with the ALs of males, but not females (Bailey & Wade 2003). At the same age, song experience results in the induction of FOS with the ALs of females, but not males (Bailey & Wade 2003). However, at P45, and in adulthood, the induction of EGR-1 and FOS within the ALs of males and females is indistinguishable (Bailey & Wade 2005; Velho et al. 2005). This developmental convergence in song responsivity within the AL is further illustrated by investigations of the mTOR signaling cascade. At P30, song experience leads to activation of the mTOR signaling cascade in the AL of P30 males, but not P30 females (Ahmadiantehrani & London 2017a). In adulthood, song experience leads to activation of the mTOR signaling cascade in the AL in both sexes (Ahmadiantehrani et al. 2018). Generally, it is

assumed that measures of responsivity to song that become inducible by song during development, and remain inducible by song in adulthood, are maintained throughout that window of time. As molecular measures of song responsivity in males and females appear to become more similar across development, one could reasonably hypothesize that the male and female regulatory region accessibility profiles would also become more similar across development. However, the results from this experiment support a non-linear progression in similarity between male and female regulatory region accessibility profiles, such that rather than starting at point of greatest difference and gradually progressing towards a point of greatest similarity, they change across development leading to interspersed timepoints of differing degrees of similarity.

Among all ages assayed in this experiment, males and females differed to the greatest extent at P60. Regions of greater accessibility in each sex outnumber those identified from all other comparisons of age-matched males and females, suggesting that increased regulatory region accessibility in each sex contributes to this outcome. However, regions of greater accessibility in females at P60 were approximately 2.5 times greater than the average number of regions with greater accessibility in females across all assayed time points, whereas regions of greater accessibility in males at P60 were only 1.2 times greater than the average number of regions with greater accessibility in males across all assayed time points. At P67, the distribution of sexually dimorphic region accessibility between males and females more closely mirrors that observed at P23 and P30, in that regions of greater accessibility in males outnumber those of greater accessibility in females. The decrease in sexually dimorphic regions between P60 and P67 resulted from a reduction in both regions with greater accessibility in males and regions with greater accessibility in females, but the proportion of reduction was notably greater in females. The same trend was present for the number and identities of enriched TFBSs. Thus, the observed increase in

similarity between males and females across development is primarily at the expense of decreasing female regulatory region accessibility, an outcome reminiscent to what was observed across the onset of the CP for TSM. This finding, as well as the results from the comparison of P60 and P67 females, leads me to speculate that P60 may coincide with a unique point in female development characterized by a relatively high degree of regulatory region accessibility with the potential to influence a broad range of processes pertinent to brain development and function.

This idea is further bolstered by the results from the high-level GO term analysis, which demonstrating that all high-level GO terms associated with a greater number of TFs originating from regions of greater accessibility in P60 females than P60 males, that those originating from regions of greater accessibility in any other across sex comparison. Similarly, GO term enrichment analysis showed that commonly enriched GO terms demonstrated greater fold enrichment and lower FDR values in females than males at P60, but also identified GO terms uniquely enriched in both sexes. Terms commonly enriched in males and females highlighted themes of general cellular development and organization (e.g., Regionalization, Pattern specification process, Regulation of cell differentiation, Cell fate commitment), as well as themes more pertinent to brain development (e.g., Central nervous system development, Neurogenesis, Neuron differentiation). Terms unique to females at this age further highlighted themes of brain development (e.g., Regulation of neurogenesis, Regulation of gliogenesis, Noradrenergic neuron development), as well as steroid hormone responsivity (e.g., Response to steroid hormone, Cellular response to steroid hormone synthesis). Contrastingly terms unique to males at this age highlighted themes of endothelial and epithelial cell migration, core promoter binding, response to calcium, and associative learning. The same pattern of results does not hold true at P67. Terms commonly enriched in males and females at P67 highlighted notably different themes, including the regulation pri-miRNA transcription

(e.g., Pri-miRNA transcription by RNA polymerase II, Regulation of pri-miRNA transcription by RNA polymerase II, Positive regulation of pri-miRNA transcription by RNA polymerase II), cellular responsivity (e.g., Response to oxygen-containing compound, Response to organonitrogen compound, Response to nitrogen compound), and epithelium development (e.g., epithelial cell differentiation, Epithelium development). Terms that were commonly enriched at P60 (e.g., Pattern Specification process, Regionalization, Anterior/posterior pattern specification, central nervous system development, Cell fate commitment, Neurogenesis, Generation of neurons, Neuron differentiation) become specific to males at P67.

GO term enrichment analysis performed on gene sets ascribed to regulatory regions of greater accessibility in each sex at P60 and P67 support a similar developmental progression of sex differences between the two ages as what was observed from the transcription factor analysis. At P60 and P67, genes ascribed to regions of greater accessibility in males and females were enriched for GO terms highlighting themes of brain development and organization. Biological process terms commonly enriched in male and female gene sets at P60 and P67 included Neurogenesis, Neuron differentiation, Neuron development, Neuron projection morphogenesis, Synapse organization, and Synaptic signaling, and cellular component terms common to all gene sets included Synapse, Neuron projection, Postsynaptic density, Postsynaptic specialization, Neuron to neuron synapse, Dendrite, and Dendritic tree. Countless other common and unique terms pertaining to brain development and organization were enriched within these genes sets, but, generally, it appears that many of the same general processes are being regulated, by definitionally distinct sets of genes, between males and females at each time point.

However, there were some distinct processes emphasized in both males and female worth noting. For example, as was the case with the comparison of P60 and P67 female gene function,

genes ascribed to regions of greater accessibility in P60 females than P60 males were most highly enriched with molecular function GO terms pertaining to protein kinase binding and activity, suggesting that this distinction in function is not only developmentally relevant, but also sexually dimorphic. Interestingly, at P67 genes ascribed to regions of greater accessibility in males, but not those of greater accessibility in females, were enriched with molecular function GO terms for protein kinase binding and activity. Of additional interest, as was the case at P30, genes ascribed to regions of greater accessibility in males than females at both P60 and P67 were enriched with biological process GO terms for Ciliary neurotrophic factor-mediated signaling pathway, Regulation of Rho protein signal transduction, and Regulation of Ras protein signal transduction, suggesting that the regulation of these processes may be particularly important for function within male, but not female AL, following the onset of TSM in males.

Although to a lesser extent than at P23 and P30, a large proportion of sexually dimorphic regions were located on sex chromosomes at P67 (68.1%). However, at P60, only 39.3% of sexually dimorphic regions were located on sex chromosomes. This shift in the distribution of differential regions across autosomes and sex chromosomes was predominately the result of an increase in regulatory regions accessibility at P60 in females. Interestingly, a recent study examining sex differences in transcription within RA, the major output nucleus of the song system, at P20 and P50 identified a strikingly similar progression in the distributions of sexually dimorphic genes between autosomes and sex chromosomes to the distribution of sexually dimorphic regions observed in this study. The RNA-seq data from RA found that 87% of differentially expressed genes (DEGs) were located on sex chromosomes at P20, while only 10% of DEGs were located on sex chromosomes at P50 (Friedrich et al. 2022). As the authors of this study used the male zebra finch genome assembly for RNA-seq read alignment, the true percentage of DEGs located on sex

chromosomes is likely even higher than reported, as W-chromosome specific genes are not represented. Within RA, these DEGs associate with sex-specific developmental programs that, in part, explain the progression of RA from a sexually monomorphic to a sexually dimorphic brain region (Friedrich et al. 2022). Unlike RA, which demonstrates extreme differences in morphology, electrophysiology, and connectivity across sexes during development and in adulthood (Bottjer et al. 1985; Konishi & Akutagawa 1985; Bottjer et al. 1986; Nordeen & Nordeen 1988a; Nordeen & Nordeen 1988b; Kirn & DeVoogd 1989; Nordeen et al. 1992; Burek et al. 1994; Mooney & Rao 1994; Nixdorf-Bergweiler 1996; Adret & Margoliash 2002; Konishi & Akutagawa 2007; Ölveczky et al. 2011; Zemel et al. 2021), the AL appears far less sexually dimorphic in morphology (Krentzel & Remage-Healey 2015; Brenowitz & Remage-Healey 2016). This aligns with the knowledge that RA supports song production (Yu & Margoliash 1996; Kimpo & Doupe 1997; Hahnloser et al. 2002; Leonardo & Fee 2005), a male-specific behavior in zebra finches, while the AL likely supports sensory song learning in both sexes (Chew et al. 1996; Bailey & Wade 2003, 2005; Velho et al. 2005; Tomaszycki et al. 2006; Scully et al. 2017; Ahmadiantehrani et al. 2018). There is the potential that similar shifts in sex-specific transcriptional regulation may underlie differences in AL development between sexes that differentially influence processes of sensory song learning. However, as we have not yet been able to identify the exact complement of cells required to support sensory song learning, decern if the molecular and cellular processes necessary to support sensory song learning in each sex meaningfully differ, or if the emergence of connections projecting from AL to downstream behaviorally-relevant circuitry developmental differ between sexes, it is not yet apparent exactly which neural feature inform differences in learning potential. As the scope of potential pertinent neural processes continues to narrow, our

interpretation of the implications of sex-specific transcriptional regulation within the AL should too.

As was the case when comparing sexually dimorphic regions at P23 and P30, comparison across TFBSs enriched in sexually dimorphic regions at P60 and P67 identified a subset of TFBSs enriched in sexually dimorphic regions independent of age and sex ('core') and subsets specific to each sex ('sex-specific'). The number of core TFBSs among the P60 and P67 comparisons was more than double the number of core TFBSs among the P23 and P30 comparisons, suggesting that the number of transcription factors with increased regulatory potential at sexually dimorphic regions of greater accessibility in both males and females increase across development. A large portion (70%) of the core TFBSs at P23 and P30 persisted in at P60 and P67. Those that did not were binding sites for ZNF148, ZNF281, RFX4, RFX6, SPIB, and the AP-1 protein dimer JUN::JUNB. Those that emerged as core TFBSs among the P60 and P67 comparisons, but were absent from the core TFBSs among the P23 and P30 comparisons, included AP-1 family members (JUN, JUNB, JUND, ATF2, ATF3) and protein dimers (FOSB::JUNB, FOSL1::JUN, FOSL2::JUN, FOSL2::JUNB, FOSL2::JUND), SP/KLF family members (SP1, SP2, KLF7, KLF10, KLF15), HOX family members (HOXA1, HOXB4, ARGFX), cAMP-responsive TFS (CREM, CREB5), and others (RFX7, MEF2D, ZKSCAN5, BNC2, SPI1, ELF5, ZNF530).

In line with the breadth of biological processes with which these TF families have been associated, GO term enrichments analysis performed on these TFs highlighted broad themes of cellular regulation (e.g., Regulation of cell population proliferation, Regulation of cell differentiation) and responsivity (e.g., Cellular response to external stimulus, Cellular response to calcium ion, Response to cAMP). Notably, Learning and Associative learning were also among the terms enriched in these TFs.

Again, it is important to note that these TFBSs were derived from sexually dimorphic regions, that are, by definition, located at different genomic locations in males and females. Therefore, the TFs binding these regions are likely regulating the expression of different gene sets. Thus, even though the number of TFs with enriched binding sites in regions of greater accessibility in males and females at both P60 and P67 were greater than at P23 and P30, these overlapping sets of TFs are still likely differential influencing their associated biological processes in males and females.

Experience: Differences in regulatory region accessibility between P67 Tutored and Isolate males

Juvenile males prevented from hearing song during the CP for TSM remain capable of copying song heard after P65 (Eales 1985, 1987; Morrison & Nottebohm 1993). Thus, tutor song experience, not maturation, determines when the CP for TSM closes. To determine how experience, independent of maturation, influences regulatory region accessibility in males, I raised male birds to P67 under conditions that either lead to CP closure ('Tutored') or extension ('Isolates') and compared the regulatory region accessibility profiles of cells composing their ALs to determine how experience alone might influence neural properties regulating learning potential.

P67 Tutored and Isolate males did not significantly differ in regulatory region number, and differential analysis identified relatively few regions significantly differing in accessibility between the two conditions. This finding stands in stark contrast to previous analyses examining differences in chromatin accessibility and gene expression between P67 Tutored and Isolate males. Previously performed ChIP-Seq for H3K27me3, H3K9me3, H3K4me3, and Polymerase II (Pol2) suggests that Tutored and Isolate males possess far more different chromatin accessibility and transcriptional profiles than the results from this experiment (Kelly et al. 2018). For example, the

results of the previous experiment indicate that Tutored males had 1181 genes possessing greater enrichment for H3K27me3, while Isolates had 51 genes possessing greater enrichment for H3K27me3 (Kelly et al. 2018). Notably, results from the same experiment indicate that Tutored males had 83 genes possessing greater enrichment for H3K9me3, while Isolates had 62 genes possessing greater enrichment for H3K9me3, supporting the notion that, as expected, not all PTMs differ between Tutored and Isolate males to the same extent (Kelly et al. 2018). However, as H3K27ac and H3K27me3 are PTMs made to same residues, they are mutually exclusive, thus, introducing a discrepancy between my results and those of Kelly and colleagues. Differences in ChIP protocols and analysis pipelines likely underlie the differences between the two results. The two experiments utilized different antibodies, numbers of biological replicates, genome assemblies, bioinformatics packages, and statistical cutoffs at each stage of analysis. Collectively, these differences in experimental design are more than sufficient to explain the observed differences in results. To address this inconsistency, it will be beneficial to analyze the results of each experiment with a single analysis pipeline.

Regions demonstrating greater accessibility in Tutored males were enriched with binding sites for three TFs, ZNF708, SPIB, and ELF3. Biological process GO term enrichment analysis performed on these TFs did not yield any statistically significant GO terms and literature search aimed at identifying potential functions of these TFs within the brain were relatively uninformative. To my knowledge there are no papers directly examining the role of ZNF708 in the brain, nor any other tissues. SPIB belongs to the Ets-transcription factor family, members of which have predominately been investigated for their roles in cancer metastasis through the coordinated regulation of adhesion molecules and extracellular matrix (ECM) proteins (Maroulakou & Bowe 2000; Zhao & Kishino 2020). Belonging to the same TF family, ELF3 has

also been implicated in the regulation extracellular matrix organization and Elf3-knockdown in zebra fish embryos leads to abnormal neural development, in part due to dysregulation of genes encoding ECM proteins (Sarmah et al. 2022).

Interestingly, many of the genes ascribed to regulatory regions of greater accessibility in Tutored males influence ECM organization and cellular membrane structure, including ANK1, APBA3, BIN3, GAS2L2, KIAA0895L, MXRA7, PLEKHO1, and SNTA1. ANK1 (Ankyrin 1) links integral membrane proteins to underlying actin cytoskeleton to regulate cell motility and the maintenance of specialized membrane domains. ANK1 is required for the survival of Purkinje neurons in the cerebellum and deficiencies in its expression have been associated with neurologic dysfunction in both mice and humans (Peters et al. 1991; Stevens et al. 2022). APBA3 (Amyloid Beta Precursor Protein Binding Family A Member 3) is an adaptor scaffolding protein involved trafficking and metabolism of membrane proteins (Sumioka et al. 2008). BIN3 (Bridging Integrator 3) contributes to signal transduction pathways that regulate membrane dynamics and cytoskeletal reorganization (Aspenström 2014). GAS2L2 (Growth Arrest Specific 2 Like 2) regulated microtubule dynamics and stability through the cross linking of microtubules and microfilaments (Stroud et al. 2014). KIAA0895L (Microtubule Associated Tyrosine Carboxypeptidase 1; also commonly known as MATCAP1) is a tyrosine carboxypeptidase that critically regulates microtubule dynamics necessary for appropriate brain development and cognitive function (Landskron et al. 2022). MXRA7 (Matrix Remodeling Associated 7) is a rather poorly characterized matrix remodeling protein implicated in a breadth of matrix remodeling-associated processes following tissue damage (Zhou et al. 2019; Shen et al. 2023). PLEKHO1 (Pleckstrin Homology Domain Containing O1) interacts with actin capping protein to regulate actin cytoskeletal structure (Edwards et al. 2014; Avenarius et al. 2017). SNTA1 (Syntrophin

Alpha 1) is an adaptor protein involved in determining the subcellular localization of membrane proteins, including channels and receptors, that has been shown to play an important role in synapse formation and acetylcholine receptors organization at neuro muscular junctions (Hosaka et al. 2002).

Interestingly, many genes regulating neurite outgrowth and synapse formation, including CELSR3, DTNB, PLXNB2, PRICKLE2, SDK2, SEMA6B, were also ascribed to regulatory regions of greater accessibility in Tutored males. As such processes also rely on the dynamic regulation of cellular membrane and structure, there is the potential that these genes are working in conjunction with those influencing ECM organization and cellular membrane structure. CELSR3 (Cadherin EGF LAG Seven-Pass G-Type Receptor 3) is nonclassic-type cadherin implicated in neural progenitor cell differentiation, axon guidance, and neuronal migration (Tissir et al. 2005; Zhou et al. 2008a; Zhou et al. 2008b; Qu et al. 2010; Feng et al. 2012b; Feng et al. 2016; Thakar et al. 2017; Hakanen et al. 2022). Targeted inactivation of CELSR in the forebrain, cortex, or telencephalon results in abnormal axonal tract development and disrupted neuronal connectivity in each individual brain region (Tissir et al. 2005; Zhou et al. 2008a). DTNB (Dystrobrevin Beta) is neuronal, PSD-enriched, dystrophin-binding protein expressed in cortical and hippocampal neurons implicated in the cognitive dysfunction observed in Duchenne muscular dystrophy patients (Blake et al. 1997; Blake et al. 1998; Blake et al. 1999). PLXNB2 (Plexin B2) is a semaphorin-responsive transmembrane receptor that participates in axon guidance and cell migration (Perrot et al. 2002; Conrotto et al. 2004). PLXNB2 has been shown to influence proliferation, differentiation, and motility of cerebellar neurons and regulates proliferation and migration of neuroblasts from adult subventricular zone (Saha et al. 2012; Van Battum et al. 2021). Furthermore, PLXNB2 mediates changes to dendritic morphology and synapse density in the adult

hippocampus necessary to support fear memory recall (Simonetti et al. 2021). PRICKLE2 (Prickle Planar Cell Polarity Protein 2) functions at the PSD to influence neurite outgrowth and stability, potentially through interacts with PSD95, NMDA receptors, and other protein partners, with which PRICKLE2 physically interacts (Okuda et al. 2007; Hida et al. 2011; Nagaoka et al. 2014; Chowdhury et al. 2020). Interestingly, PRICKLE2 deficient mice present with seizure sensitivity and ASD-like behavior (Sowers et al. 2013). SDK2 (Sidekick Cell Adhesion Molecule 2), a gene also ascribed to a region of greater accessibility in P67 males than P60 males, is a neural adhesion molecule that promotes the formation of synapses between retinal neurons necessary for the detection of motion (Yamagata et al. 2002; Krishnaswamy et al. 2015). SEMA6B (Semaphorin 6B) belongs to a family of proteins that critically regulate axon guidance, has been shown to function as a repellent for developing hippocampal neurons, and has been implicated in regulating the progressive myoclonic epilepsy (Tawarayama et al. 2010; Hamanaka et al. 2020; Herzog et al. 2021). Collectively, the genes discussed have been implicated in regulating cellular membrane structure and stability, as well as synapse formation and function. Maintaining neural architecture via the prevention of neurite shrinkage and shortening is necessary to prevent brain atrophy and memory decline associated with aging and neurodegenerative disease (Pakkenberg et al. 2003; Fox & Schott 2004; Freeman et al. 2008). There is the potential that regulation of these genes may be relevant for bringing about a state of neural stability that prevents further alteration to the template acquired during the CP for TSM.

Regions demonstrating greater accessibility in Isolate males were enriched with binding sites for TFs that highlight themes of cellular responsivity (e.g., Response to calcium ion, Response to metal ion, Response to oxygen-containing compound) and generalized cellular processes (e.g., Bile acid and bile salt transport, Cholesterol metabolic process, Monocarboxylic acid transport).

TFBSs enriched in regions with greater accessibility in Isolate males, were for transcription factors belonging to the AP-1/ATF transcription factor family (ATF2, FOSL2::JUND, FOSB::JUNB, FOSL2::JUNB, FOS::JUND). As discussed previously, members of AP-1/ATF transcription factor family are integral for regulating gene expression in response to internal and external cues, including those influencing learning and memory formation (Herdegen 1996; Alberini 2009; Bejjani et al. 2019). This result aligns with previous reports of overrepresentation of FOS and AP-1 binding sites in genomic regions with greater accessibility in Isolates than Tutored males (Kelly et al. 2018). Furthermore, hearing song induces FOS expression in the AL of adult males, supporting the notion that maintained accessibility at these genomic locations may support maintained receptivity in males isolated from hearing song during the CP for TSM (Velho et al. 2005).

Many of remaining TFs with enriched binding sites in regions of greater accessibility in Isolate males have been implicated in the regulation of various stages of neural development, but the regulatory roles they might be playing within the AL are less apparent. For example, VSX1 and VSX2 are crucial in regulating neuronal differentiation and patterning of the retina, but there are no reports of either playing a similar role in other regions of the developing nervous system (Passini et al. 1997; Decembrini et al. 2006). Dynamic shift in SP5 expression during murine brain development support patterning of the neuroectoderm and mesoderm and region-specific SP5 immunoreactivity has been documented in human cerebellum, but, to the best of my knowledge a functional role for SP5 in the adult brain has not yet been identified (Honer et al. 1993; Treichel et al. 2001). Similarly, limited investigations in THAP11 (also commonly known as Ronin), a TF essential for embryonic development, support a role for THAP11 in regulating the survival of cerebellar neurons, as its overexpression results in cerebellar degeneration, but its functional role

within such neurons under normal biological conditions is less apparent (Dejosez et al. 2008; Zwaka et al. 2021). The regulatory role of HES6 in supporting brain development has potentially been the most well studied among these TFs. HES6 expression succeeds that of neurogenins, TFs involved in specifying neurons from precursor cells, and promotes cortical neurogenesis and differentiation (Koyano-Nakagawa et al. 2000; Gratton et al. 2003; Jhas et al. 2006; Murai et al. 2011). Thus, while many of the TFs with binding sites enriched in regions of greater accessibility in Isolate males have been shown to influence neuron development and function, it is not entirely apparent what biological roles these TFs might be playing in Isolate AL.

Genes ascribed to regions of greater accessibility in Isolate males were enriched for cellular component GO terms highlighting chromosomal regions (e.g., Kinetochore, Chromosome, centromeric region) and the only significant molecular function identified was Cell adhesion molecule binding, which was associated with SEPTIN7 and PTPRF. SEPTIN7 (Septin 7) is a cytoskeletal GTPase required for actin cytoskeleton organization necessary for neuronal spine morphogenesis and dendritic branching (Tada et al. 2007; Xie et al. 2007; Wang et al. 2018). Interestingly, work in breast cancer cells indicates that SEPTIN7 may be a downstream target of the ERK signaling cascade, as ERK activation positively correlated with SEPTIN7 expression in these cells (Zhang et al. 2016). PTPRF (Protein Tyrosine Phosphatase Receptor Type F) is a neural phosphatase implicated in a breadth of neurodevelopmental processes including neuronal adhesion to the ECM, neurite outgrowth, hippocampal neurogenesis, and cholinergic neuron differentiation and innervation in the forebrain and hippocampus (Yeo et al. 1997; Van Lieshout et al. 2001; Dunah et al. 2005; Bernabeu et al. 2006).

Other genes with notable neuronal functions ascribed to regions of greater accessibility in Isolate males included BEND5, DYNC1I1, and PHF21B. BEND5 (BEN Domain Containing 5) is

a neuron-specific transcriptional repressor expressed in Cortical neurons (Dai et al. 2013). DYNC1I1 (Dynein Cytoplasmic 1 Intermediate Chain 1) is a neuron-specific component of the dynein 1 complex, which promotes movement of vesicles and organelles along axonal microtubules (Vallee et al. 1989; Susalka & Pfister 2000; Myers et al. 2007). DYNC1I1 deficiency results in atrophy of hippocampal neurons that can be rescued via activation of the ERK signaling cascade, suggesting that both limit neural atrophy (Liu et al. 2016b). PHF21B (PHD Finger Protein 21B) is an epigenetic reader highly expressed during cortical neurogenesis, that, when depleted leads to the accumulation of neural progenitor cells in proliferative zones, impaired social memory, reduced synaptic protein expression, and impaired hippocampal LTP (Basu et al. 2020; Chin et al. 2022). Similar to genes attributed to regions of greater accessibility in Tutored males, genes attributed to regions of greater accessibility in Isolate males have also been implicated in the regulation of neuron development processes, including neurogenesis, neuronal differentiation, synaptic protein localization, and nerite growth. These finding stand to support the notion that regulation of the same neural properties, by different sets of TFs and genes, may lead to the closure or prolongation of the CP for TSM.

Chapter IV: Future directions

Introduction

The work performed in pursuit of this dissertation was motivated by the prospect of identifying the cellular subtypes composing the AL that distinguish birds capable and incapable of sensory song learning. While substantial progress has been made towards identifying the cell types comprising the AL and their relationships to measures of song responsivity (Mello et al. 1998; Pinaud et al. 2004; Pinaud et al. 2006; Pinaud & Mello 2007; Pinaud et al. 2008; Dagostin et al. 2012; Velho et al. 2012; Dai et al. 2018; Lee et al. 2018), the exact complement of cell types required to support sensory song learning remains unknown. Cells of differing types, as well as individual cells of differing subtypes, possess different combinations of accessible regulatory regions that mediate cell-type-specific transcriptional responses to intracellular and extracellular stimuli (Heintzman et al. 2009; Visel et al. 2009; Thurman et al. 2012). Within a given cell, exposure to an appropriate stimulus induces cellular signaling cascades that lead to increased TF expression and activity. TFs induced by internal or external stimuli predominantly recognize and bind regions of the genome that already exhibit enhancer-typical PTMs and are bound by other TFs (Barish et al. 2010; John et al. 2011). Thus, defining a cell's type based on its regulatory region accessibility profile has emerged as a common practice among researchers aiming to disentangle the cellular composition of characteristically complex tissues, in which alternative classification strategies (i.e., cellular morphology, cellular connectivity, gene expression) are insufficient (Von Bartheld et al. 2016). By leveraging the results from large-scale sequencing experiments, comparable to the experiment outlined in this dissertation, researchers have utilized enhancers, differentially accessible between individual cortical brain regions, as a means to identify the

cellular subtypes composing those regions with a considerably greater degree of specificity than achievable by other methods (Blankvoort et al. 2018; Blankvoort et al. 2020; Nair et al. 2020). The researchers who initially developed this strategy refer to the technique as Enhancer Driven Gene Expression (EDGE) (Blankvoort et al. 2018). With the goals of further defining the cellular subtypes composing the AL, determining which influence the potential for sensory song learning, and examining how these cell populations change across development as a function of age, sex, and experience, I sought to leverage the EDGE strategy in combination with the London laboratory's established protocol for in vivo electroporation (Ahmadiantehrani & London 2017a).

Methods

Animals and housing

All procedures were conducted in accordance with the NIH guidelines for the care and use of animals for experimentation and were approved by the University of Chicago Institutional Animal Care and Use Committee (ACUP no. 72220). All chicks were hatched in the London lab laboratory breeding colony at the University of Chicago. Animals were housed on a 14:10 h light/dark cycle. Food and water were provided ad libitum. Following electroporation, chicks were returned to their nests, where they received parental care until their collection date. Electroporations were performed at P3, and collections were performed at various ages to assess electroporation success and determine the capability with which regulatory regions could drive fluorescent protein gene expression.

Plasmids

The Super PiggyBac Transposase Expression Vector (PB210PA-1, was obtained from System Biosciences; Palo Alto, CA). The pPB-CAG-eGFP, -mRFP, and -CFP plasmids, containing green (G), red (R), and cyan (C) fluorescent proteins (FPs) flanked by piggyBac-

specific ITRs were gifted to the London laboratory from the LoTurco laboratory (University of Connecticut). The AAV-MEC13-53-pTRE3G-GFP, -HSV-TK-GFP, and -FGF4-GFP plasmids, containing minimal promoter sequences driving GFP expression, were gifted to the London laboratory from the Kentros laboratory (Norwegian University of Science and Technology). The pPB-mFGF4-mRFP, -eGFP, -and -CFP plasmids were constructed by polymerase chain reaction (PCR) cloning the Fibroblast Growth Factor 4 minimal promoter (mFGF4; 210 bp) from the AAV-MEC13-53-pFGF4-GFP plasmid using the following primers: mFGF4 fwd, 5′-ACATACTAGTATCTGAGCTCTTACGCGTG-3’; mFGF4 rev, 5′-ACATGAATTCGACCGGTGGATCTTGCG-3’. The mFGF4 minimal promoter sequence was then subcloned into the SpeI/EcoRI sites of pPB-CAG-mRFP, -eGFP, and -CFP. The pPB-mTK-mRFP plasmid was constructed by PCR-cloning the Thymidine Kinase minimal promoter (mTK; 63 bp) from the AAV-MEC13-53-HSV-TK-GFP plasmid using the following primers: mTK fwd, 5′-ACATACTAGTATCTGAGCTCTTACGCGT-3’; mTK rev, 5′-ACATGAATTCCGACCGGTGGATCTTGC-3’. The TK minimal promoter sequence was then subcloned into the SpeI/EcoRI sites of pPB-CAG-mRFP. I did not generate a pPB-mCMV-FP plasmid. The cytomegalovirus (CMV) minimal promoter sequence contained multiple SpeI restriction enzyme (RE) recognition sites, and the SpeI RE site within the pPB-CAG-FP was the only suitable location for minimal promoter insertion. The pPB-CaMKIIa-mRFP plasmid was constructed by PCR-cloning the Calcium/Calmodulin Dependent Protein Kinase II Alpha promoter (CaMKIIa; 1289 bp) from the AAV-CaMKIIa-GCaMP6f-P2A-nls-dTomato plasmid (Addgene plasmid #51087) using the following primers: CaMKIIa fwd, 5′-ACATACTAGTGCGGCCGCACGCGTTTAATT-3’; CaMKIIa rev, 5′-ACATGAATTCCCGGATCCCCCGCTGCCC -3’. The CaMKIIa promoter sequence was then

subcloned into the SpeI/EcoRI sites of pPB-CAG-mRFP. The pPB-mDlx-mFGF4-mRFP plasmid was constructed by PCR-cloning the Distal-Less Homeobox 1 enhancer (mDlx; 530 bp) from the pAAV-mDlx-GFP-Fishell-1 plasmid (Addgene plasmid #83900) using the following primers: mDlx fwd, 5′-ACATACTAGTGCCGCACGCGTTTAATTAAGAAT-3’; mDlx rev, 5′-ACATACTAGTGCCCTGACTTTTATGCCCAG-3’. The mDlx enhancer sequence was then subcloned into the SpeI site of pPB-mfgf4-eGFP. Putative differentially accessible regulatory regions were selected with the aim of exploring the influence of age and sex on AL cell type composition. Those aimed at elucidating the effects of age were derived from the comparison of P23 males and P30 males. In contrast, those aimed at elucidating the effects of sex were derived from the comparison of P30 males and P30 females. Putative regulatory regions were PCR amplified from genomic DNA using primer sets designed to limit off-target sequence recognition using the NCBI tool Primer-BLAST (Ye et al. 2012). Two primer sets were created for each target sequence in case of unresolvable primer failure or off-target amplification (Table 3). Putative regulatory region sequences were PCR-cloned into the SpeI site of pPB-mfgf4-mRFP. To confirm that newly generated plasmids were constructed appropriately, all plasmids were sequenced at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility. Simplified linearized plasmid maps depicting the plasmid constructs discussed above are displayed in Figure 30.

Comp.	Regulatory Region	F' (5'->3')	R' (5'->3')
P23MvsP30M	chr1A:47593251-47593816	ACATGAATTCGAGCACGAACCCAAACACAC	ACATACTAGTCCAATCATAGGCACGGCTCT
		ACATGAATTCTGATTGGTCGTTCTACCCGC	ACATACTAGTCAACCAATCATAGGCACGGC
	chr18:4142980-4143383	ACATACTAGTAGGGCATCATGTGACAGTGAAA	ACATACTAGTGGCCATCACCGATCCCAC
		ACATACTAGTCACGTGATGGGGTGCAACG	ACATACTAGTGATCCCACCCCTCGCACAAA
	chr13:18267849-18268266	ACATACTAGTCTGCCCGGTGTCGAATAAAC	ACATACTAGTGGCGCTGGGGGTATTACATC
		ACATACTAGTTCCGTCACAGTTGGATATCGG	ACATACTAGTGGCGCTGGGGGTATTACAT
	chr7:38044409-38044808	ACATACTAGTAATTGCACAAACAGGCGCTC	ACATACTAGTGAACAAGGCAAATATGGCGG
		-	-
	chr23:1304-1693	ACATACTAGTGGCGCTCAGGGTAGTACATC	ACATACTAGTAAATGCACAAACAGGCGCTC
		ACATACTAGTTTGCAAAGATGGCGGCAAAT	ACATACTAGTCCGTCACAGTTGGATATCGGT
P30MvsP30F	chrZ:1811687-1812086	ACATACTAGTTGCAATCCTCAGGCAACTAACA	ACATACTAGTATCCATCCGGGATTGGCAG
		ACATACTAGTCAATCCTCAGGCAACTAACACCTG	ACATACTAGTTGGTGGCCAAGATATGTTCTCT
	chrZ:21098193-21098592	ACATACTAGTGAAGGTGAGATGGGCCTTTGTTA	ACATACTAGTTTAAGCCAGGATTGAGCAGACA
		ACATACTAGTAGAACTTAAGTGATGCGAAGGTG	ACATACTAGTTTTCACAAGGCGGATACAGCAA
	chrZ:5455710-5456109	ACATACTAGTCTGAACTGCAGCAAGCTAACC	ACATACTAGTTTGCCTGACTGTCTTTTACCCA
		ACATACTAGTGCAAGCTAACCCACAAATGTCTT	ACATACTAGTCGGTACTTCCATTTGACGTGAT
	chr1A:19666163-19666562	ACATACTAGTGGTGCAAAGCCTCCTACTTTG	ACATACTAGTTTATGGGGACAGTGCTGTTACAC
		ACATACTAGTGCCAGGAGTTAGAGCAACATCT	ACATACTAGTACACAGGGCTCTTGTATGTTAG
	chr1A:28580958-28581357	ACATACTAGTCCAGCAGGAAATGTGCCAAC	ACATACTAGTTGCCTCAGGTTGTGCTGTAG
		ACATACTAGTTTTTAAGTCACCCCACCCCC	ACATACTAGTTGCTTGGACAGTACTCGCAG
	chr5:4095837-4096236	ACATACTAGTTCCCCAGACCTCTATCCTCTCT	ACATACTAGTCAACAGCAATTAGGTCCACAGATT
		ACATACTAGTCTGAGCATGTGGAGCTGTGA	ACATACTAGTTGGTTCAGACTGTTTCCTGGG
	chr1A:64201115-64201514	ACATACTAGTCTTGGAAGCAGAGTGCCAGA	ACATACTAGTGTGCCTTTGCTGCCACATTT
		ACATACTAGTAAAGAGCCCCTTGGAAGCAG	ACATACTAGTAATGCTGGCACTCCTACGTG
	chr1:22129554-22129953	ACATACTAGTTACAATGGGGCGTATCGTTCATT	ACATACTAGTACTGGTGAGGTCATAGGCAA
		ACATACTAGTACAATGGGGCGTATCGTTCA	ACATACTAGTACACGCTATCCTCTGTCCCT
	chr36:928628-929027	ACATACTAGTCTAAAATGGCGGACGAAGCG	ACATACTAGTGAGGGGGTGGGGCTATCTAT
		ACATACTAGTAAGCGCTTCCGGGCTATC	ACATACTAGTTGAGTAGCCACGCCCATTAT

Table 3. List of primers used to generate electroporated plasmids. Comparison (Comp.) indicates the comparisons from which the differentially accessible putative regulatory region was derived. Regulatory region indicates the genomic location of the amplified region. F' and R' indicate the sequences of the primer pairs used for region amplification in the 5'->3' orientation. This table only contains information about primer sets that were successfully used to generate electroporated plasmids.

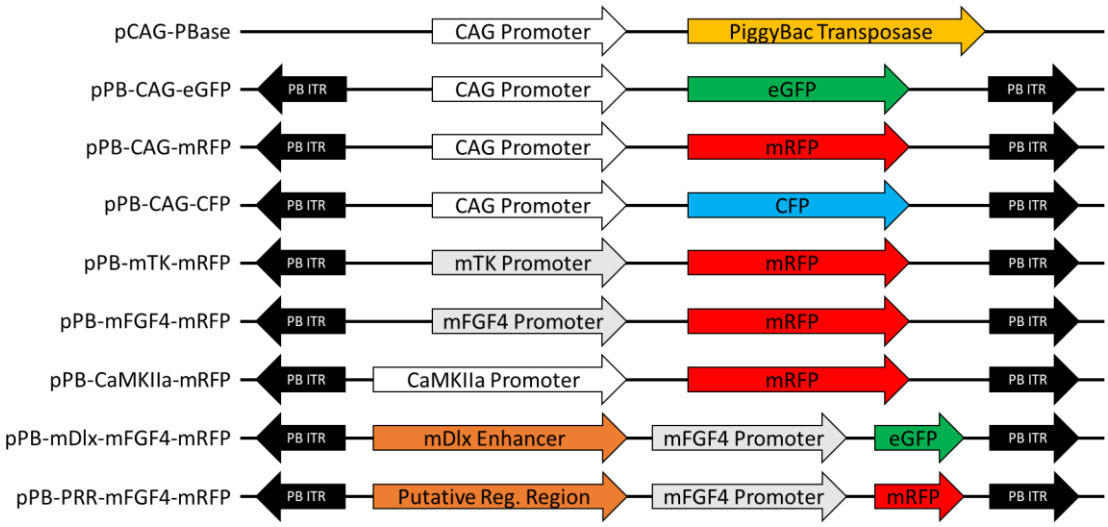

Figure 30. Simplified plasmid maps. pPB plasmids contain inverted terminal repeats (ITRs) flanking transgene expression cassettes, each containing a promoter (CAG, mTK, mFGF4, or CamKIIa), potentially paired with an enhancer (mDlx or putative regulatory regions), and a fluorescent protein transgene (eGFP, mRFP, or CFP). Figure adapted from Figure 1a in Ahmadiantehrani and London, 2017.

Electroporation solution preparation

Each electroporation solution contained Super PiggyBac Transposase Expression Vector, an experimental plasmid, and a positive control plasmid (pPB-CAG-eGFP, -mRFP, or -CFP), each at final concentrations of 500 ng/µl. The electroporation solution volume was adjusted using ddH20, and no more than 0.5% of the solution consisted of Fast-Green dye to assist with visualization. High concentrations of each plasmid were prepared from glycerol stocks stored at -80˚C since the initial synthesis of the plasmid. Briefly, using a toothpick, a small scraping was collected from a glycerol stock and placed in Falcon 2059 containing ~6mL of Luria Broth (LB) with ampicillin at a final concentration of 100 ug/mL. Tubes were incubated in a rotating mixer at 37°C and 250 rpm overnight (approx. 16 hours). Plasmid DNA was isolated from the bacteria growth medium using the QIAprep Spin Miniprep Kit (Qiagen, Cat# 27104). Plasmid DNA was

then purified and concentrated using a standard ethanol precipitation procedure, and concentrations were determined using a NanoDrop ND-1000.

In vivo electroporation

Prior to electroporation, P3 chicks were examined to ensure that they were of the appropriate size and appeared healthy enough to survive the electroporation procedure. Chicks were removed from their nests and anesthetized with isoflurane soaking a small piece of cotton at the bottom of a 1.5 mL tube. Light pinches to the feet were used to assess the degree of anesthetization before beginning the procedure, and breathing was monitored throughout. A cotton swab soaked in Nolvasan (0.1%) was used to clean the chick's scalp and clear away feathers from the midline. A small (~3 mm) midline incision was made along the anterior-posterior axis of the scalp using forceps and small spring scissors (Fine Science Tools Cat# 11003-12, and 15018-10, respectively). Bupivacaine (0.2%) was applied to the wound with a cotton swab to numb the incision. Using a pulled-glass capillary fixed to a micropipette via a trimmed pipette tip connecter (Figure 31A), the electroporation solution was bilaterally injected into the ventricles. Approximately 2 µls of electroporation solution was injected into each ventricle (Figure 31B). A second injection was immediately performed if this was insufficient to fill the ventricular space or the ventricle did not fill appropriately. An ELP-01D cell and tissue electroporator (NPI Electronic; Tamm, Germany) connected to custom-shaped tri-electrode gold-plated paddles (Figure 31C) wet with 0.1 M phosphate-buffered saline (PBS) was used to deliver seven 80V pulses, each lasting 100 ms with an inter-pulse interval of 900 ms. Positively charged paddles were placed on either side of the head, targeting the posterior portions of the ventricles. A single negatively charged paddle was placed on top of the head, directly over the posterior portions of the ventricles. Following electroporation, the incision was sealed with Vetbond (3 M; St. Paul, Minnesota), and

the chicks were moved to a heating pad, where they were monitored until normal begging behaviors resumed. Chicks were then returned to their home nest until their date of collection. The entire procedure, spanning chick removal to return, was conducted in no more than 30 minutes. Chicks were closely observed over the following 2-3 days to ensure they received proper parental care. In instances of chick rejection, the chick was relocated to a different nest containing nestlings of similar age. In instances of parental neglect, the chick was handfed and cared for until the parents either resumed parental care or the chick old enough to care for itself.

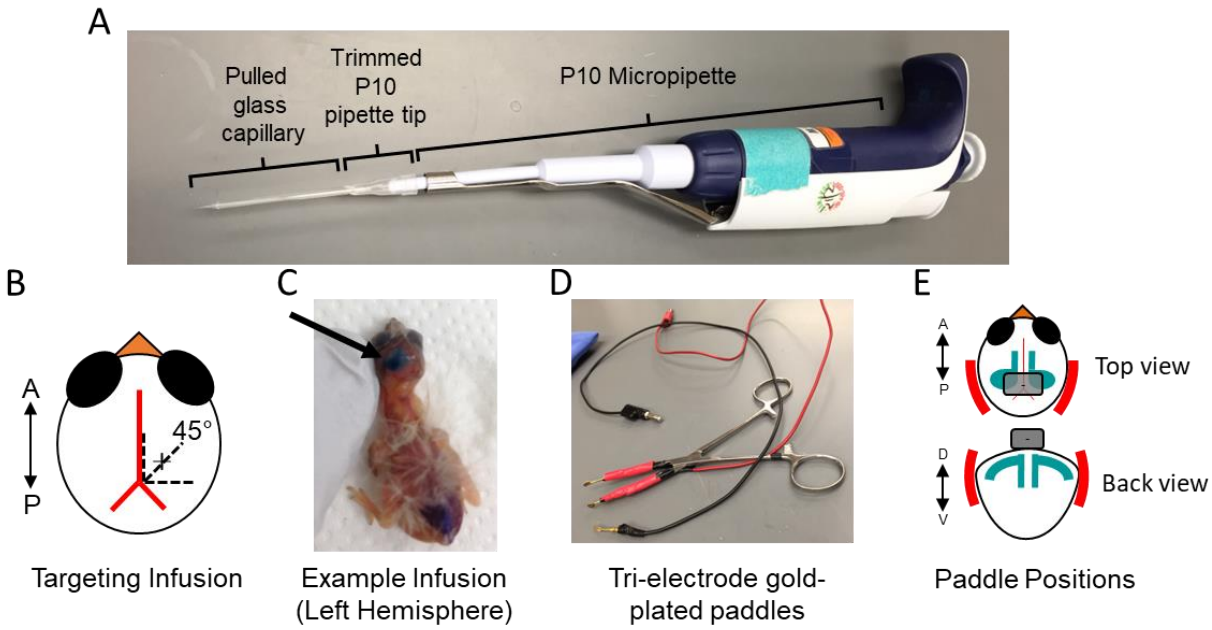

Figure 31. Depictions of electroporation tools and injection targets. (A) Photo of pulled-glass capillary fixed to a micropipette via a trimmed pipette tip connecter. (B) Diagram of the injection target location. Injections were targeted along a 45° angle to the point at which the midline and Y0 (the anterior-most boundary of the cerebellum) meet. (C) Photo of a successful injection into a chick's left ventricle (arrow). Fast green dye allows for visual confirmation that the plasmid solution has filled the entire ventricle. (D) Image of the tri-electrode gold-plated paddles fixed to a hemostat with electrical tape. (D) Diagram of paddle positions, as seen from the top and back views of the chick's head. Placement of the positively-charged paddles is indicated by red bars, and placement of the negatively-charged paddle is indicated by the black bar. Figure adapted from Figure 1b in Ahmadiantehrani and London, 2017.

Tissue processing and imaging

Brains of P20 or younger birds were removed and drop-fixed in 4% paraformaldehyde (PFA) in 0.025 M PBS overnight at 4 °C. P20, or older birds were intracardially perfused with 0.1 M PBS and 4% PFA. Their brains were removed by dissection and stored in PFA overnight at 4 °C. All brains were embedded in gelatin (8% in 0.1 M PBS) and stored in PFA overnight at 4 °C. Excess gelatin was trimmed away using a razor blade, and the gelatin-embedded brains were stored in sucrose (30% in 0.1 M PBS) overnight at 4 °C to cryoprotect the tissue. Brains were sectioned at 50 μm with a cryostat, mounted on Superfrost Plus slides (Fisher), dried overnight in the dark, and coverslipped with 2.5% polyvinyl alcohol (PVA) containing 0.5% 1,4-diazabicyclo[2.2.2]octane (DABCO). Images were captured using the 5, 10, 20, 40, and 63X objectives of a Zeiss Axio Imager Widefield Fluorescence Microscope (Zeiss, White Plains, NY).

Immunohistochemistry for GAD67 and parvalbumin

To determine if the mDlx enhancer could be used to selectively label subtypes of inhibitory neurons, I performed immunohistochemistry for GAD67 and parvalbumin on a subset of sections from a brain expressing GFP and RFP that had been electroporated with pPB-CAG-mRFP and pPB-mDlx-eGFP plasmids. Sections were permeabilized with 0.1 M PBS containing 0.03% Triton-X on a rotator at room temperature (RT) for 30 minutes (m) and then washed with 0.1 M PBS containing 0.5% Tween-20 (PBST) for 10 minutes at RT three times. Sections were blocked for 1 hour (h) at RT in 2% normal goat serum (NGS; Cat# NC9270494, Vector Laboratories, Burlingame, CA, USA). A monoclonal mouse IgG anti-GAD67 primary antibody (1:1,000 in 1% NGS; #MAB5406, EMD Millipore, Billerica, MA) or a monoclonal mouse IgG anti-parvalbumin primary antibody (1:1,000 in 1% NGS; #A-11005, EMD Millipore, Billerica, MA) was applied to the sections for overnight incubation (~16 h) at 4°C. Sections were then washed with PBST for 10

minutes at RT three times and incubated in Alexa Fluor 594 Goat anti-mouse IgG secondary (1:200 in 1% NGS; Cat# A-11005, Thermo Fisher Scientific, Waltham, MA) for 1 h at RT. Sections were washed with PBST for 10 minutes at RT three times, mounted on Superfrost Plus slides (Fisher), dried overnight in the dark, and coverslipped with 2.5% PVA containing 0.5% DABCO.

Results

mFGF4, but not mTK, drives minimal FP expression in AL cells

Enhancers alone cannot drive gene expression. Enhancers increase gene expression through interactions with promoters and other transcriptional machinery (i.e., RNA Pol II, basic TFs) but cannot initiate transcription independent of these cofactors. Thus, it was necessary to synthesize and test plasmids containing minimal promoter sequences to determine which would be suitable to pair with putative enhancers to drive reporter gene expression within the AL. A minimal promoter should drive FP expression to a lesser degree than a full-length promoter sequence, and when paired with an appropriate enhancer, should increase FP expression to a degree greater than that driven by the minimal promoter alone. To this end, I generated and electroporated two constructs incorporating minimal promoter sequences used in previous EDGE experiments: pPB-mFGF4-mRFP and pPB-mTK-mRFP. Electroporation of the pPB-mFGF4-mRFP, but not the pPB-mTK-mRFP, plasmid successfully drove FP expression, at a distinguishably lower level than the pPB-CAG-eGFP in P5 birds (Figure 32A, 32B). The observed pattern of minimal FP expression persisted at least through P23 (Figure 32C).

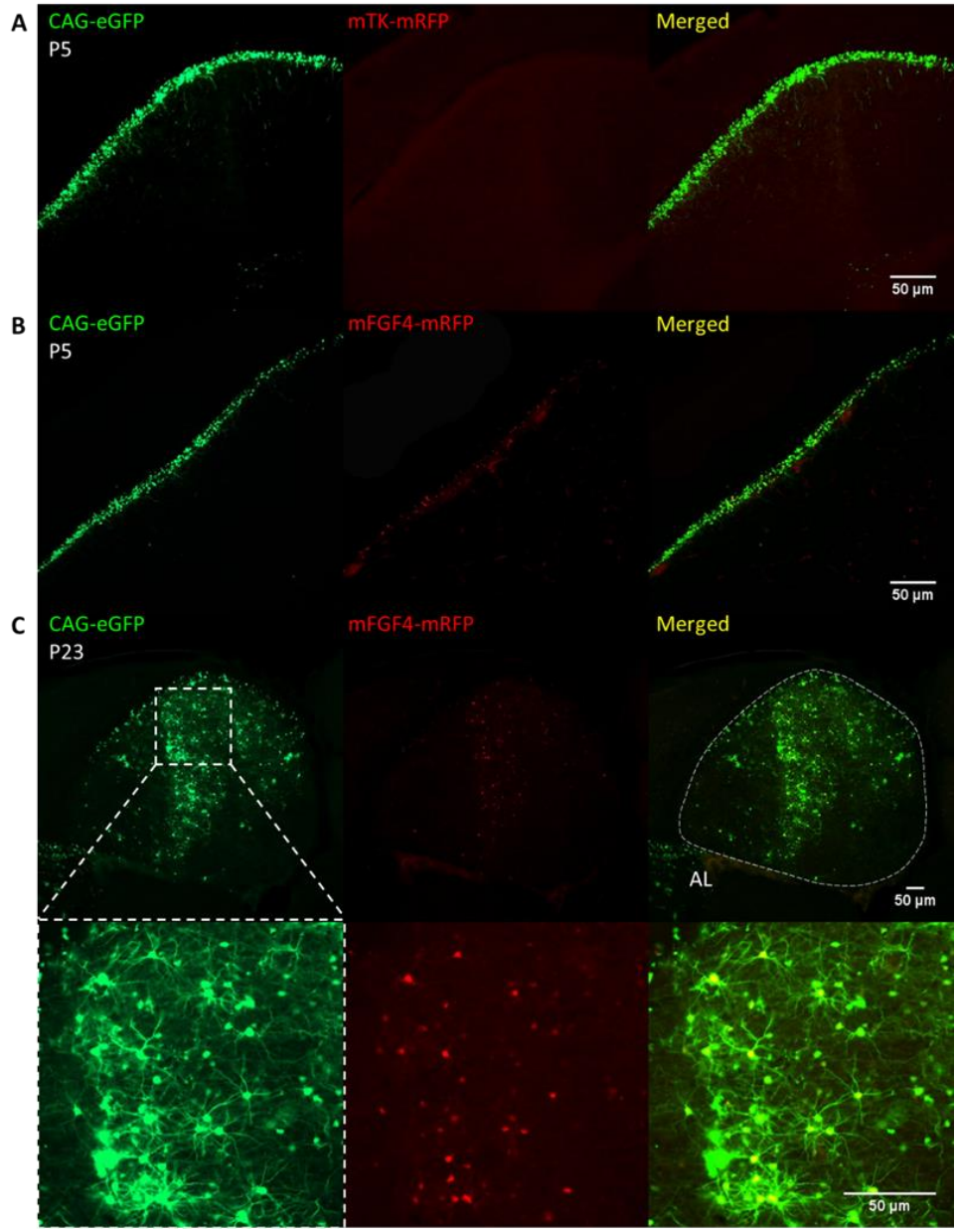

Figure 32. Representative sagittal plane images from minimal promoter plasmid electroporations. (A) Electroporation of pPB-CAG-eGFP and pPB-mTK-mRFP plasmids followed by collection at P5. Left panel: CAG-driven eGFP+ cells. Middle panel: Absence of mTK-driven mRFP+ cells. Right panel: Left and middle panels merged. (B) Electroporation of pPB-CAG-eGFP and pPB-mFGF4-mRFP plasmids followed by collection at P5. Left panel: CAG-driven eGFP+ cells. Middle panel: mFGF4-driven mRFP+ cells. Right panel: Left and middle panels merged. (C) Electroporation of pPB-CAG-eGFP and pPB-mTK-mRFP plasmids followed by collection at P23. Left panels: CAG-driven eGFP+ cells. Middle panels: mFGF4-driven mRFP+ cells. Right panels: Left and middle panels merged. The lower series of panels depicts higher magnification images taken of the same section. The white dashed box (upper left panel) indicates the location from which the higher magnification images were derived. The white dashed outline (upper right panel) encompasses the AL.

mDlx drives FP expression exclusively in GABAergic interneurons composing AL

In addition to allowing us to test putative enhancers derived from the ChIP-Seq experiment I performed, minimal promoter plasmids enable us to leverage conserved enhancers from other model organisms to drive cell-type-specific FP expression in the AL. mDlx is an enhancer for the distal-less homeobox 5 and 6 (Dlx5/6) genes, which are specifically expressed by all forebrain GABAergic interneurons (Zerucha et al. 2000; Ghanem et al. 2003). The mDlx enhancer has been repeatedly utilized to label GABAergic interneurons in accordance with their native expression of Dlx5/6 in both embryonic and adult forebrain (Zerucha et al. 2000; Stühmer et al. 2002; Stenman et al. 2003). Looking to leverage this well-establish method of targeting and labeling GABAergic interneurons, I constructed and electroporated a pPB-mDlx1-mFGF4-eGFP plasmid targeting the zebra finch AL. To confirm that the observed FP expression was isolated to GABAergic interneurons, I performed immunohistochemistry for GAD67 and parvalbumin, markers for distinct subsets of inhibitory cell populations. Although no quantification of colocalization was performed, the distribution of Dlx-driven GFP expression within the AL appeared to more closely overlap with GAD67+ cells than parvalbumin+ cells, providing support for the utility of enhancers in disambiguating the cellular subtypes composing the AL (Figure 33).

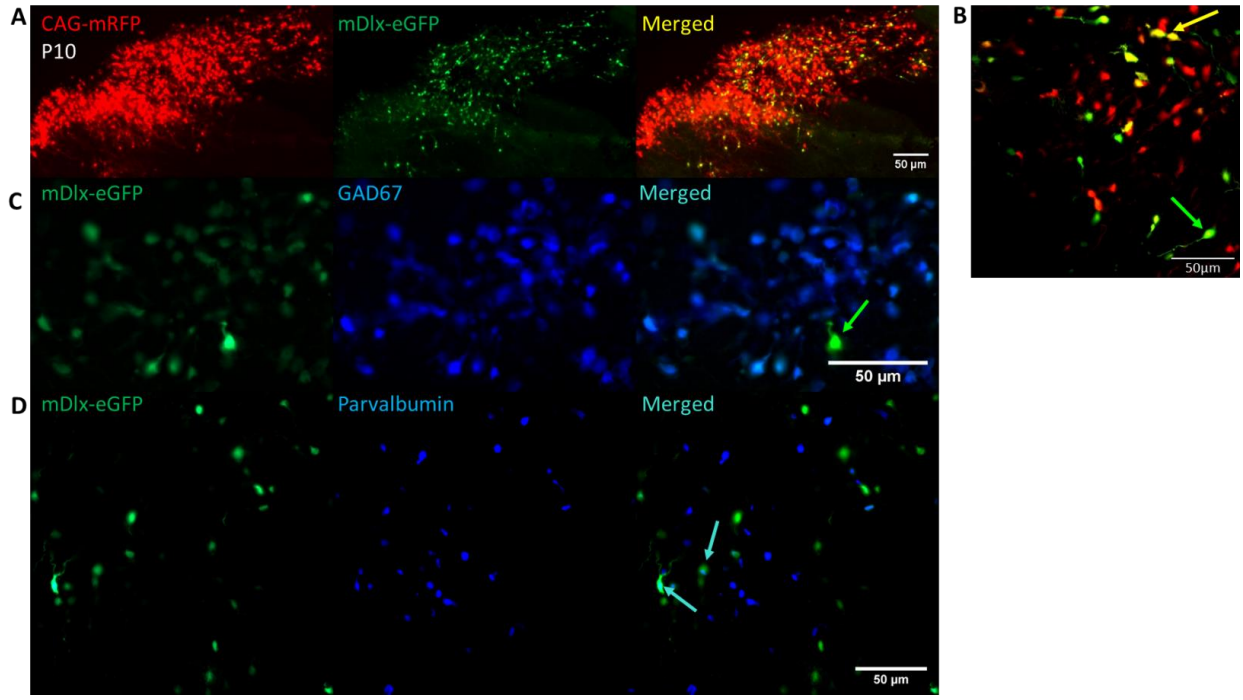

Figure 33. Representative sagittal plane images from mDlx plasmid electroporations followed by immunohistochemistry for GAD67 and parvalbumin. (A) Electroporation of pPB-CAG-mRFP and pPB-mDlx-eGFP plasmids followed by collection at P10. Right panel: CAG-driven mRFP+ cells. Middle panel: mDlx-driven eGFP+ cells. Right panel: Left and middle panels merged. In line with expectations, mRFP+ cells greatly outnumbered eGFP+ cells, and there was partial colocalization in GFP and RFP expression. (B) Higher magnification image depicting partial colocalization between mDlx-driven eGFP expression and CAG-driven mRFP expression. (C) Right panel: mDlx-driven eGFP+ cells. Middle panel: GAD67+ cells. Right panel: Left and middle panels merged. mDlx-driven eGFP expression highly colocalized with staining for GAD67. (D) Right panel: mDlx-driven eGFP+ cells. Middle panel: Parvalbumin+ cells. Right panel: Left and middle panels merged. mDlx-driven eGFP expression demonstrates little colocalization with staining for parvalbumin, demonstrating the utility of enhancers for labeling the cellular subtypes composing the AL. Figure adapted from Figure 3c-l in London, 2020.

CAMKIIa drives FP expression in excitatory neurons composing AL

Calcium/calmodulin-dependent protein kinase II alpha (CAMK2A), a highly abundant serine/threonine kinase involved in the regulation of synaptic plasticity and learning, is predominately expressed in excitatory neurons in the cortex and hippocampus (Liu & Jones 1996; Sík et al. 1998; Wang et al. 2013). The CAMK2A promoter sequence has been repeatedly utilized to label excitatory neurons in developing and adult brain regions (Basu et al. 2008; Wang et al. 2013; Scheyltjens et al. 2015; Egashira et al. 2018; Spool et al. 2021). To complement the mDlx construct and support investigations of excitatory and inhibitory cells within the AL, I constructed and electroporated a pPB-CaMKIIa-mRFP plasmid targeting the zebra finch AL (Figure 34). Unlike with the mDlx construct, I did not perform the requisite immunohistochemistry to confirm that the CAMK2A promoter drove FP expression exclusively in excitatory cells. However, researchers using an identical CAMK2A promoter sequence to label neuronal subtypes in zebra finch AL confirmed that CAMK2A-driven FP expression colocalized with CAMK2A-positive cells labeled via immunohistochemistry (Spool et al. 2021).

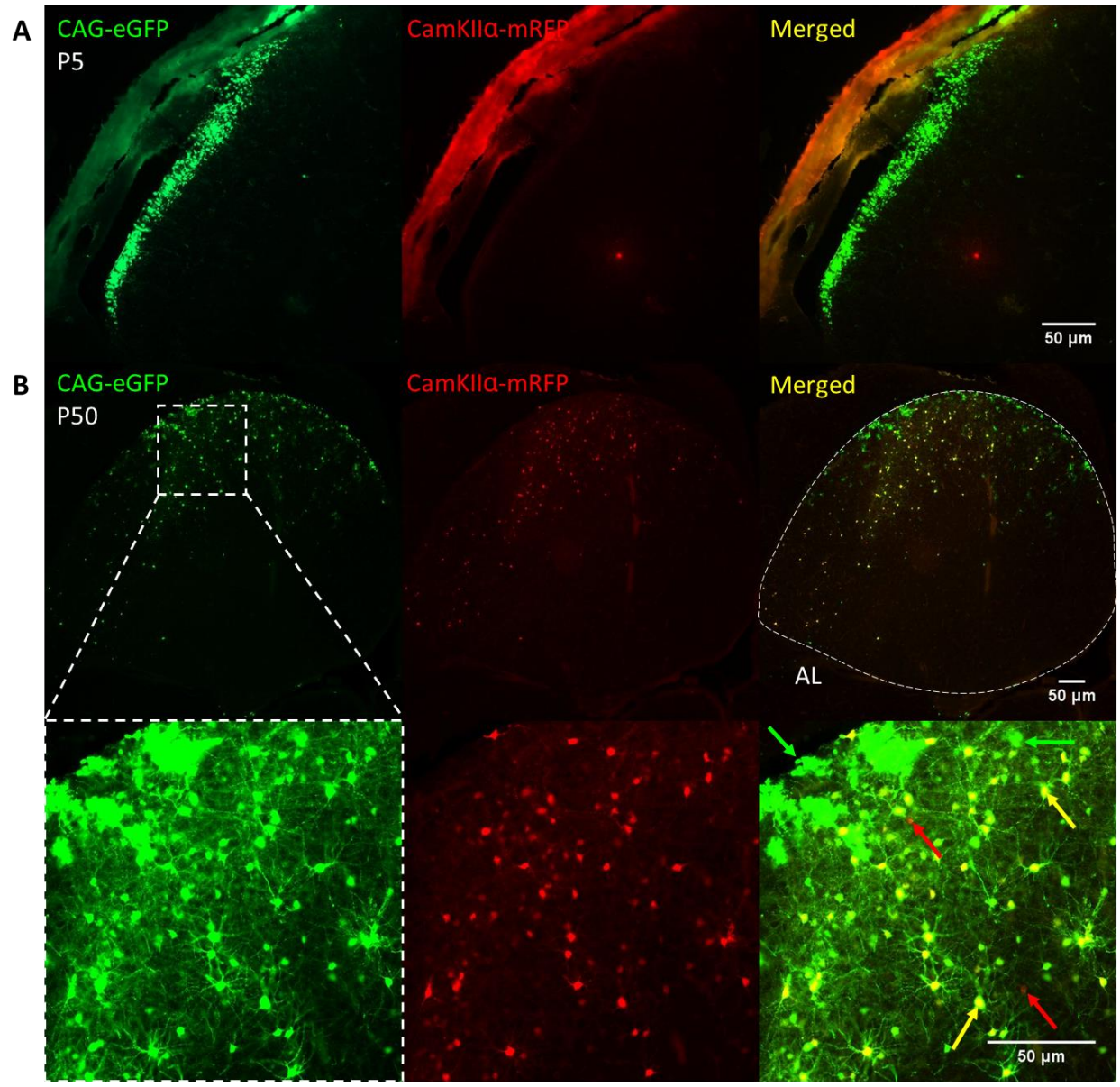

Figure 34. Representative sagittal plane images from CamKIIa plasmid electroporations. (A) Electroporation of pPB-CAG-eGFP and pPB-CamKIIa-mRFP plasmids followed by collection at P5. Left panel: CAG-driven eGFP+ cells. Middle panel: Absence of CamKIIa-driven mRFP+ cells. The lack of CamKIIa-driven mRFP agrees with the GPF+ cells being neural progenitor cells yet to migrate away from the lateral ventricle or differentiate into mature excitatory neurons. Right panel: Left and middle panels merged. (B) Electroporation of pPB-CAG-eGFP and pPB-CamKIIa-mRFP plasmids followed by collection at P50. Left panels: CAG-driven eGFP+ cells. Middle panels: CamKIIa-driven mRFP+ cells. Right panels: Left and middle panels merged. The lower series of panels depicts higher magnification images taken of the same section. The white dashed box (upper left panel) indicates the location from which the higher magnification images were derived. The white dashed outline (upper right panel) encompasses the AL.

Experimental regulatory regions did not drive FP expression in the AL

The end goal of this project was to identify the cellular subtypes composing the AL that support sensory song learning and to investigate how these cell populations change across development as a function of age, sex, and experience. With this aim in mind, I designed twenty primer sets to PCR-clone putative regulatory regions differing in accessibility between males spanning the onset of the CP for TSM (P23 males vs. P30 males) and between males and females at the onset of the CP for TSM (P30 males vs. P30 females) into the pPB-mFGF4-mRFP plasmid backbone. Using the primer sets I had designed, I successfully constructed fourteen plasmids containing a single putative regulatory region, a minimal FGF4 promoter, and the mRFP reporter gene. Each of these plasmids was co-electroporated with the pPB-CAG-CFP plasmid, which was used to assess electroporation success, and the pPB-mFGF4-eGFP plasmid, which was used as a comparison to determine if the putative regulatory region increased FP expression over that of the minimal promoter alone. None of the putative regulatory regions were sufficient to increase FP expression to a level distinguishable from that driven by the minimal promoter alone. As all tested putative regulatory regions were more accessible in P30 males than either P23 males or P30 females, all collections were performed at P30. To demonstrate that a putative regulatory region was insufficient to increase FP expression over that of the minimal promoter alone, I achieved reporter gene expression in a minimum of three P30 males.

Discussion

Plasmid construction, followed by in vivo electroporation, is a promising avenue for identifying and exploring the functional roles of the different cell types composing the AL. Plasmid construction is a relatively streamlined process, the product of which can utilize to label specific cell populations based on transcriptomic identity and investigate the potential roles of putative

regulatory regions within a given tissue. Furthermore, the "plug and play" nature of plasmid construction allows for a great degree of versatility but also permits the side-by-side production of numerous constructs when utilizing the same plasmid backbone, a feature particularly appealing for screening large sets of putative regulatory regions. In vivo electroporation allows for the simultaneous incorporation of multiple constructs, thereby potentiating modular and complex experimental designs necessary for distinguishing between numerous cell types belonging to a single brain region. Cargo size limitations are of little concern, as the PiggyBac transposase system can be used to integrate DNA sequences of virtually any size into the genome. Furthermore, in vivo electroporation presents an alternative to viral trans-gene delivery, a strategy that has not always been reliable in zebra finches (Haesler et al. 2007; Schulz et al. 2010; Yip et al. 2012; Heston & White 2017). Lastly, AL cells stably express electroporated transgenes through to at least P50 (expression likely persists beyond P50, but the experiments necessary to demonstrate such have not yet been performed), allowing one to perform genetic manipulations to elucidate the potential mechanisms of developmental song learning (Ahmadiantehrani & London 2017b).

The work outlined here represents only the early stages of what may be achieved through utilizing these experimental tools. Utilizing a CamKIIa promoter and an mDlx enhancer, I was able to generate plasmids that can be used to effectively drive FP expression in excitatory and inhibitory cell types, respectively. Furthermore, utilizing immunohistochemistry, I demonstrated the promise of using enhancers as a means to further distinguish between cell populations of the same general type. While these are, in no way, novel uses for these regulatory regions, this work further expands the molecular tool kit accessible to birdsong researchers. In the same vein, the successful integration of the minimal FGF4 promoter into a plasmid containing the ITRs recognized by the PiggyBac transposase will hopefully reduce the burden for future London

laboratory members and other bird song researchers aiming to use electroporation as a means to investigate regulatory region function within the brain. Plasmid construct generation and electroporation are well-situated to bolster functional gene manipulation experiments, as these can help facilitate the implementation of CRISPR/Cas9 gene editing, optogenetics, and Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools (Ahmadiantehrani & London 2017b).

Unfortunately, none of the experimentally identified putative regulatory regions I tested were sufficient to increase FP expression to a level distinguishable from that driven by the minimal promoter alone. As I focused on building plasmids that incorporated putative regulatory regions differing in accessibility between birds of different ages or sex, I did not select the regulatory regions with the greatest degree of accessibility among those identified from my ChIP-Seq experiment. Instead, I selected those with the greatest degree of difference in accessibility between the compared groups. Had I the forethought to consider such a possible outcome, I would have also selected a subset of the highly accessible non-differential regulatory regions from my experiment to screen using the same methodology. While non-differentially accessible regulatory regions would likely be of little use for identifying the cells within the AL that support sensory song learning, such confirmation would still be beneficial and could still facilitate new lines of inquiry into the transcriptomic landscape of the AL.

There are many potential explanations as to why the selected differentially accessible putative regulatory regions were insufficient to drive an increase in FP expression. These regions were derived from a purely bioinformatic experiment. Although stringent statistical cutoffs were applied at every step of the processing pipeline, investigations of this nature maintain the tendency to generate some false positive results. Thus, the outcomes from my electroporation experiments

may reflect a need to increase the number of putative regulatory regions being screened, as was the case with the initial experiment piloting the EDGE technique (Blankvoort et al. 2018). As multiple enhancers can functionally regulate the expression of a single gene, screening putative enhancers in a combinatorial fashion may be a suitable approach for increasing experimental throughput and successfully labeling the cell populations supporting sensory song learning in the AL. Notably, this approach is limited by our current inability to bioinformatically link putative enhancers and target genes, leading to uncertainty in which enhancer sequences to group and precludes the possibility of attributing an increase in FP expression to a single enhancer without further experimentation. Thus, an argument can be made that individually screening putative enhancers is the most reasonable approach, a process that could be expedited by the generation of long custom oligos containing putative enhancer sequences in place of the PCR amplification approach I took. Albeit far more costly, this would eliminate the need for primer design, thereby eliminating the possibility of failed or off-target PCR amplification and decreasing the number of steps needed to generate individual plasmids.

Chapter V: Loose ends and miscellaneous retrospections

Introduction

The purpose of this final chapter is to discuss a few interesting findings and ideas that emerged during the analysis of this data that did not sensibly fit into another section of this dissertation and to reflect on some of the lessons learned while developing the skill set necessary to analyze and interpret data originating from a large-scale genomic investigation, such as the one discussed throughout this dissertation. As such, each section of this brief chapter is effectively written to stand alone but references findings from my analyses and choices I made while building the bioinformatic pipeline utilized to perform the aforementioned analyses.

Notable TF families poised for investigation within auditory forebrain

Comparisons across experimental groups differing in either age or sex led to the repeated detection of enrichment of binding sites for TFs belonging to TF families that have been heavily implicated in the regulation of brain development and memory formation but have received little to no attention in investigations of zebra finch AL biology. Perhaps this is because there has been little data implicating these TFs in AL transcriptional regulation up to this point. I would argue that these TF families may be particularly well poised for investigation within the AL as there is the potential to draw on large bodies of literature, albeit originating from different organisms and brain regions, to inform investigation methodology and results interpretation.

The MEF2 family is one such example. Binding sites for all MEF2 TF family members (MEF2A, -B, -C, -D) were enriched in multiple sets of regions differing in accessibility between birds of different ages and sex. Binding sites for both MEF2A and MEF2B were enriched in regions differing in accessibility between males spanning the onset of the CP for TSM and the

closure of the CP for TSM. Furthermore, binding sites for MEF2 family members were regularly among those with the greatest degree of enrichment in sexually dimorphic differentially accessible regions across development. MEF2 TF family members, predominately MEF2A, -C, -D, have been shown to regulate an extensive number of biological processes instrumental for appropriate brain development and organization. These include promoting neuron survival (Gaudilliere et al. 2002; Gong et al. 2003; Wang et al. 2009; Akhtar et al. 2012; Salma & McDermott 2012), regulating neuron differentiation (Ikeshimaa et al. 1995; Lyons et al. 1995; Skerjanc & Wilton 2000; Heidenreich & Linseman 2004; Lam & Chawla 2007; Li et al. 2008; Cho et al. 2011), and influencing synapse number and stability (Barbosa et al. 2008; Flavell et al. 2008; Pfeiffer et al. 2010; Vetere et al. 2011; Akhtar et al. 2012; Cole et al. 2012; Rashid et al. 2014); all processes with definitive implications in learning, memory, and neural plasticity. Additionally, MEF2 TF dysfunction has been implicated in the emergence of a number of human neurodevelopmental diseases including epilepsy, intellectual disability, and lack of speech onset (Novara et al. 2010; Zweier et al. 2010; Zweier & Rauch 2012; Bienvenu et al. 2013). Due to the breadth of neuronal development processes under the regulation of MEF2 family members and their high degree of binding site enrichment within differentially accessible regions stemming from this experiment, it is exciting to speculate about the potential regulatory roles of these TFs within AL. However, substantial work will likely be required to determine exactly which, if any, biological processes are under their regulation with the AL and the extent to which those processes dictate differences in learning capability.

The RFX family of TFs is another example of a TF family poised for investigation within the AL. As was the case with MEF2 family members, binding sites for RFX TF family members 1-7 were enriched in multiple sets of regions differing in accessibility between birds of different

ages and sex, and were commonly among those with the greatest degree of enrichment in sexually dimorphic differentially accessible regions across development. RFX family member expression has been reported in several tissues including the brain, where they function as master regulators of central nervous system development and ciliogenesis (Sugiaman-Trapman et al. 2018; Lemeille et al. 2020). In the brain, cilia function as important determinants of neurogenesis, neuronal survival, and neural patterning (Youn & Han 2018). In humans, deleterious mutations of RFX family members associate with neurobehavioral phenotypes characteristic of autism spectrum disorder (ASD), intellectual disability, and attention-deficit/hyperactivity disorder (ADHD) (Harris et al. 2021), demonstrating that these TFs have the capacity to influence aspects of brain development required to support higher-level cognitive processes. Characterizations of RFX family members are still in the early stages and many of the results implicating them as important regulators of brain development and disease have only emerged in recent years (Sugiaman-Trapman et al. 2018; Lemeille et al. 2020; Harris et al. 2021). As such, investigating the functional roles of RFX TFs in zebra finch AL could meaningfully contribute to the actively expanding body of RFX TF literature while also informing questions of AL development and function.

Large numbers of sexually dimorphic regions attributed to individual genes maintain across development

Differentially accessible regions from all comparisons were assigned putative target genes of regulation based on the nearest TSS. In some cases, annotation of sexually dimorphic regions, obtained through the comparison of age-matched males and females, resulted in up to 50 differential regions of greater accessibility in a single sex being annotated to the same gene. Interestingly, genes to which a relatively high number of regulatory regions were associated demonstrated a similar pattern of results at each timepoint assayed in this experiment, suggesting

that regulation of these genes within the AL may be sexually dimorphic independent of age. For example, CELF4 (CUGBP Elav-like family member 4), an RNA-binding protein primarily expressed in excitatory neurons, whose mRNA targets encode a variety of proteins critically involved in neuron development and function (Wagnon et al. 2012), was associated with 49 regions of greater accessibility in males at P23, 37 regions of greater accessibility in males at P30, 50 regions of greater accessibility in males at P60, and 32 regions of greater accessibility in males at P67. Contrastingly, no regions of greater accessibility in females at any of the time points assayed were annotated to CELF4.

Genes displaying a similar pattern of male-specificity and high differential region association included NRG1, NTRK2, TSPAN3, and CAMK4. NRG1 (Neuregulin 1), a member of the epidermal growth factor family of receptor tyrosine kinase proteins, influences neuronal proliferation, migration, and differentiation (Anton et al. 1997; Bermingham-McDonogh et al. 1997; Rio et al. 1997; Rieff et al. 1999; Schmid et al. 2003) and functions as an important regulator of synapse formation and activity-dependent synaptic plasticity during development and in adulthood (Ozaki et al. 1997; Yang et al. 1998; Rieff et al. 1999; Liu et al. 2001; Roysommuti et al. 2003). NTRK2 (Neurotrophic Receptor Tyrosine Kinase 2; commonly referred to as TRKB in zebra finch literature) is a receptor tyrosine kinase activated by the binding of neurotrophic factors including brain-derived neurotrophic factor (BDNF), that plays a critical role in supporting neuronal cell survival and differentiation (Klein et al. 1991; Huang & Reichardt 2001; Deinhardt & Chao 2014; Miranda et al. 2019). BDNF is important for supporting the male-specific development of song system nuclei, including HVC and RA (Johnson et al. 1997; Dittrich et al. 1999; Rasika et al. 1999; Li et al. 2000; Tang & Wade 2013; Beach et al. 2016). The masculinizing effect of BDNF appears heavily reliant on binding to NTRK2, as inhibiting NTRK2 during

juvenile development in males through siRNA treatment results in a dramatic demasculinization of HVC and RA in terms of cell number, but not cell size (Beach et al. 2016). In zebra finches, NTRK2 is a Z-linked gene and is expressed at higher levels in males than in females throughout the whole telencephalon as early as P6 and in adulthood, although not at P1 or P10 (Chen et al. 2005). NTRK2 expression was greater in male HVC than in surrounding telencephalon, suggesting that it may be particularly important for driving sexually dimorphic development of HVC (Chen et al. 2005). This observation does not alter the possibility that sexually-dimorphic NTRK2 expression could be masculinizing other telencephalonic brain regions in biologically and behaviorally meaningful ways. TSPAN3 (Tetraspanin 3) is a transmembrane protein that, through interactions with other proteins, including integrins, cell adhesion proteins, growth factors, and other tetrespanins, mediates signal transduction events that play a role in the regulation of cell development, activation, growth, and motility (Todd et al. 1998; Boucheix & Rubinstein 2001; Hemler 2003). CAMK4 (Calcium/Calmodulin Dependent Protein Kinase IV) is a nuclear calcium/calmodulin dependent protein kinase that influences neuronal transmission, synaptic plasticity, and neuronal gene expression during brain development and in adulthood (Krebs et al., 1998). In response to calcium influx, CAMK4 phosphorylates several IEG TFs, including cyclic AMP-responsive element binding protein (CREB), which then leads to the induction of FOS and BDNF (Krebs 1998; Shieh & Ghosh 1999; Ho et al. 2000). The activation of this molecular cascade appears critically important for regulating memory formation and maintenance (Ho et al. 2000; Wei et al. 2002; Fukushima et al. 2008; Kokubo et al. 2009). Interestingly, NRG1, NTRK2, and CAMK4 have been linked to the activation of the MAPK/ERK signaling cascade (Agell et al. 2002; Ieguchi et al. 2010; Long et al. 2022), suggesting that the regulation of these genes may underlie developmental differences in EGR-1 molecular responsivity within AL.

Many of the genes displaying a pattern of female-specific regulation and high region association were for genes that have not yet been characterized. Those that have been characterized included UBE2R2, BTF3, ZSWIM6, and AARDC3. Belonging to the E2 ubiquitin-conjugating enzyme family, UBE2R2 (Ubiquitin-conjugating Enzyme E2 R2) promotes protein degradation by tagging target proteins with ubiquitin molecules (Semplici et al. 2002). As far as I was able to determine, the role of UBE2R2 in the brain has not yet been characterized, but current evidence suggests it may limit the adherens junction formation by enhancing beta-catenin degradation, thereby influencing cell-cell signaling and organization (Semplici et al. 2002). BTF3 (Basic Transcription Factor 3) is one of the proteins that forms a stable complex with RNA polymerase II to initiate transcription (Zheng et al. 1987). ZSWIM6 (Zinc Finger SWIM-type Containing 6) is a protein whose function is largely unknown. Mutations of the ZSWIM6 gene in humans have been extensively implicated in the development of neurological diseases including schizophrenia and intellectual disability (Ripke et al. 2013; Lencz et al. 2014; Palmer et al. 2017) and ZSWIM6 is currently among the top five schizophrenia-associated genes involved in MAPK signaling (Pers et al. 2016). In mice, ZSWIM6 is expressed at high levels in developing and adult mouse forebrain (Lein et al. 2007; Chang et al. 2021), where, if knocked out leads to reduces striatum volume and altered medium spiny neuron morphology (Tischfield et al. 2017). AARDC3 (Arrestin Domain Containing 3) is an adaptor protein that promotes the ubiquitination, endocytosis, and subsequent degradation of adrenergic receptors (Nabhan et al. 2010; Han et al. 2013). Song-responsive neurons in AL, specifically NCM, express adrenergic receptors and auditory-evoked EGR-1 induction in AL is reduced following local treatment with adrenergic receptor antagonists (Velho et al. 2012). Furthermore, norepinephrine infusion into NCM during song exposure enhances song responsivity of NCM neurons in both males and females through different mechanisms (Lee et al.

2018). Perhaps differences in regulation and expression of genes, such AARDC3, and potentially even other, yet-to-be-characterized genes emerging from this analysis, are contributing to the documented differences in the influence of norepinephrine on song responsivity of male and female NCM neurons.

Continuous implication of individual TFs in biological processes supporting brain development and function

As one might sensibly assume, a complete characterization of all TF function across all cell and tissue types has not yet been achieved. Such a task is further complicated by fact that both TF expression and function are modulated by internal and external environment factors in cell-type- and tissue-type-specific manner. Nevertheless, the ever-expanding number of carefully curated scientific experiments being performed is continually attributing novel regulatory roles to already well-studied TFs. This continuous progression in our understanding of various TF functions has been particularly exciting, as I have been able to readily adjust my interpretations of my data in accordance with the publication of novel findings. For example, WT1 (now officially named WT1 Transcription Factor, previously named Wilms Tumor 1) is a TF that plays critical roles in regulating cancer progression and organ development (Scharnhorst et al. 2001; Qi et al. 2015). Publications demonstrating as much were all I was able to initially find when first aiming to understand WT's biological functions, making it difficult to infer how WT1 might be influencing AL development or function. However, more recently, support for WT1 playing essential regulatory roles in brain development and mature brain function has come to light. Brain-specific WT1 deletion during embryonic neurogenesis results in enhanced neural progenitor cell proliferation, reduced neuronal differentiation, and abnormal cell patterning, as well (Ji et al. 2021). In the mature brain, WT1 appears to regulate memory flexibility, a process critically

important for learning from new experiences by balancing mechanisms of neural plasticity and stability (Mariottini et al. 2019). WT1 is induced in the hippocampus under conditions sufficient to drive LTP and learning, its knockdown enhances hippocampal neuron excitability, and its overexpression decreases memory retention (Mariottini et al. 2019). Furthermore, WT1 was identified, alongside other TFs, including EGR family members (EGR-1, -2, and -3), as a contributor to the regulation of gene networks that underlie age-related cognitive impairment and memory deficits (Emanetci & Çakır 2021). While these are notably fewer studies than those that have been performed on other well-established neural TFs, these studies put forth sufficient evidence to support further investigations into WT1 function within the brain and provide critical information to researchers such as myself looking to comprehend large networks of TFs working in concert. Excitingly, initial characterizations of WT1 expression within the AL, support its presence (Figure 35) (Kunzelman and London, unpublished). Further work will be required to determine if WT1 functionally influences mechanisms of sensory song learning in AL and how WT1 expression and function might differ across development or between sexes.

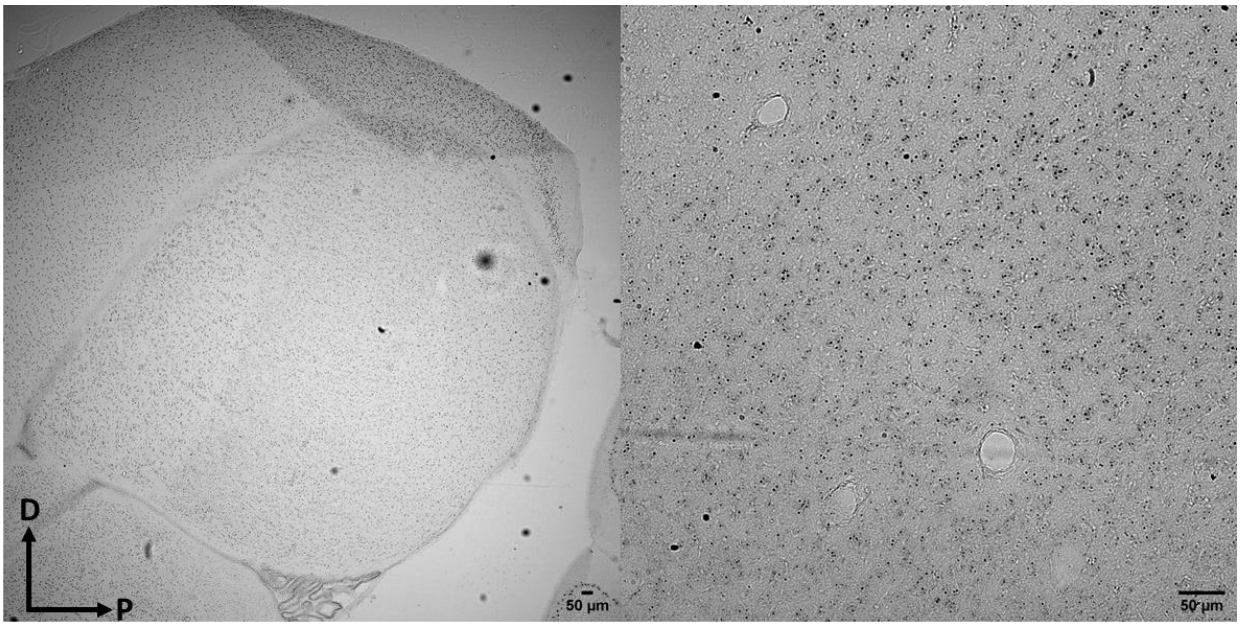

Figure 35. Representative sagittal plane images from WT1 immunohistochemistry pilot experiments. Images show WT1+ cells throughout the entire AL and surround brain tissue. The axis denotes that dorsal (D) is up and posterior (P) is to the right. Left panel: Lower magnification (5X) image of WT1+ cells in the AL. Right panel: Higher magnification (20X) image of WT1+ cells in the AL.

Need for increased accessibility of tools supporting bioinformatic analyses in non-model organisms

Over the course of the last decade, the use of sequencing technologies as a means to investigate the coordinated regulation and expression of gene networks has become increasingly common among researchers aiming to address questions surrounding nearly every area of biology (Bansal & Boucher 2019; McCombie et al. 2019). The use of such technologies has been further bolstered by significant advancements in throughput and decreasing sequencing costs (Bansal & Boucher 2019; McCombie et al. 2019). These advancements, in combination with the exponentially increasing number of publicly available high-quality genome assemblies, have made

the use of sequencing technologies accessible to a greater number of researchers performing work in a greater number of organisms than ever before. In the process of assembling the bioinformatic analysis pipeline used to generate the work discussed in this dissertation, it has become my opinion that many bioinformatic tools commonly used in the analysis of sequencing data still favor a small subset of model organisms, such that certain package features or the entire package can only be utilized if data was obtained from one of those organisms. For example, Genomic Regions Enrichment of Annotations Tool (GREAT) is a package commonly used in the processing of ChIP-Seq data to assign biological roles to regulatory regions of the genome by analyzing the annotations of the nearby genes (McLean et al. 2010). Currently, the only genome assemblies that can be utilized by GREAT are for humans, mice, and zebrafish, thus making it a tool inaccessible to a large portion of the scientific community. Other tools, including some utilized in the bioinformatic analysis pipeline I put together, such as DiffBind and ChIPseeker, possess built-in features supporting analyses in a subset of model organisms but also provide the option to utilize the tool for analyses pertaining to any organism (for which a genome assembly exists) through a few extra programmatic steps (Stark & Brown 2011; Ross-Innes et al. 2012; Yu et al. 2015). While I recognize that it is not the responsibility of programmers to ensure that their packages are accessible to all researchers and that some package features rely on data sources, often supplied, and maintained by parties external to those who developed the program, it is my hope that quality bioinformatic analysis packages will become increasingly accessible to researchers working with less common research organisms throughout coming years.

Variability in output from comparable bioinformatic tools

Generally, there are many different tools to choose from when looking to perform any particular bioinformatic process. In some cases, labs have painstakingly curated reliable and

efficient pipelines that take into consideration the species they are working in, their computing resources, the quality of their data, and the suite of tools available to them. A tool's inclusion or exclusion might be further influenced by the programming language in which the package was coded, the file formats the package can input and output, the package's computational requirements, the availability of package resources (such as individual package features, vignettes, and tutorial), community engagement with the package, as well as countless other package characteristics. For processes such as file conversion, manipulation, and visualization, package selection typically has a negligible impact on data analysis or interpretation. However, for more complex steps of a bioinformatic analysis pipeline, such as peak calling, differential region identification, and motif enrichment analysis, appropriate package selection is of great importance, as it can drastically impact the results obtained at each stage of analysis. Furthermore, decisions regarding analysis parameters and statistical cutoffs have equivalent potential to influence results. Through the careful examination of published ChIP-Seq analysis pipelines, the completion of online training materials (such as those available through Harvard Chan Bioinformatics Core and Bioinformatics Team (BioITeam) at the University of Texas), communications with another researcher in the field, and consultation with package creators, I took great care in selecting the programs and parameters utilized at every stage of analysis while building the bioinformatic analysis pipeline used to generate the work discussed in this dissertation. That having been said, I still think it is imperative to recognize that small alterations to this pipeline can, and likely would (as was my experience while testing different package options and parameters), result in meaningful alteration to the obtained results and interpretations. While differences in output from bioinformatic packages performing the same type of analysis are typically attributable to variations in program algorithms, statistical models, and normalization methods, it is important to recognize

that these choices inherently influence what data is obtained, how it is interpreted, and how it is contextualized in relation to data emerging from differing pipelines. In conclusion, while bioinformatic analyses have drastically expedited the rate at which researchers are capable of generating and interpreting data pertaining to genome regulation and expression, variability among program outputs emphasizes the need for careful results considerations (well beyond the use of statistical criteria alone) and highlights the importance of confirming results from in silico analyses with in vivo experiments performed in the organism from which the data was derived.

Shortcomings of H3K27ac ChIP-Seq analysis

In this experiment, I utilized ChIP-Seq for H3K27ac as a means to identify putative differentially accessible regulatory regions of the genome within the ALs of birds differing in age, sex, and experience. I then performed TFBS enrichment analysis to determine which, if any, TFs have increased potential to influence transcription by binding these regulatory regions. Lastly, I utilized GO term enrichment analysis to identify biological themes of regulation overrepresented among these TFs in comparison to the protein-coding genes of the genome. It is of the utmost importance to recognize that these are simply enriched sequences of nucleotides and that there is still substantial work required to confirm that the corresponding TFs are in fact expressed within the AL, bind to the identified putative differentially accessible regions, and that their binding leads to functional differences in gene expression. Of course, experiments aimed at demonstrating that a TF meets such criteria are also limited, in that they necessitate a focus on a single TF, rather than taking a broad approach, as is more characteristic of large-scale bioinformatic experiments. In sum, while this experiment has further propped opened the door for future lines of inquiry into general mechanisms of AL function, as well as those that might help explain maturation-, experience-, and sex-dependent biological processes supporting learning and memory formation,

there remains a significant need to corroborate these findings with data from other experimental measures.

References

Abbott, G. W. (2016). KCNE1 and KCNE3: The yin and yang of voltage-gated K+ channel regulation. *Gene*, *576*(1), 1-13.

Abel, T., Martin, K. C., Bartsch, D., & Kandel, E. R. (1998). Memory suppressor genes: inhibitory constraints on the storage of long-term memory. *Science*, *279*(5349), 338-341.

Abraham, W., Mason, S., Demmer, J., Williams, J., Richardson, C., Tate, W., Lawlor, P., & Dragunow, M. (1993). Correlations between immediate early gene induction and the persistence of long-term potentiation. *Neuroscience*, *56*(3), 717-727.

Adams, M. M., Fink, S. E., Janssen, W. G., Shah, R. A., & Morrison, J. H. (2004). Estrogen modulates synaptic N-methyl-D-aspartate receptor subunit distribution in the aged hippocampus. *Journal of Comparative Neurology*, *474*(3), 419-426.

Adret, P. (2004a). In search of the song template. *Annals of the New York Academy of Sciences*, *1016*(1), 303-324.

Adret, P. (2004b). Vocal imitation in blindfolded zebra finches (Taeniopygia guttata) is facilitated in the presence of a non-singing conspecific female. *Journal of ethology*, *22*, 29-35.

Adret, P., & Margoliash, D. (2002). Metabolic and neural activity in the song system nucleus robustus archistriatalis: effect of age and gender. *Journal of Comparative Neurology*, *454*(4), 409-423.

Adret, P., Meliza, C. D., & Margoliash, D. (2012). Song tutoring in presinging zebra finch juveniles biases a small population of higher-order song-selective neurons toward the tutor song. *Journal of neurophysiology*, *108*(7), 1977-1987.

Aftab, S., Semenec, L., Chu, J. S.-C., & Chen, N. (2008). Identification and characterization of novel human tissue-specific RFX transcription factors. *BMC evolutionary biology*, *8*(1), 1-11.

Agell, N., Bachs, O., Rocamora, N., & Villalonga, P. (2002). Modulation of the Ras/Raf/MEK/ERK pathway by Ca2+, and calmodulin. *Cellular Signalling*, *14*(8), 649-654.

Ahmadiantehrani, S., Gores, E. O., & London, S. E. (2018). A complex mTOR response in habituation paradigms for a social signal in adult songbirds. *Learn Mem*, *25*(6), 273-282. https://doi.org/10.1101/lm.046417.117

Ahmadiantehrani, S., & London, S. E. (2017a). Bidirectional manipulation of mTOR signaling disrupts socially mediated vocal learning in juvenile songbirds. *Proc Natl Acad Sci U S A*, *114*(35), 9463-9468. https://doi.org/10.1073/pnas.1701829114

Ahmadiantehrani, S., & London, S. E. (2017b). A reliable and flexible gene manipulation strategy in posthatch zebra finch brain. *Scientific Reports*, *7*(1), 1-16.

Akhtar, M. W., Kim, M.-S., Adachi, M., Morris, M. J., Qi, X., Richardson, J. A., Bassel-Duby, R., Olson, E. N., Kavalali, E. T., & Monteggia, L. M. (2012). In vivo analysis of MEF2 transcription factors in synapse regulation and neuronal survival. *PloS one*, *7*(4), e34863.

Albéri, L., Sgadò, P., & Simon, H. H. (2004). Engrailed genes are cell-autonomously required to prevent apoptosis in mesencephalic dopaminergic neurons.

Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiological reviews*, *89*(1), 121-145.

Aldavert-Vera, L., Huguet, G., Costa-Miserachs, D., de Ortiz, S. P., Kádár, E., Morgado-Bernal, I., & Segura-Torres, P. (2013). Intracranial self-stimulation facilitates active-avoidance retention and induces expression of c-Fos and Nurr1 in rat brain memory systems. *Behavioural brain research*, *250*, 46-57.

Allfrey, V. G., Faulkner, R., & Mirsky, A. E. (1964). ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS. *Proc Natl Acad Sci U S A*, *51*(5), 786-794. https://doi.org/10.1073/pnas.51.5.786

Alves dos Santos, M. T., & Smidt, M. P. (2011). En1 and Wnt signaling in midbrain dopaminergic neuronal development. *Neural development*, *6*, 1-15.

Anton, E., Marchionni, M., Lee, K., & Rakic, P. (1997). Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development*, *124*(18), 3501-3510.

Antunes, C., Sousa, N., Pinto, L., & Marques, C. J. (2019). TET enzymes in neurophysiology and brain function. *Neurosci Biobehav Rev*, *102*, 337-344. https://doi.org/10.1016/j.neubiorev.2019.05.006

Aronov, D., Andalman, A. S., & Fee, M. S. (2008). A specialized forebrain circuit for vocal babbling in the juvenile songbird. *Science*, *320*(5876), 630-634.

Ashique, A. M., Choe, Y., Karlen, M., May, S. R., Phamluong, K., Solloway, M. J., Ericson, J., & Peterson, A. S. (2009). The Rfx4 transcription factor modulates Shh signaling by regional control of ciliogenesis. *Science signaling*, *2*(95), ra70-ra70.

Aspenström, P. (2014). BAR domain proteins regulate Rho GTPase signaling. *Small GTPases*, *5*(2), e972854.

Assali, A., Harrington, A. J., & Cowan, C. W. (2019). Emerging roles for MEF2 in brain development and mental disorders. *Current opinion in neurobiology*, *59*, 49-58.

Avenarius, M. R., Krey, J. F., Dumont, R. A., Morgan, C. P., Benson, C. B., Vijayakumar, S., Cunningham, C. L., Scheffer, D. I., Corey, D. P., & Müller, U. (2017). Heterodimeric capping protein is required for stereocilia length and width regulation. *Journal of Cell Biology*, *216*(11), 3861-3881.

Bailey, D. J., & Wade, J. (2003). Differential expression of the immediate early genes FOS and ZENK following auditory stimulation in the juvenile male and female zebra finch. *Molecular Brain Research*, *116*(1-2), 147-154.

Bailey, D. J., & Wade, J. (2005). FOS and ZENK responses in 45-day-old zebra finches vary with auditory stimulus and brain region, but not sex. *Behavioural brain research*, *162*(1), 108-115.

Bailey, T. L., & Grant, C. E. (2021). SEA: Simple Enrichment Analysis of motifs. *bioRxiv*, 2021.2008.2023.457422. https://doi.org/10.1101/2021.08.23.457422

Bailey, T. L., Johnson, J., Grant, C. E., & Noble, W. S. (2015). The MEME Suite. *Nucleic Acids Res*, *43*(W1), W39-49. https://doi.org/10.1093/nar/gkv416

Bakke, J., Wright, W. C., Zamora, A. E., Ong, S. S., Wang, Y.-M., Hoyer, J. D., Brewer, C. T., Thomas, P. G., & Chen, T. (2017). Transcription factor ZNF148 is a negative regulator of human muscle differentiation. *Scientific Reports*, *7*(1), 8138.

Baldelli, P., & Meldolesi, J. (2015). The transcription repressor REST in adult neurons: physiology, pathology, and diseases. *ENeuro*, *2*(4).

Ballas, N., Grunseich, C., Lu, D. D., Speh, J. C., & Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell*, *121*(4), 645-657.

Balmer, T. S., Carels, V. M., Frisch, J. L., & Nick, T. A. (2009). Modulation of perineuronal nets and parvalbumin with developmental song learning. *Journal of Neuroscience*, *29*(41), 12878-12885.

Banerjee, S. B., Gutzeit, V. A., Baman, J., Aoued, H. S., Doshi, N. K., Liu, R. C., & Ressler, K. J. (2017). Perineuronal nets in the adult sensory cortex are necessary for fear learning. *Neuron*, *95*(1), 169-179. e163.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Res*, *21*(3), 381-395. https://doi.org/10.1038/cr.2011.22

Bansal, V., & Boucher, C. (2019). Sequencing technologies and analyses: where have we been and where are we going? In (Vol. 18, pp. 37-41): Elsevier.

Baran, N. M., Peck, S. C., Kim, T. H., Goldstein, M. H., & Adkins-Regan, E. (2017). Early life manipulations of vasopressin-family peptides alter vocal learning. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1859), 20171114.

Barbosa, A. C., Kim, M.-S., Ertunc, M., Adachi, M., Nelson, E. D., McAnally, J., Richardson, J. A., Kavalali, E. T., Monteggia, L. M., & Bassel-Duby, R. (2008). MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function. *Proceedings of the National Academy of Sciences*, *105*(27), 9391-9396.

Barish, G. D., Ruth, T. Y., Karunasiri, M., Ocampo, C. B., Dixon, J., Benner, C., Dent, A. L., Tangirala, R. K., & Evans, R. M. (2010). Bcl-6 and NF-κB cistromes mediate opposing regulation of the innate immune response. *Genes & development*, *24*(24), 2760-2765.

Bartsch, D., Ghirardi, M., Skehel, P. A., Karl, K. A., Herder, S. P., Chen, M., Bailey, C. H., & Kandel, E. R. (1995). Aplysia CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. *Cell*, *83*(6), 979-992.

Basham, M., Nordeen, E., & Nordeen, K. (1996). Blockade of NMDA receptors in the anterior forebrain impairs sensory acquisition in the zebra finch (Poephila guttata). *Neurobiology of learning and memory*, *66*(3), 295-304.

Basu, A., Mestres, I., Sahu, S. K., Tiwari, N., Khongwir, B., Baumgart, J., Singh, A., Calegari, F., & Tiwari, V. K. (2020). Phf21b imprints the spatiotemporal epigenetic switch essential for neural stem cell differentiation. *Genes & development*, *34*(17-18), 1190-1209.

Basu, K., Gravel, C., Tomioka, R., Kaneko, T., Tamamaki, N., & Sík, A. (2008). Novel strategy to selectively label excitatory and inhibitory neurons in the cerebral cortex of mice. *Journal of neuroscience methods*, *170*(2), 212-219.

Bauer, E. E., Coleman, M. J., Roberts, T. F., Roy, A., Prather, J. F., & Mooney, R. (2008). A synaptic basis for auditory–vocal integration in the songbird. *Journal of Neuroscience*, *28*(6), 1509-1522.

Beach, L. Q., Tang, Y. P., Kerver, H., & Wade, J. (2016). Inhibition of TrkB limits development of the zebra finch song system. *Brain research*, *1642*, 467-477.

Bejjani, F., Evanno, E., Zibara, K., Piechaczyk, M., & Jariel-Encontre, I. (2019). The AP-1 transcriptional complex: Local switch or remote command? *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1872*(1), 11-23.

Bengoetxea, H., Ortuzar, N., Bulnes, S., Rico-Barrio, I., Lafuente, J. V., & Argandona, E. G. (2012). Enriched and deprived sensory experience induces structural changes and rewires connectivity during the postnatal development of the brain. *Neural plasticity*, *2012*.

Bentrop, D., Beyermann, M., Wissmann, R., & Fakler, B. (2001). NMR Structure of the "Ball-and-chain" Domain of KCNMB2, the β2-Subunit of Large Conductance Ca2+-and Voltage-activated Potassium Channels* 210. *Journal of Biological Chemistry*, *276*(45), 42116-42121.

Berglund, E. O., Murai, K. K., Fredette, B., Sekerková, G., Marturano, B., Weber, L., Mugnaini, E., & Ranscht, B. (1999). Ataxia and abnormal cerebellar microorganization in mice with ablated contactin gene expression. *Neuron*, *24*(3), 739-750.

Bermingham-McDonogh, O., Xu, Y.-T., Marchionni, M. A., & Scherer, S. S. (1997). Neuregulin expression in PNS neurons: isoforms and regulation by target interactions. *Molecular and Cellular Neuroscience*, *10*(3-4), 184-195.

Bernabeu, R., Yang, T., Xie, Y., Mehta, B., Ma, S. Y., & Longo, F. M. (2006). Downregulation of the LAR protein tyrosine phosphatase receptor is associated with increased dentate gyrus neurogenesis and an increased number of granule cell layer neurons. *Molecular and Cellular Neuroscience*, *31*(4), 723-738.

Bernard, D., Prasanth, K. V., Tripathi, V., Colasse, S., Nakamura, T., Xuan, Z., Zhang, M. Q., Sedel, F., Jourdren, L., & Coulpier, F. (2010). A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *The EMBO journal*, *29*(18), 3082-3093.

Bienvenu, T., Diebold, B., Chelly, J., & Isidor, B. (2013). Refining the phenotype associated with MEF2C point mutations. *Neurogenetics*, *14*, 71-75.

Blake, D. J., Hawkes, R., Benson, M. A., & Beesley, P. W. (1999). Different dystrophin-like complexes are expressed in neurons and glia. *The Journal of cell biology*, *147*(3), 645-658.

Blake, D. J., Nawrotzki, R., Loh, N., & Davies, K. (1997). A novel dystrophin-associated protein in brain. *American Journal of Human Genetics*, *61*(4), A8-A8.

Blake, D. J., Nawrotzki, R., Loh, N. Y., Górecki, D. C., & Davies, K. E. (1998). β-Dystrobrevin, a member of the dystrophin-related protein family. *Proceedings of the National Academy of Sciences*, *95*(1), 241-246.

Blankvoort, S., Descamps, L. A., & Kentros, C. (2020). Enhancer-Driven Gene Expression (EDGE) enables the generation of cell type specific tools for the analysis of neural circuits. *Neuroscience research*, *152*, 78-86.

Blankvoort, S., Witter, M. P., Noonan, J., Cotney, J., & Kentros, C. (2018). Marked Diversity of Unique Cortical Enhancers Enables Neuron-Specific Tools by Enhancer-Driven Gene Expression. *Curr Biol*, *28*(13), 2103-2114.e2105. https://doi.org/10.1016/j.cub.2018.05.015

Böhner, J. (1983). Song learning in the zebra finch (Taeniopygia guttata): selectivity in the choice of a tutor and accuracy of song copies. *Animal Behaviour*, *31*(1), 231-237.

Böhner, J. (1990). Early acquisition of song in the zebra finch, Taeniopygia guttata. *Animal Behaviour*, *39*(2), 369-374.

Bolhuis, J. J., & Gahr, M. (2006). Neural mechanisms of birdsong memory. *Nature reviews neuroscience*, *7*(5), 347-357.

Bolhuis, J. J., Hetebrij, E., Den Boer-Visser, A. M., De Groot, J. H., & Zijlstra, G. G. (2001). Localized immediate early gene expression related to the strength of song learning in socially reared zebra finches. *European Journal of Neuroscience*, *13*(11), 2165-2170.

Bolhuis, J. J., Zijlstra, G. G., den Boer-Visser, A. M., & Van der Zee, E. A. (2000). Localized neuronal activation in the zebra finch brain is related to the strength of song learning. *Proceedings of the National Academy of Sciences*, *97*(5), 2282-2285.

Bornschein, G., & Schmidt, H. (2019). Synaptotagmin Ca2+ sensors and their spatial coupling to presynaptic Cav channels in central cortical synapses. *Frontiers in molecular neuroscience*, *11*, 494.

Böse, C. M., Qiu, D., Bergamaschi, A., Gravante, B., Bossi, M., Villa, A., Rupp, F., & Malgaroli, A. (2000). Agrin controls synaptic differentiation in hippocampal neurons. *Journal of Neuroscience*, *20*(24), 9086-9095.

Bottjer, S. W., Glaessner, S. L., & Arnold, A. P. (1985). Ontogeny of brain nuclei controlling song learning and behavior in zebra finches. *Journal of Neuroscience*, *5*(6), 1556-1562.

Bottjer, S. W., Miesner, E. A., & Arnold, A. P. (1984). Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science*, *224*(4651), 901-903.

Bottjer, S. W., Miesner, E. A., & Arnold, A. P. (1986). Changes in neuronal number, density and size account for increases in volume of song-control nuclei during song development in zebra finches. *Neuroscience letters*, *67*(3), 263-268.

Boucheix, C., & Rubinstein, E. (2001). Tetraspanins. *Cellular and Molecular Life Sciences CMLS*, *58*, 1189-1205.

Boudry-Labis, E., Demeer, B., Le Caignec, C., Isidor, B., Mathieu-Dramard, M., Plessis, G., George, A. M., Taylor, J., Aftimos, S., & Wiemer-Kruel, A. (2013). A novel microdeletion syndrome at 9q21. 13 characterised by mental retardation, speech delay, epilepsy and characteristic facial features. *European Journal of Medical Genetics*, *56*(3), 163-170.

Bowe, M. A., Deyst, K. A., Leszyk, J. D., & Fallon, J. R. (1994). Identification and purification of an agrin receptor from Torpedo postsynaptic membranes: a heteromeric complex related to the dystroglycans. *Neuron*, *12*(5), 1173-1180.

Braaten, R. F. (2010). Song recognition in zebra finches: Are there sensitive periods for song memorization? *Learning and Motivation*, *41*(3), 202-212.

Braaten, R. F., & Reynolds, K. (1999). Auditory preference for conspecific song in isolation-reared zebra finches. *Animal Behaviour*, *58*(1), 105-111.

Brenowitz, E. A., & Remage-Healey, L. (2016). It takes a seasoned bird to be a good listener: communication between the sexes. *Current opinion in neurobiology*, *38*, 12-17.

Bridi, M. S., & Abel, T. (2013). The NR4A orphan nuclear receptors mediate transcription-dependent hippocampal synaptic plasticity. *Neurobiology of learning and memory*, *105*, 151-158.

Burek, M., Nordeen, K., & Nordeen, E. (1994). Ontogeny of sex differences among newly-generated neurons of the juvenile avian brain. *Developmental brain research*, *78*(1), 57-64.

Burgess, D. L., Davis, C. F., Gefrides, L. A., & Noebels, J. L. (1999). Identification of three novel Ca2+ channel γ subunit genes reveals molecular diversification by tandem and chromosome duplication. *Genome research*, *9*(12), 1204-1213.

Carcea, I., & Froemke, R. C. (2013). Cortical plasticity, excitatory–inhibitory balance, and sensory perception. *Progress in brain research*, *207*, 65-90.

Carroll, T. S., Liang, Z., Salama, R., Stark, R., & de Santiago, I. (2014). Impact of artifact removal on ChIP quality metrics in ChIP-seq and ChIP-exo data [Technology Report]. *Frontiers in Genetics*, *5*. https://doi.org/10.3389/fgene.2014.00075

Castro-Mondragon, J. A., Riudavets-Puig, R., Rauluseviciute, I., Lemma, R. B., Turchi, L., Blanc-Mathieu, R., Lucas, J., Boddie, P., Khan, A., Manosalva Pérez, N., Fornes, O., Leung, T. Y., Aguirre, A., Hammal, F., Schmelter, D., Baranasic, D., Ballester, B., Sandelin, A., Lenhard, B., . . . Mathelier, A. (2022). JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Res*, *50*(D1), D165-d173. https://doi.org/10.1093/nar/gkab1113

Chang, B. H., Mukherji, S., & Soderling, T. R. (1998). Characterization of a calmodulin kinase II inhibitor protein in brain. *Proceedings of the National Academy of Sciences*, *95*(18), 10890-10895.

Chang, C.-C., Kuo, H.-Y., Chen, S.-Y., Lin, W.-T., Lu, K.-M., Saito, T., & Liu, F.-C. (2021). Developmental Characterization of Schizophrenia-Associated Gene Zswim6 in Mouse Forebrain. *Frontiers in Neuroanatomy*, *15*, 669631.

Chang, F., Steelman, L. S., Lee, J. T., Shelton, J. G., Navolanic, P. M., Blalock, W. L., Franklin, R. A., & McCubrey, J. A. (2003a). Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. *Leukemia (08876924)*, *17*(7).

Chang, F., Steelman, L. S., Shelton, J. G., Lee, J. T., Navolanic, P. M., Blalock, W. L., Franklin, R., & McCubrey, J. A. (2003b). Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway. *International journal of oncology*, *22*(3), 469-480.

Chen, C., Meng, Q., Xia, Y., Ding, C., Wang, L., Dai, R., Cheng, L., Gunaratne, P., Gibbs, R. A., & Min, S. (2018). The transcription factor POU3F2 regulates a gene coexpression

network in brain tissue from patients with psychiatric disorders. *Science translational medicine*, *10*(472), eaat8178.

Chen, L., Chatterjee, M., & Li, J. Y. (2010). The mouse homeobox gene Gbx2 is required for the development of cholinergic interneurons in the striatum. *Journal of Neuroscience*, *30*(44), 14824-14834.

Chen, X., Agate, R. J., Itoh, Y., & Arnold, A. P. (2005). Sexually dimorphic expression of trkB, a Z-linked gene, in early posthatch zebra finch brain. *Proceedings of the National Academy of Sciences*, *102*(21), 7730-7735.

Chen, Y., Clark, O., & Woolley, S. C. (2017). Courtship song preferences in female zebra finches are shaped by developmental auditory experience. *Proc Biol Sci*, *284*(1855). https://doi.org/10.1098/rspb.2017.0054

Chen, Y., Matheson, L. E., & Sakata, J. T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *Proc Natl Acad Sci U S A*, *113*(24), 6641-6646. https://doi.org/10.1073/pnas.1522306113

Chen, Y., Pei, M., Li, J., Wang, Z., Liu, S., Xiang, L., Zhang, J., Hong, L., Lin, J., & Dai, W. (2022). Disruption of the CCDC43-FHL1 interaction triggers apoptosis in gastric cancer cells. *Experimental Cell Research*, *415*(1), 113107.

Cheng, H.-Y., & Clayton, D. F. (2004). Activation and habituation of extracellular signal-regulated kinase phosphorylation in zebra finch auditory forebrain during song presentation. *Journal of Neuroscience*, *24*(34), 7503-7513.

Cheng, Y., Geng, H., Cheng, S. H., Liang, P., Bai, Y., Li, J., Srivastava, G., Ng, M. H., Fukagawa, T., & Wu, X. (2010). KRAB Zinc Finger Protein ZNF382 Is a Proapoptotic Tumor Suppressor That Represses Multiple Oncogenes and Is Commonly Silenced in Multiple CarcinomasMethylation of ZNF382 in Multiple Carcinomas. *Cancer research*, *70*(16), 6516-6526.

Chew, S. J., Mello, C., Nottebohm, F., Jarvis, E., & Vicario, D. S. (1995). Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. *Proceedings of the National Academy of Sciences*, *92*(8), 3406-3410.

Chew, S. J., Vicario, D. S., & Nottebohm, F. (1996). A large-capacity memory system that recognizes the calls and songs of individual birds. *Proceedings of the National Academy of Sciences*, *93*(5), 1950-1955.

Chin, E. W., Ma, Q., Ruan, H., Chin, C., Somasundaram, A., Zhang, C., Liu, C., Lewis, M. D., White, M., & Smith, T. L. (2022). The epigenetic reader PHF21B modulates murine social memory and synaptic plasticity–related genes. *JCI insight*, *7*(14).

Cho, E.-G., Zaremba, J. D., McKercher, S. R., Talantova, M., Tu, S., Masliah, E., Chan, S. F., Nakanishi, N., Terskikh, A., & Lipton, S. A. (2011). MEF2C enhances dopaminergic

neuron differentiation of human embryonic stem cells in a parkinsonian rat model. *PloS one*, *6*(8), e24027.

Cho, J., Yu, N.-K., Choi, J.-H., Sim, S.-E., Kang, S. J., Kwak, C., Lee, S.-W., Kim, J.-i., Choi, D. I., & Kim, V. N. (2015). Multiple repressive mechanisms in the hippocampus during memory formation. *Science*, *350*(6256), 82-87.

Chong, J. A., Tapia-Ramirez, J., Kim, S., Toledo-Aral, J. J., Zheng, Y., Boutros, M. C., Altshuller, Y. M., Frohman, M. A., Kraner, S. D., & Mandel, G. (1995). REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell*, *80*(6), 949-957.

Chowdhury, M. I. H., Nishioka, T., Mishima, N., Ohtsuka, T., Kaibuchi, K., & Tsuboi, D. (2020). Prickle2 and Igsf9b coordinately regulate the cytoarchitecture of the axon initial segment. *Cell structure and function*, *45*(2), 143-154.

Chung, M.-I., Peyrot, S. M., LeBoeuf, S., Park, T. J., McGary, K. L., Marcotte, E. M., & Wallingford, J. B. (2012). RFX2 is broadly required for ciliogenesis during vertebrate development. *Developmental biology*, *363*(1), 155-165.

Clayton, D., Arnold, A. P., Warren, W., & Dodgson, J. (2005). Proposal for construction of a physical map of the genome of the Zebra Finch (Taeniopygia guttata). In: University of California Los Angeles, USA.

Clayton, D. F. (2000). The genomic action potential. *Neurobiology of learning and memory*, *74*(3), 185-216.

Clayton, D. F., Anreiter, I., Aristizabal, M., Frankland, P. W., Binder, E. B., & Citri, A. (2020). The role of the genome in experience-dependent plasticity: Extending the analogy of the genomic action potential. *Proceedings of the National Academy of Sciences*, *117*(38), 23252-23260.

Clayton, D. F., & London, S. E. (2014). Advancing avian behavioral neuroendocrinology through genomics. *Front Neuroendocrinol*, *35*(1), 58-71. https://doi.org/10.1016/j.yfrne.2013.09.004

Clayton, N. (1987). Song tutor choice in zebra finches. *Animal Behaviour*, *35*(3), 714-721.

Clayton, N. (1988). Song discrimination learning in zebra finches. *Animal Behaviour*, *36*(4), 1016-1024.

Çolakoğlu, G., Bergstrom-Tyrberg, U., Berglund, E. O., & Ranscht, B. (2014). Contactin-1 regulates myelination and nodal/paranodal domain organization in the central nervous system. *Proceedings of the National Academy of Sciences*, *111*(3), E394-E403.

Cole, C. J., Mercaldo, V., Restivo, L., Yiu, A. P., Sekeres, M. J., Han, J.-H., Vetere, G., Pekar, T., Ross, P. J., & Neve, R. L. (2012). MEF2 negatively regulates learning-induced structural plasticity and memory formation. *Nature neuroscience*, *15*(9), 1255-1264.

Collins, S. (2004). Vocal fighting and flirting: the functions of birdsong. *Nature's music: the science of birdsong*, 39-79.

Colombo, E., Collombat, P., Colasante, G., Bianchi, M., Long, J., Mansouri, A., Rubenstein, J. L., & Broccoli, V. (2007). Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *Journal of Neuroscience*, *27*(17), 4786-4798.

Conrotto, P., Corso, S., Gamberini, S., Comoglio, P. M., & Giordano, S. (2004). Interplay between scatter factor receptors and B plexins controls invasive growth. *Oncogene*, *23*(30), 5131-5137.

Cornez, G., Jonckers, E., Ter Haar, S. M., Van der Linden, A., Cornil, C. A., & Balthazart, J. (2018). Timing of perineuronal net development in the zebra finch song control system correlates with developmental song learning. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 20180849.

Creighton, S. D., Stefanelli, G., Reda, A., & Zovkic, I. B. (2020). Epigenetic Mechanisms of Learning and Memory: Implications for Aging. *Int J Mol Sci*, *21*(18). https://doi.org/10.3390/ijms21186918

Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, B. W., Steine, E. J., Hanna, J., Lodato, M. A., Frampton, G. M., Sharp, P. A., Boyer, L. A., Young, R. A., & Jaenisch, R. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A*, *107*(50), 21931-21936. https://doi.org/10.1073/pnas.1016071107

Dagostin, A. A., Mello, C. V., & Leão, R. M. (2012). Increased bursting glutamatergic neurotransmission in an auditory forebrain area of the zebra finch (Taenopygia guttata) induced by auditory stimulation. *Journal of Comparative Physiology A*, *198*, 705-716.

Dai, J. B., Chen, Y., & Sakata, J. T. (2018). EGR-1 expression in catecholamine-synthesizing neurons reflects auditory learning and correlates with responses in auditory processing areas. *Neuroscience*, *379*, 415-427.

Dai, Q., Ren, A., Westholm, J. O., Serganov, A. A., Patel, D. J., & Lai, E. C. (2013). The BEN domain is a novel sequence-specific DNA-binding domain conserved in neural transcriptional repressors. *Genes & development*, *27*(6), 602-614.

Daly, G., Hawi, Z., Fitzgerald, M., & Gill, M. (1999). Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. *Molecular psychiatry*, *4*(2), 192-196.

Davenport, M., Choe, H. N., Matsunami, H., & Jarvis, E. D. (2021). A sex chromosome drives the emergence of vocal learning following hormonal manipulation in female zebra finches. *bioRxiv*, 2021.2007. 2012.452102.

Davidson, D., Graham, E., Sime, C., & Hill, R. (1988). A gene with sequence similarity to Drosophila engrailed is expressed during the development of the neural tube and vertebrae in the mouse. *Development*, *104*(2), 305-316.

Decembrini, S., Andreazzoli, M., Vignali, R., Barsacchi, G., & Cremisi, F. (2006). Timing the generation of distinct retinal cells by homeobox proteins. *PLoS biology*, *4*(9), e272.

Decressac, M., Volakakis, N., Björklund, A., & Perlmann, T. (2013). NURR1 in Parkinson disease—from pathogenesis to therapeutic potential. *Nature Reviews Neurology*, *9*(11), 629-636.

Deinhardt, K., & Chao, M. V. (2014). Trk receptors. *Neurotrophic factors*, 103-119.

Deinhardt, K., Kim, T., Spellman, D. S., Mains, R. E., Eipper, B. A., Neubert, T. A., Chao, M. V., & Hempstead, B. L. (2011). Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Science signaling*, *4*(202), ra82-ra82.

Dejosez, M., Krumenacker, J. S., Zitur, L. J., Passeri, M., Chu, L.-F., Songyang, Z., Thomson, J. A., & Zwaka, T. P. (2008). Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells. *Cell*, *133*(7), 1162-1174.

Di Nardo, A. A., Nedelec, S., Trembleau, A., Volovitch, M., Prochiantz, A., & Montesinos, M. L. (2007). Dendritic localization and activity-dependent translation of Engrailed1 transcription factor. *Molecular and Cellular Neuroscience*, *35*(2), 230-236.

Diddens, J., Coussement, L., Frankl-Vilches, C., Majumdar, G., Steyaert, S., Ter Haar, S. M., Galle, J., De Meester, E., De Keulenaer, S., & Van Criekinge, W. (2021). DNA methylation regulates transcription factor-specific neurodevelopmental but not sexually dimorphic gene expression dynamics in zebra finch telencephalon. *Frontiers in Cell and Developmental Biology*, *9*, 583555.

Diez, A., Cui, A., & MacDougall-Shackleton, S. A. (2019). The neural response of female zebra finches (Taeniopygia guttata) to conspecific, heterospecific, and isolate song depends on early-life song exposure. *Behavioural Processes*, *163*, 37-44.

Dittrich, F., Feng, Y., Metzdorf, R., & Gahr, M. (1999). Estrogen-inducible, sex-specific expression of brain-derived neurotrophic factor mRNA in a forebrain song control nucleus of the juvenile zebra finch. *Proceedings of the National Academy of Sciences*, *96*(14), 8241-8246.

Dong, S., Replogle, K. L., Hasadsri, L., Imai, B. S., Yau, P. M., Rodriguez-Zas, S., Southey, B. R., Sweedler, J. V., & Clayton, D. F. (2009). Discrete molecular states in the brain accompany changing responses to a vocal signal. *Proceedings of the National Academy of Sciences*, *106*(27), 11364-11369.

Doupe, A. J., & Solis, M. M. (1997). Song-and order-selective neurons develop in the songbird anterior forebrain during vocal learning. *Journal of neurobiology*, *33*(5), 694-709.

Duclot, F., & Kabbaj, M. (2017). The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. *Front Behav Neurosci, 11*, 35. https://doi.org/10.3389/fnbeh.2017.00035

Dugas-Ford, J., Rowell, J. J., & Ragsdale, C. W. (2012). Cell-type homologies and the origins of the neocortex. *Proceedings of the National Academy of Sciences, 109*(42), 16974-16979.

Dunah, A. W., Hueske, E., Wyszynski, M., Hoogenraad, C. C., Jaworski, J., Pak, D. T., Simonetta, A., Liu, G., & Sheng, M. (2005). LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses. *Nature neuroscience, 8*(4), 458-467.

Duncan, K. A., & Carruth, L. L. (2007). The sexually dimorphic expression of L7/SPA, an estrogen receptor coactivator, in zebra finch telencephalon. *Developmental neurobiology, 67*(14), 1852-1866.

Eales, L. A. (1985). Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Animal Behaviour, 33*(4), 1293-1300.

Eales, L. A. (1987). Song learning in female-raised zebra finches: another look at the sensitive phase. *Animal Behaviour, 35*(5), 1356-1365.

Edwards, M., Zwolak, A., Schafer, D. A., Sept, D., Dominguez, R., & Cooper, J. A. (2014). Capping protein regulators fine-tune actin assembly dynamics. *Nature reviews Molecular cell biology, 15*(10), 677-689.

Egashira, Y., Mori, Y., Yanagawa, Y., & Takamori, S. (2018). Development of lentiviral vectors for efficient glutamatergic-selective gene expression in cultured hippocampal neurons. *Scientific Reports, 8*(1), 15156.

Emanetci, E., & Çakır, T. (2021). Network-Based Analysis of Cognitive Impairment and Memory Deficits from Transcriptome Data. *Journal of Molecular Neuroscience, 71*(11), 2415-2428.

Faber, E. L., & Sah, P. (2003). Calcium-activated potassium channels: multiple contributions to neuronal function. *The Neuroscientist, 9*(3), 181-194.

Feng, J., Liu, T., Qin, B., Zhang, Y., & Liu, X. S. (2012a). Identifying ChIP-seq enrichment using MACS. *Nat Protoc, 7*(9), 1728-1740. https://doi.org/10.1038/nprot.2012.101

Feng, J., Xian, Q., Guan, T., Hu, J., Wang, M., Huang, Y., So, K.-F., Evans, S. M., Chai, G., & Goffinet, A. M. (2016). Celsr3 and Fzd3 organize a pioneer neuron scaffold to steer growing thalamocortical axons. *Cerebral cortex, 26*(7), 3323-3334.

Feng, J., Xu, Y., Wang, M., Ruan, Y., So, K.-F., Tissir, F., Goffinet, A., & Zhou, L. (2012b). A role for atypical cadherin Celsr3 in hippocampal maturation and connectivity. *Journal of Neuroscience, 32*(40), 13729-13743.

Feng, J., Zhou, Y., Campbell, S. L., Le, T., Li, E., Sweatt, J. D., Silva, A. J., & Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat Neurosci*, *13*(4), 423-430. https://doi.org/10.1038/nn.2514

Ferreira, A. (1999). Abnormal synapse formation in agrin-depleted hippocampal neurons. *Journal of Cell Science*, *112*(24), 4729-4738.

Flavell, S. W., Cowan, C. W., Kim, T.-K., Greer, P. L., Lin, Y., Paradis, S., Griffith, E. C., Hu, L. S., Chen, C., & Greenberg, M. E. (2006). Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science*, *311*(5763), 1008-1012.

Flavell, S. W., Kim, T.-K., Gray, J. M., Harmin, D. A., Hemberg, M., Hong, E. J., Markenscoff-Papadimitriou, E., Bear, D. M., & Greenberg, M. E. (2008). Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. *Neuron*, *60*(6), 1022-1038.

Fox, N. C., & Schott, J. M. (2004). Imaging cerebral atrophy: normal ageing to Alzheimer's disease. *The Lancet*, *363*(9406), 392-394.

Freeman, S. H., Kandel, R., Cruz, L., Rozkalne, A., Newell, K., Frosch, M. P., Hedley-Whyte, E. T., Locascio, J. J., Lipsitz, L. A., & Hyman, B. T. (2008). Preservation of neuronal number despite age-related cortical brain atrophy in elderly subjects without Alzheimer disease. *Journal of Neuropathology & Experimental Neurology*, *67*(12), 1205-1212.

Friedrich, S. R., Nevue, A. A., Andrade, A. L., Velho, T. A., & Mello, C. V. (2022). Emergence of sex-specific transcriptomes in a sexually dimorphic brain nucleus. *Cell reports*, *40*(5), 111152.

Friocourt, G., & Parnavelas, J. G. (2011). Identification of Arx targets unveils new candidates for controlling cortical interneuron migration and differentiation. *Frontiers in cellular neuroscience*, *5*, 28.

Fu, Y., Liu, H., Ng, L., Kim, J.-W., Hao, H., Swaroop, A., & Forrest, D. (2014). Feedback induction of a photoreceptor-specific isoform of retinoid-related orphan nuclear receptor β by the rod transcription factor NRL. *Journal of Biological Chemistry*, *289*(47), 32469-32480.

Fukushima, H., Maeda, R., Suzuki, R., Suzuki, A., Nomoto, M., Toyoda, H., Wu, L.-J., Xu, H., Zhao, M.-G., & Ueda, K. (2008). Upregulation of calcium/calmodulin-dependent protein kinase IV improves memory formation and rescues memory loss with aging. *Journal of Neuroscience*, *28*(40), 9910-9919.

Garza-Lombó, C., & Gonsebatt, M. E. (2016). Mammalian target of rapamycin: its role in early neural development and in adult and aged brain function. *Frontiers in cellular neuroscience*, *10*, 157.

Gaudilliere, B., Shi, Y., & Bonni, A. (2002). RNA interference reveals a requirement for myocyte enhancer factor 2A in activity-dependent neuronal survival. *Journal of Biological Chemistry*, *277*(48), 46442-46446.

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*, *36*(8), 2628-2629. https://doi.org/10.1093/bioinformatics/btz931

Gerlai, R., Roder, J. C., & Hampson, D. R. (1998). Altered spatial learning and memory in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *Behavioral neuroscience*, *112*(3), 525.

Ghanem, N., Jarinova, O., Amores, A., Long, Q., Hatch, G., Park, B. K., Rubenstein, J. L., & Ekker, M. (2003). Regulatory roles of conserved intergenic domains in vertebrate Dlx bigene clusters. *Genome research*, *13*(4), 533-543.

Giamanco, K., Morawski, M., & Matthews, R. (2010). Perineuronal net formation and structure in aggrecan knockout mice. *Neuroscience*, *170*(4), 1314-1327.

Giovannini, M. G., & Lana, D. (2016). mTOR involvement in the mechanisms of memory: An overview of animal studies. *Molecules to Medicine with mTOR*, 169-184.

Gobes, S. M., Zandbergen, M. A., & Bolhuis, J. J. (2010). Memory in the making: localized brain activation related to song learning in young songbirds. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1698), 3343-3351.

Gogola, J. V., Gores, E. O., & London, S. E. (2019). Inhibitory cell populations depend on age, sex, and prior experience across a neural network for Critical Period learning. *Scientific Reports*, *9*(1), 19867.

Gong, X., Tang, X., Wiedmann, M., Wang, X., Peng, J., Zheng, D., Blair, L. A., Marshall, J., & Mao, Z. (2003). Cdk5-mediated inhibition of the protective effects of transcription factor MEF2 in neurotoxicity-induced apoptosis. *Neuron*, *38*(1), 33-46.

Govek, E.-E., Newey, S. E., & Van Aelst, L. (2005). The role of the Rho GTPases in neuronal development. *Genes & development*, *19*(1), 1-49.

Graber, T. E., McCamphill, P. K., & Sossin, W. S. (2013). A recollection of mTOR signaling in learning and memory. *Learning & memory*, *20*(10), 518-530.

GrandPré, T., Li, S., & Strittmatter, S. M. (2002). Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature*, *417*(6888), 547-551.

Gratton, M.-O., Torban, E., Jasmin, S. B., Theriault, F. M., German, M. S., & Stifani, S. (2003). Hes6 promotes cortical neurogenesis and inhibits Hes1 transcription repression activity by multiple mechanisms. *Molecular and cellular biology*, *23*(19), 6922-6935.

Griguoli, M., Sgritta, M., & Cherubini, E. (2016). Presynaptic BK channels control transmitter release: physiological relevance and potential therapeutic implications. *The Journal of physiology*, *594*(13), 3489-3500.

Gu, N., Vervaeke, K., & Storm, J. F. (2007). BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. *The Journal of physiology*, *580*(3), 859-882.

Gunaratne, P. H., Lin, Y.-C., Benham, A. L., Drnevich, J., Coarfa, C., Tennakoon, J. B., Creighton, C. J., Kim, J. H., Milosavljevic, A., & Watson, M. (2011). Song exposure regulates known and novel microRNAs in the zebra finch auditory forebrain. *BMC genomics*, *12*(1), 1-14.

Haesler, S., Rochefort, C., Georgi, B., Licznerski, P., Osten, P., & Scharff, C. (2007). Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus Area X. *PLoS biology*, *5*(12), e321.

Hagan, N., Guarente, J., Ellisor, D., & Zervas, M. (2017). The temporal contribution of the Gbx2 lineage to cerebellar neurons. *Frontiers in Neuroanatomy*, *11*, 50.

Hahnloser, R. H., Kozhevnikov, A. A., & Fee, M. S. (2002). An ultra-sparse code underliesthe generation of neural sequences in a songbird. *Nature*, *419*(6902), 65-70.

Hakanen, J., Parmentier, N., Sommacal, L., Garcia-Sanchez, D., Aittaleb, M., Vertommen, D., Zhou, L., Ruiz-Reig, N., & Tissir, F. (2022). The Celsr3-Kif2a axis directs neuronal migration in the postnatal brain. *Progress in neurobiology*, *208*, 102177.

Hamanaka, K., Imagawa, E., Koshimizu, E., Miyatake, S., Tohyama, J., Yamagata, T., Miyauchi, A., Ekhilevitch, N., Nakamura, F., & Kawashima, T. (2020). De novo truncating variants in the last exon of SEMA6B cause progressive myoclonic epilepsy. *The American Journal of Human Genetics*, *106*(4), 549-558.

Han, S. O., Kommaddi, R. P., & Shenoy, S. K. (2013). Distinct roles for β-arrestin2 and arrestin-domain-containing proteins in β2 adrenergic receptor trafficking. *EMBO reports*, *14*(2), 164-171.

Hansen, N., & Manahan-Vaughan, D. (2014). Dopamine D1/D5 receptors mediate informational saliency that promotes persistent hippocampal long-term plasticity. *Cerebral cortex*, *24*(4), 845-858.

Harris, C. J., Scheibe, M., Wongpalee, S. P., Liu, W., Cornett, E. M., Vaughan, R. M., Li, X., Chen, W., Xue, Y., Zhong, Z., Yen, L., Barshop, W. D., Rayatpisheh, S., Gallego-Bartolome, J., Groth, M., Wang, Z., Wohlschlegel, J. A., Du, J., Rothbart, S. B., . . . Jacobsen, S. E. (2018). A DNA methylation reader complex that enhances gene transcription. *Science*, *362*(6419), 1182-1186. https://doi.org/10.1126/science.aar7854

Harris, H. K., Nakayama, T., Lai, J., Zhao, B., Argyrou, N., Gubbels, C. S., Soucy, A., Genetti, C. A., Suslovitch, V., & Rodan, L. H. (2021). Disruption of RFX family transcription

factors causes autism, attention-deficit/hyperactivity disorder, intellectual disability, and dysregulated behavior. *Genetics in Medicine*, *23*(6), 1028-1040.

Harsing, L., Zsilla, G., Matyus, P., Nagy, K., Marko, B., Gyarmati, Z., & Timar, J. (2012). Interactions between glycine transporter type 1 (GlyT-1) and some inhibitor molecules—glycine transporter type 1 and its inhibitors. *Acta Physiologica Hungarica*, *99*(1), 1-17.

Harvey, R. J., & Yee, B. K. (2013). Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. *Nature reviews Drug discovery*, *12*(11), 866-885.

Hauber, M. E., Woolley, S. M., Cassey, P., & Theunissen, F. E. (2013). Experience dependence of neural responses to different classes of male songs in the primary auditory forebrain of female songbirds. *Behavioural brain research*, *243*, 184-190.

Hawk, J. D., Florian, C., & Abel, T. (2011). Post-training intrahippocampal inhibition of class I histone deacetylases enhances long-term object-location memory. *Learning & memory*, *18*(6), 367-370.

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes & development*, *18*(16), 1926-1945.

Heidenreich, K. A., & Linseman, D. A. (2004). Myocyte enhancer factor-2 transcription factors in neuronal differentiation and survival. *Molecular neurobiology*, *29*, 155-165.

Heintzman, N. D., Hon, G. C., Hawkins, R. D., Kheradpour, P., Stark, A., Harp, L. F., Ye, Z., Lee, L. K., Stuart, R. K., & Ching, C. W. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*, *459*(7243), 108-112.

Heintzman, N. D., Stuart, R. K., Hon, G., Fu, Y., Ching, C. W., Hawkins, R. D., Barrera, L. O., Van Calcar, S., Qu, C., Ching, K. A., Wang, W., Weng, Z., Green, R. D., Crawford, G. E., & Ren, B. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet*, *39*(3), 311-318. https://doi.org/10.1038/ng1966

Hemler, M. E. (2003). Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annual review of cell and developmental biology*, *19*(1), 397-422.

Herb, A., Wisden, W., Catania, M. V., Maréchal, D., Dresse, A., & Seeburg, P. H. (1997). Prominent dendritic localization in forebrain neurons of a novel mRNA and its product, dendrin. *Molecular and Cellular Neuroscience*, *8*(5), 367-374.

Herdegen, T. (1996). REVIEW■: Jun, Fos, and CREB/ATF Transcription Factors in the Brain: Control of Gene Expression under Normal and Pathophysiological Conditions. *The Neuroscientist*, *2*(3), 153-161.

Herdegen, T., & Waetzig, V. (2001). AP-1 proteins in the adult brain: facts and fiction about effectors of neuroprotection and neurodegeneration. *Oncogene*, *20*(19), 2424-2437.

Herzog, R., Hellenbroich, Y., Brüggemann, N., Lohmann, K., Grimmel, M., Haack, T. B., von Spiczak, S., & Münchau, A. (2021). Zonisamide-responsive myoclonus in SEMA6B-associated progressive myoclonic epilepsy. *Annals of Clinical and Translational Neurology*, *8*(7), 1524-1527.

Hess, E. H. (1959). Imprinting. *Science*, *130*(3377), 733. https://doi.org/10.1126/science.130.3377.733

Heston, J. B., & White, S. A. (2017). To transduce a zebra finch: interrogating behavioral mechanisms in a model system for speech. *Journal of Comparative Physiology A*, *203*, 691-706.

Hida, Y., Fukaya, M., Hagiwara, A., Deguchi-Tawarada, M., Yoshioka, T., Kitajima, I., Inoue, E., Watanabe, M., & Ohtsuka, T. (2011). Prickle2 is localized in the postsynaptic density and interacts with PSD-95 and NMDA receptors in the brain. *The journal of biochemistry*, *149*(6), 693-700.

Ho, N., Liauw, J. A., Blaeser, F., Wei, F., Hanissian, S., Muglia, L. M., Wozniak, D. F., Nardi, A., Arvin, K. L., & Holtzman, D. M. (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. *Journal of Neuroscience*, *20*(17), 6459-6472.

Hoeffer, C. A., & Klann, E. (2010). mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in neurosciences*, *33*(2), 67-75.

Holveck, M.-J., & Riebel, K. (2014). Female zebra finches learn to prefer more than one song and from more than one tutor. *Animal Behaviour*, *88*, 125-135.

Honer, W. G., Hu, L., & Davies, P. (1993). Human synaptic proteins with a heterogeneous distribution in cerebellum and visual cortex. *Brain research*, *609*(1-2), 9-20.

Hosaka, Y., Yokota, T., Miyagoe-Suzuki, Y., Yuasa, K., Imamura, M., Matsuda, R., Ikemoto, T., Kameya, S., & Takeda, S. i. (2002). α1-Syntrophin–deficient skeletal muscle exhibits hypertrophy and aberrant formation of neuromuscular junctions during regeneration. *The Journal of cell biology*, *158*(6), 1097-1107.

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: A review of animal models and human studies across development. *Psychological bulletin*, *140*(1), 256.

Hu, H., Shao, L.-R., Chavoshy, S., Gu, N., Trieb, M., Behrens, R., Laake, P., Pongs, O., Knaus, H. G., & Ottersen, O. P. (2001). Presynaptic Ca2+-activated K+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *Journal of Neuroscience*, *21*(24), 9585-9597.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annual review of neuroscience*, *24*(1), 677-736.

Hubel, D. H., & Wiesel, T. N. (1964). Effects of Monocular Deprivation in Kittens. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol*, *248*, 492-497. https://doi.org/10.1007/BF00348878

Huisinga, K. L., Brower-Toland, B., & Elgin, S. C. (2006). The contradictory definitions of heterochromatin: transcription and silencing. *Chromosoma*, *115*(2), 110-122. https://doi.org/10.1007/s00412-006-0052-x

Ieguchi, K., Fujita, M., Ma, Z., Davari, P., Taniguchi, Y., Sekiguchi, K., Wang, B., Takada, Y. K., & Takada, Y. (2010). Direct binding of the EGF-like domain of neuregulin-1 to integrins (αvβ3 and α6β4) is involved in neuregulin-1/ErbB signaling. *Journal of Biological Chemistry*, *285*(41), 31388-31398.

Ignatieva, E. V., Levitsky, V. G., & Kolchanov, N. A. (2015). Human genes encoding transcription factors and chromatin-modifying proteins have low levels of promoter polymorphism: a study of 1000 genomes project data. *International journal of genomics*, *2015*.

Ikeshimaa, H., Imai, S.-i., Shimoda, K., Hata, J.-i., & Takano, T. (1995). Expression of a MADS box gene, MEF2D, in neurons of the mouse central nervous system: implication of its binary function in myogenic and neurogenic cell lineages. *Neuroscience letters*, *200*(2), 117-120.

Immelmann, K. (1969). Bird vocalizations. *(No Title)*, 61.

Itoh, Y., Melamed, E., Yang, X., Kampf, K., Wang, S., Yehya, N., Van Nas, A., Replogle, K., Band, M. R., & Clayton, D. F. (2007). Dosage compensation is less effective in birds than in mammals. *Journal of biology*, *6*(1), 1-15.

Itoh, Y., Replogle, K., Kim, Y.-H., Wade, J., Clayton, D. F., & Arnold, A. P. (2010). Sex bias and dosage compensation in the zebra finch versus chicken genomes: general and specialized patterns among birds. *Genome research*, *20*(4), 512-518.

Jabaudon, D., J. Shnider, S., J. Tischfield, D., J. Galazo, M., & Macklis, J. D. (2012). ROR β induces barrel-like neuronal clusters in the developing neocortex. *Cerebral cortex*, *22*(5), 996-1006.

Jansen, S., Collins, A., Yang, C., Rebowski, G., Svitkina, T., & Dominguez, R. (2011). Mechanism of actin filament bundling by fascin. *Journal of Biological Chemistry*, *286*(34), 30087-30096.

Jarvis, E. D., & Nottebohm, F. (1997). Motor-driven gene expression. *Proceedings of the National Academy of Sciences*, *94*(8), 4097-4102.

Jeong, J. K., Burrows, K., Tremere, L. A., & Pinaud, R. (2011). Neurochemical organization and experience-dependent activation of estrogen-associated circuits in the songbird auditory forebrain. *European Journal of Neuroscience*, *34*(2), 283-291.

Jhas, S., Ciura, S., Belanger-Jasmin, S., Dong, Z., Llamosas, E., Theriault, F. M., Joachim, K., Tang, Y., Liu, L., & Liu, J. (2006). Hes6 inhibits astrocyte differentiation and promotes neurogenesis through different mechanisms. *Journal of Neuroscience*, *26*(43), 11061-11071.

Ji, F., Wang, W., Feng, C., Gao, F., & Jiao, J. (2021). Brain-specific Wt1 deletion leads to depressive-like behaviors in mice via the recruitment of Tet2 to modulate Epo expression. *Molecular psychiatry*, *26*(8), 4221-4233.

Ji, R., Shi, Q., Cao, Y., Zhang, J., Zhao, C., Zhao, H., Sayyed, Y., Fu, L., & Li, L.-Y. (2022). Alternative splicing of the human rhomboid family-1 gene RHBDF1 inhibits epidermal growth factor receptor activation. *Journal of Biological Chemistry*, *298*(6).

Jia, C., Brown, R. W., Malone, H. M., Burgess, K. C., Gill, W. D., Keasey, M. P., & Hagg, T. (2019). Ciliary neurotrophic factor is a key sex-specific regulator of depressive-like behavior in mice. *Psychoneuroendocrinology*, *100*, 96-105.

Jia, L., Oh, E. C., Ng, L., Srinivas, M., Brooks, M., Swaroop, A., & Forrest, D. (2009). Retinoid-related orphan nuclear receptor RORβ is an early-acting factor in rod photoreceptor development. *Proceedings of the National Academy of Sciences*, *106*(41), 17534-17539.

Jin, H., & Clayton, D. F. (1997). Localized changes in immediate-early gene regulation during sensory and motor learning in zebra finches. *Neuron*, *19*(5), 1049-1059.

Jin, W., Sugaya, A., Tsuda, T., Ohguchi, H., & Sugaya, E. (2000). Relationship between large conductance calcium-activated potassium channel and bursting activity. *Brain research*, *860*(1-2), 21-28.

Jo, A.-Y., Kim, M.-Y., Lee, H.-S., Rhee, Y.-H., Lee, J.-E., Baek, K.-H., Park, C.-H., Koh, H.-C., Shin, I., & Lee, Y.-S. (2009). Generation of dopamine neurons with improved cell survival and phenotype maintenance using a degradation-resistant nurr1 mutant. *Stem Cells*, *27*(9), 2238-2246.

John, S., Sabo, P. J., Thurman, R. E., Sung, M.-H., Biddie, S. C., Johnson, T. A., Hager, G. L., & Stamatoyannopoulos, J. A. (2011). Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nature genetics*, *43*(3), 264-268.

Johnson, F., Hohmann, S. E., DiStefano, P. S., & Bottjer, S. W. (1997). Neurotrophins suppress apoptosis induced by deafferentation of an avian motor-cortical region. *Journal of Neuroscience*, *17*(6), 2101-2111.

Jones, M., Errington, M. L., French, P. J., Fine, A., Bliss, T. V., Garel, S., Charnay, P., Bozon, B., Laroche, S., & Davis, S. (2001). A requirement for the immediate early gene Zif268

in the expression of late LTP and long-term memories. *Nature neuroscience*, *4*(3), 289-296.

Joseph, D. J., Von Deimling, M., Hasegawa, Y., Cristancho, A. G., Ahrens-Nicklas, R. C., Rogers, S. L., Risbud, R., McCoy, A. J., & Marsh, E. D. (2021). Postnatal Arx transcriptional activity regulates functional properties of PV interneurons. *iScience*, *24*(1), 101999. https://doi.org/10.1016/j.isci.2020.101999

Kadakkuzha, B. M., Liu, X.-A., McCrate, J., Shankar, G., Rizzo, V., Afinogenova, A., Young, B., Fallahi, M., Carvalloza, A. C., & Raveendra, B. (2015). Transcriptome analyses of adult mouse brain reveal enrichment of lncRNAs in specific brain regions and neuronal populations. *Frontiers in cellular neuroscience*, *9*, 63.

Kadkhodaei, B., Ito, T., Joodmardi, E., Mattsson, B., Rouillard, C., Carta, M., Muramatsu, S.-I., Sumi-Ichinose, C., Nomura, T., & Metzger, D. (2009). Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. *Journal of Neuroscience*, *29*(50), 15923-15932.

Kandel, E. R. (2001). The molecular biology of memory storage: a dialog between genes and synapses. *Biosci Rep*, *21*(5), 565-611. https://doi.org/10.1023/a:1014775008533

Karten, H. J. (1968). The ascending auditory pathway in the pigeon (Columba livia) II. Telencephalic projections of the nucleus ovoidalis thalami. *Brain research*, *11*(1), 134-153.

Kawata, A., Iida, J., Ikeda, M., Sato, Y., Mori, H., Kansaku, A., Sumita, K., Fujiwara, N., Rokukawa, C., & Hamano, M. (2006). CIN85 is localized at synapses and forms a complex with S-SCAM via dendrin. *Journal of biochemistry*, *139*(5), 931-939.

Kazdoba, T., Leach, P., & Crawley, J. (2016). Behavioral phenotypes of genetic mouse models of autism. *Genes, Brain and Behavior*, *15*(1), 7-26.

Kellis, M., Wold, B., Snyder, M. P., Bernstein, B. E., Kundaje, A., Marinov, G. K., Ward, L. D., Birney, E., Crawford, G. E., & Dekker, J. (2014). Defining functional DNA elements in the human genome. *Proceedings of the National Academy of Sciences*, *111*(17), 6131-6138.

Kelly, T. K., Ahmadiantehrani, S., Blattler, A., & London, S. E. (2018). Epigenetic regulation of transcriptional plasticity associated with developmental song learning. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1878), 20180160.

Kim, S., & Kaang, B. K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. *Exp Mol Med*, *49*(1), e281. https://doi.org/10.1038/emm.2016.140

Kimpo, R. R., & Doupe, A. J. (1997). FOS is induced by singing in distinct neuronal populations in a motor network. *Neuron*, *18*(2), 315-325.

Kirn, J. R., & DeVoogd, T. J. (1989). Genesis and death of vocal control neurons during sexual differentiation in the zebra finch. *Journal of Neuroscience*, *9*(9), 3176-3187.

Kitamura, K., Itou, Y., Yanazawa, M., Ohsawa, M., Suzuki-Migishima, R., Umeki, Y., Hohjoh, H., Yanagawa, Y., Shinba, T., & Itoh, M. (2009). Three human ARX mutations cause the lissencephaly-like and mental retardation with epilepsy-like pleiotropic phenotypes in mice. *Human molecular genetics*, *18*(19), 3708-3724.

Kitamura, K., Yanazawa, M., Sugiyama, N., Miura, H., Iizuka-Kogo, A., Kusaka, M., Omichi, K., Suzuki, R., Kato-Fukui, Y., & Kamiirisa, K. (2002). Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nature genetics*, *32*(3), 359-369.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., & Barbacid, M. (1991). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell*, *66*(2), 395-403.

Klose, R. J., Sarraf, S. A., Schmiedeberg, L., McDermott, S. M., Stancheva, I., & Bird, A. P. (2005). DNA binding selectivity of MeCP2 due to a requirement for A/T sequences adjacent to methyl-CpG. *Mol Cell*, *19*(5), 667-678. https://doi.org/10.1016/j.molcel.2005.07.021

Knapska, E., & Kaczmarek, L. (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Progress in neurobiology*, *74*(4), 183-211.

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *J Cogn Neurosci*, *16*(8), 1412-1425. https://doi.org/10.1162/0898929042304796

Koemeter-Cox, A. I., Sherwood, T. W., Green, J. A., Steiner, R. A., Berbari, N. F., Yoder, B. K., Kauffman, A. S., Monsma, P. C., Brown, A., & Askwith, C. C. (2014). Primary cilia enhance kisspeptin receptor signaling on gonadotropin-releasing hormone neurons. *Proceedings of the National Academy of Sciences*, *111*(28), 10335-10340.

Kokubo, M., Nishio, M., Ribar, T. J., Anderson, K. A., West, A. E., & Means, A. R. (2009). BDNF-mediated cerebellar granule cell development is impaired in mice null for CaMKK2 or CaMKIV. *Journal of Neuroscience*, *29*(28), 8901-8913.

Kondoh, H., & Kamachi, Y. (2016). Sox2–partner factor interactions and enhancer regulation. In *Sox2* (pp. 131-144). Elsevier.

Konishi, M. (1965). The role of auditory feedback in the control of vocalization in the white-crowned sparrow 1. *Zeitschrift für Tierpsychologie*, *22*(7), 770-783.

Konishi, M., & Akutagawa, E. (1985). Neuronal growth, atrophy and death in a sexually dimorphic song nucleus in the zebra finch brain. *Nature*, *315*, 145-147.

Konishi, M., & Akutagawa, E. (2007). Hormonal control of cell death in a sexually dimorphic song nucleus in the zebra finch. Ciba Foundation Symposium 126-Selective Neuronal Death: Selective Neuronal Death: Ciba Foundation Symposium 126,

Konno, D., Iwashita, M., Satoh, Y., Momiyama, A., Abe, T., Kiyonari, H., & Matsuzaki, F. (2012). The mammalian DM domain transcription factor Dmrta2 is required for early embryonic development of the cerebral cortex.

Korosi, A., Naninck, E., Oomen, C., Schouten, M., Krugers, H., Fitzsimons, C., & Lucassen, P. (2012). Early-life stress mediated modulation of adult neurogenesis and behavior. *Behavioural brain research*, *227*(2), 400-409.

Kouwenhoven, W. M., Veenvliet, J. V., van Hooft, J. A., Van Der Heide, L., & Smidt, M. P. (2016). Engrailed 1 shapes the dopaminergic and serotonergic landscape through proper isthmic organizer maintenance and function. *Biology open*, *5*(3), 279-288.

Koyano-Nakagawa, N., Kim, J., Anderson, D., & Kintner, C. (2000). Hes6 acts in a positive feedback loop with the neurogenins to promote neuronal differentiation. *Development*, *127*(19), 4203-4216.

Krebs, J. (1998). Calmodulin-dependent protein kinase IV: regulation of function and expression. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1448*(2), 183-189.

Kremerskothen, J., Kindler, S., Finger, I., Veltel, S., & Barnekow, A. (2006). Postsynaptic recruitment of Dendrin depends on both dendritic mRNA transport and synaptic anchoring. *Journal of neurochemistry*, *96*(6), 1659-1666.

Krentzel, A. A., & Remage-Healey, L. (2015). Sex differences and rapid estrogen signaling: a look at songbird audition. *Frontiers in neuroendocrinology*, *38*, 37-49.

Krishnaswamy, A., Yamagata, M., Duan, X., Hong, Y. K., & Sanes, J. R. (2015). Sidekick 2 directs formation of a retinal circuit that detects differential motion. *Nature*, *524*(7566), 466-470.

Krueger, F. (2012). *Trim Galore: A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files*. In http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/

Kustanovich, V., Ishii, J., Crawford, L., Yang, M., McGough, J., McCracken, J., Smalley, S., & Nelson, S. (2004). Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. *Molecular psychiatry*, *9*(7), 711-717.

Kwok, J. C., Carulli, D., & Fawcett, J. W. (2010). In vitro modeling of perineuronal nets: hyaluronan synthase and link protein are necessary for their formation and integrity. *Journal of neurochemistry*, *114*(5), 1447-1459.

Lam, B. Y. H., & Chawla, S. (2007). MEF2D expression increases during neuronal differentiation of neural progenitor cells and correlates with neurite length. *Neuroscience letters*, *427*(3), 153-158.

Lana, D., Di Russo, J., Mello, T., Wenk, G., & Giovannini, M. (2017). Rapamycin inhibits mTOR/p70S6K activation in CA3 region of the hippocampus of the rat and impairs long term memory. *Neurobiology of learning and memory*, *137*, 15-26.

Landskron, L., Bak, J., Adamopoulos, A., Kaplani, K., Moraiti, M., van den Hengel, L. G., Song, J.-Y., Bleijerveld, O. B., Nieuwenhuis, J., & Heidebrecht, T. (2022). Posttranslational modification of microtubules by the MATCAP detyrosinase. *Science*, *376*(6595), eabn6020.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods*, *9*(4), 357-359. https://doi.org/10.1038/nmeth.1923

Lauay, C., Gerlach, N. M., Adkins-Regan, E., & DeVoogd, T. J. (2004). Female zebra finches require early song exposure to prefer high-quality song as adults. *Animal Behaviour*, *68*(6), 1249-1255.

Le, W.-d., Conneely, O. M., Zou, L., He, Y., Saucedo-Cardenas, O., Jankovic, J., Mosier, D. R., & Appel, S. H. (1999). Selective agenesis of mesencephalic dopaminergic neurons in Nurr1-deficient mice. *Experimental neurology*, *159*(2), 451-458.

Ledger, M. L., Kaare, M., Mailo, J. A., & Jain-Ghai, S. (2023). Phenotype expansion and neurological manifestations of neurobehavioural disease caused by a variant in RFX7. *European Journal of Medical Genetics*, *66*(1), 104657.

Lee, K., Ireland, K., Bleeze, M., & Shoubridge, C. (2017). ARX polyalanine expansion mutations lead to migration impediment in the rostral cortex coupled with a developmental deficit of calbindin-positive cortical GABAergic interneurons. *Neuroscience*, *357*, 220-231.

Lee, V., Pawlisch, B. A., Macedo-Lima, M., & Remage-Healey, L. (2018). Norepinephrine enhances song responsiveness and encoding in the auditory forebrain of male zebra finches. *Journal of neurophysiology*, *119*(1), 209-220.

Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., & Byrnes, E. J. (2007). Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, *445*(7124), 168-176.

Lemeille, S., Paschaki, M., Baas, D., Morlé, L., Duteyrat, J.-L., Ait-Lounis, A., Barras, E., Soulavie, F., Jerber, J., & Thomas, J. (2020). Interplay of RFX transcription factors 1, 2 and 3 in motile ciliogenesis. *Nucleic acids research*, *48*(16), 9019-9036.

Lencz, T., Knowles, E., Davies, G., Guha, S., Liewald, D. C., Starr, J. M., Djurovic, S., Melle, I., Sundet, K., & Christoforou, A. (2014). Molecular genetic evidence for overlap between

general cognitive ability and risk for schizophrenia: a report from the Cognitive Genomics consorTium (COGENT). *Molecular psychiatry*, *19*(2), 168-174.

Lensjø, K. K., Lepperød, M. E., Dick, G., Hafting, T., & Fyhn, M. (2017). Removal of perineuronal nets unlocks juvenile plasticity through network mechanisms of decreased inhibition and increased gamma activity. *Journal of Neuroscience*, *37*(5), 1269-1283.

Leonardo, A., & Fee, M. S. (2005). Ensemble coding of vocal control in birdsong. *Journal of Neuroscience*, *25*(3), 652-661.

Lepicard, E. M., Mizuno, K., Antunes-Martins, A., Von Hertzen, L. S., & Giese, K. P. (2006). An endogenous inhibitor of calcium/calmodulin-dependent kinase II is up-regulated during consolidation of fear memory. *European Journal of Neuroscience*, *23*(11), 3063-3070.

Li, C., Brake, W. G., Romeo, R. D., Dunlop, J. C., Gordon, M., Buzescu, R., Magarinos, A. M., Allen, P. B., Greengard, P., & Luine, V. (2004). Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. *Proceedings of the National Academy of Sciences*, *101*(7), 2185-2190.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & Genome Project Data Processing, S. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078-2079. https://doi.org/10.1093/bioinformatics/btp352

Li, H., Radford, J. C., Ragusa, M. J., Shea, K. L., McKercher, S. R., Zaremba, J. D., Soussou, W., Nie, Z., Kang, Y.-J., & Nakanishi, N. (2008). Transcription factor MEF2C influences neural stem/progenitor cell differentiation and maturation in vivo. *Proceedings of the National Academy of Sciences*, *105*(27), 9397-9402.

Li, X.-C., Jarvis, E. D., Alvarez-Borda, B., Lim, D. A., & Nottebohm, F. (2000). A relationship between behavior, neurotrophin expression, and new neuron survival. *Proceedings of the National Academy of Sciences*, *97*(15), 8584-8589.

Li, Z., Massengill, J., O'Dowd, D., & Smith, M. (1997). Agrin gene expression in mouse somatosensory cortical neurons during development in vivo and in cell culture. *Neuroscience*, *79*(1), 191-201.

Liang, F., Ren, C., Wang, J., Wang, S., Yang, L., Han, X., Chen, Y., Tong, G., & Yang, G. (2019). The crosstalk between STAT3 and p53/RAS signaling controls cancer cell metastasis and cisplatin resistance via the Slug/MAPK/PI3K/AKT-mediated regulation of EMT and autophagy. *Oncogenesis*, *8*(10), 59.

Lin, X., Shah, S., & Bulleit, R. F. (1996). The expression of MEF2 genes is implicated in CNS neuronal differentiation. *Molecular Brain Research*, *42*(2), 307-316.

Lisek, M., Przybyszewski, O., Zylinska, L., Guo, F., & Boczek, T. (2023). The Role of MEF2 Transcription Factor Family in Neuronal Survival and Degeneration. *International Journal of Molecular Sciences*, *24*(4), 3120.

Liu, A., & Joyner, A. L. (2001). EN and GBX2 play essential roles downstream of FGF8 in patterning the mouse mid/hindbrain region. *Development*, *128*(2), 181-191.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., & Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, *277*(5332), 1659-1662. https://doi.org/10.1126/science.277.5332.1659

Liu, F., Day, M., Muniz, L. C., Bitran, D., Arias, R., Revilla-Sanchez, R., Grauer, S., Zhang, G., Kelley, C., & Pulito, V. (2008). Activation of estrogen receptor-β regulates hippocampal synaptic plasticity and improves memory. *Nature neuroscience*, *11*(3), 334-343.

Liu, H., Kim, S.-Y., Fu, Y., Wu, X., Ng, L., Swaroop, A., & Forrest, D. (2013). An isoform of retinoid-related orphan receptor β directs differentiation of retinal amacrine and horizontal interneurons. *Nature communications*, *4*(1), 1813.

Liu, L., Zang, J., Chen, X., Yang, G., Zhu, Y., Wu, Y., & Li, T. (2016a). Role of miR-124 and miR-141 in the regulation of vascular reactivity and the relationship to RhoA and Rac1 after hemorrhage and hypoxia. *American Journal of Physiology-Heart and Circulatory Physiology*, *310*(2), H206-H216.

Liu, X.-B., & Jones, E. G. (1996). Localization of alpha type II calcium calmodulin-dependent protein kinase at glutamatergic but not gamma-aminobutyric acid (GABAergic) synapses in thalamus and cerebral cortex. *Proceedings of the National Academy of Sciences*, *93*(14), 7332-7336.

Liu, X. B., & Murray, K. D. (2012). Neuronal excitability and calcium/calmodulin-dependent protein kinase type II: location, location, location. *Epilepsia*, *53*, 45-52.

Liu, Y., Ford, B., Mann, M. A., & Fischbach, G. D. (2001). Neuregulins increase α7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus. *Journal of Neuroscience*, *21*(15), 5660-5669.

Liu, Z.-D., Zhang, S., Hao, J.-J., Xie, T.-R., & Kang, J.-S. (2016b). Cellular model of neuronal atrophy induced by DYNC1I1 deficiency reveals protective roles of RAS-RAF-MEK signaling. *Protein & cell*, *7*(9), 638-650.

London, S. E. (2019). Developmental song learning as a model to understand neural mechanisms that limit and promote the ability to learn. *Behavioural Processes*, *163*, 13-23.

London, S. E., Boulter, J., & Schlinger, B. A. (2003). Cloning of the zebra finch androgen synthetic enzyme CYP17: a study of its neural expression throughout posthatch development. *Journal of Comparative Neurology*, *467*(4), 496-508.

London, S. E., & Clayton, D. F. (2008). Functional identification of sensory mechanisms required for developmental song learning. *Nat Neurosci*, *11*(5), 579-586. https://doi.org/10.1038/nn.2103

London, S. E., Dong, S., Replogle, K., & Clayton, D. F. (2009). Developmental shifts in gene expression in the auditory forebrain during the sensitive period for song learning. *Developmental neurobiology*, *69*(7), 437-450.

Long, A., Crouse, A., Kesterson, R. A., Might, M., & Wallis, D. (2022). Functional characterization and potential therapeutic avenues for variants in the NTRK2 gene causing developmental and epileptic encephalopathies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *189*(1-2), 37-47.

Lorenzo, P. I., Juárez-Vicente, F., Cobo-Vuilleumier, N., García-Domínguez, M., & Gauthier, B. R. (2017). The diabetes-linked transcription factor PAX4: from gene to functional consequences. *Genes*, *8*(3), 101.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Lowe, N., Kirley, A., Hawi, Z., Sham, P., Wickham, H., Kratochvil, C. J., Smith, S. D., Lee, S. Y., Levy, F., & Kent, L. (2004). Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes. *The American Journal of Human Genetics*, *74*(2), 348-356.

Luger, K., Dechassa, M. L., & Tremethick, D. J. (2012). New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat Rev Mol Cell Biol*, *13*(7), 436-447. https://doi.org/10.1038/nrm3382

Lyons, G. E., Micales, B., Schwarz, J., Martin, J., & Olson, E. (1995). Expression of mef2 genes in the mouse central nervous system suggests a role in neuronal maturation. *Journal of Neuroscience*, *15*(8), 5727-5738.

Ma, J., Hu, Z., Yue, H., Luo, Y., Wang, C., Wu, X., Gu, Y., & Wang, L. (2023). GRM2 regulates functional integration of adult-born DGCs by paradoxically modulating MEK/ERK1/2 pathway. *Journal of Neuroscience*, *43*(16), 2822-2836.

Macdougall-Shackleton, S. A. (1997). Sexual selection and the evolution of song repertoires. *Current ornithology*, 81-124.

Maddox, S. A., & Schafe, G. E. (2011). Epigenetic alterations in the lateral amygdala are required for reconsolidation of a Pavlovian fear memory. *Learn Mem*, *18*(9), 579-593. https://doi.org/10.1101/lm.2243411

Majumder, P., Chanda, K., Das, D., Singh, B. K., Chakrabarti, P., Jana, N. R., & Mukhopadhyay, D. (2021). A nexus of miR-1271, PAX4 and ALK/RYK influences the cytoskeletal

architectures in Alzheimer's Disease and Type 2 Diabetes. *Biochemical Journal*, *478*(17), 3297-3317.

Mann, N., & Slater, P. (1995). Song tutor choice by zebra finches in aviaries. *Animal Behaviour*, *49*(3), 811-820.

Mao, Z., Bonni, A., Xia, F., Nadal-Vicens, M., & Greenberg, M. E. (1999). Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science*, *286*(5440), 785-790.

Marcello, E., Saraceno, C., Musardo, S., Vara, H., de La Fuente, A. G., Pelucchi, S., Di Marino, D., Borroni, B., Tramontano, A., & Pérez-Otaño, I. (2013). Endocytosis of synaptic ADAM10 in neuronal plasticity and Alzheimer's disease. *The Journal of clinical investigation*, *123*(6), 2523-2538.

Margoliash, D. (1986). Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. *Journal of Neuroscience*, *6*(6), 1643-1661.

Margoliash, D., & Konishi, M. (1985). Auditory representation of autogenous song in the song system of white-crowned sparrows. *Proceedings of the National Academy of Sciences*, *82*(17), 5997-6000.

Mariottini, C., Munari, L., Gunzel, E., Seco, J. M., Tzavaras, N., Hansen, J., Stern, S. A., Gao, V., Aleyasin, H., & Sharma, A. (2019). Wilm's tumor 1 promotes memory flexibility. *Nature communications*, *10*(1), 3756.

Marler, P. (1970). A comparative approach to vocal learning: song development in white-crowned sparrows. *Journal of comparative and physiological psychology*, *71*(2p2), 1.

Maroulakou, I. G., & Bowe, D. B. (2000). Expression and function of Ets transcription factors in mammalian development: a regulatory network. *Oncogene*, *19*(55), 6432-6442.

Martin, L. (2018). Characterization of the roles of neuronal cholesterol biosynthesis and ORP4 in hippocampal neurons and glia.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12.

Maston, G. A., Evans, S. K., & Green, M. R. (2006). Transcriptional regulatory elements in the human genome. *Annu Rev Genomics Hum Genet*, *7*, 29-59. https://doi.org/10.1146/annurev.genom.7.080505.115623

Matsuya, S., Sakagami, H., Tohgo, A., Owada, Y., Shin, H. W., Takeshima, H., Nakayama, K., Kokubun, S., & Kondo, H. (2005). Cellular and subcellular localization of EFA6C, a third member of the EFA6 family, in adult mouse Purkinje cells. *Journal of neurochemistry*, *93*(3), 674-685.

Matthews, E. A., Linardakis, J. M., & Disterhoft, J. F. (2009). The fast and slow afterhyperpolarizations are differentially modulated in hippocampal neurons by aging and learning. *Journal of Neuroscience*, *29*(15), 4750-4755.

Matthews, R. T., Kelly, G. M., Zerillo, C. A., Gray, G., Tiemeyer, M., & Hockfield, S. (2002). Aggrecan glycoforms contribute to the molecular heterogeneity of perineuronal nets. *Journal of Neuroscience*, *22*(17), 7536-7547.

McCombie, W. R., McPherson, J. D., & Mardis, E. R. (2019). Next-generation sequencing technologies. *Cold Spring Harbor perspectives in medicine*, *9*(11), a036798.

McCroskery, S., Bailey, A., Lin, L., & Daniels, M. P. (2009). Transmembrane agrin regulates dendritic filopodia and synapse formation in mature hippocampal neuron cultures. *Neuroscience*, *163*(1), 168-179.

McCrossan, Z. A., Lewis, A., Panaghie, G., Jordan, P. N., Christini, D. J., Lerner, D. J., & Abbott, G. W. (2003). MinK-related peptide 2 modulates Kv2. 1 and Kv3. 1 potassium channels in mammalian brain. *Journal of Neuroscience*, *23*(22), 8077-8091.

McLean, C. Y., Bristor, D., Hiller, M., Clarke, S. L., Schaar, B. T., Lowe, C. B., Wenger, A. M., & Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology*, *28*(5), 495-501.

McNulty, S. E., Barrett, R. M., Vogel-Ciernia, A., Malvaez, M., Hernandez, N., Davatolhagh, M. F., Matheos, D. P., Schiffman, A., & Wood, M. A. (2012). Differential roles for Nr4a1 and Nr4a2 in object location vs. object recognition long-term memory. *Learning & memory*, *19*(12), 588-592.

McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., Alenghat, T., Mullican, S. E., Jones, S., Rusche, J. R., & Lazar, M. A. (2011). HDAC3 is a critical negative regulator of long-term memory formation. *Journal of Neuroscience*, *31*(2), 764-774.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science*, *239*(4841 Pt 1), 766-768. https://doi.org/10.1126/science.3340858

Mello, C., Nottebohm, F., & Clayton, D. (1995). Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. *Journal of Neuroscience*, *15*(10), 6919-6925.

Mello, C., Pinaud, R., & Ribeiro, S. (1998). Noradrenergic system of the zebra finch brain: Immunocytochemical study of dopamine-β-hydroxylase. *Journal of Comparative Neurology*, *400*(2), 207-228.

Mello, C. V., & Clayton, D. F. (1994). Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. *Journal of Neuroscience*, *14*(11), 6652-6666.

Mello, C. V., Velho, T. A., & Pinaud, R. (2004). Song-induced gene expression: a window on song auditory processing and perception. *Annals of the New York Academy of Sciences*, *1016*(1), 263-281.

Mello, C. V., Vicario, D. S., & Clayton, D. F. (1992). Song presentation induces gene expression in the songbird forebrain. *Proceedings of the National Academy of Sciences*, *89*(15), 6818-6822.

Mercer, T. R., Dinger, M. E., Sunkin, S. M., Mehler, M. F., & Mattick, J. S. (2008). Specific expression of long noncoding RNAs in the mouse brain. *Proceedings of the National Academy of Sciences*, *105*(2), 716-721.

Merkulova, T., Ananko, E., Ignatieva, E., & Kolchanov, N. (2013). Transcription regulatory codes of eukaryotic genomes. *Russian Journal of Genetics*, *49*, 29-45.

Meyer, C. E., Boroda, E., & Nick, T. A. (2014). Sexually dimorphic perineuronal net expression in the songbird. *Basal Ganglia*, *3*(4), 229-237.

Miao, C., Du, L., Zhang, Y., Jia, F., & Shan, L. (2023). Novel de novo ZNF148 truncating variant causing autism spectrum disorder, attention deficit hyperactivity disorder, and intellectual disability. *Clinical Genetics*, *103*(3), 364-368.

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron*, *53*(6), 857-869. https://doi.org/10.1016/j.neuron.2007.02.022

Miller, D. B. (1979a). The acoustic basis of mate recognition by female zebra finches (Taeniopygia guttata). *Animal Behaviour*, *27*, 376-380.

Miller, D. B. (1979b). Long-term recognition of father's song by female zebra finches. *Nature*, *280*(5721), 389-391.

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. *Frontiers in cellular neuroscience*, 363.

Mochida, G. H., Ganesh, V. S., Felie, J. M., Gleason, D., Hill, R. S., Clapham, K. R., Rakiec, D., Tan, W.-H., Akawi, N., & Al-Saffar, M. (2010). A homozygous mutation in the tight-junction protein JAM3 causes hemorrhagic destruction of the brain, subependymal calcification, and congenital cataracts. *The American Journal of Human Genetics*, *87*(6), 882-889.

Mooney, R., & Rao, M. (1994). Waiting periods versus early innervation: the development of axonal connections in the zebra finch song system. *Journal of Neuroscience*, *14*(11), 6532-6543.

Moore, A. K., Weible, A. P., Balmer, T. S., Trussell, L. O., & Wehr, M. (2018). Rapid Rebalancing of Excitation and Inhibition by Cortical Circuitry. *Neuron*, *97*(6), 1341-1355.e1346. https://doi.org/10.1016/j.neuron.2018.01.045

Mori, C., & Wada, K. (2015). Audition-independent vocal crystallization associated with intrinsic developmental gene expression dynamics. *Journal of Neuroscience*, *35*(3), 878-889.

Morris, J. A., Jordan, C. L., & Breedlove, S. M. (2004). Sexual differentiation of the vertebrate nervous system. *Nature neuroscience*, *7*(10), 1034-1039.

Morrison, R. G., & Nottebohm, F. (1993). Role of a telencephalic nucleus in the delayed song learning of socially isolated zebra finches. *Journal of neurobiology*, *24*(8), 1045-1064.

Murai, K., Philpott, A., & Jones, P. H. (2011). Hes6 is required for the neurogenic activity of neurogenin and NeuroD. *PloS one*, *6*(11), e27880.

Myers, K. R., Lo, K. W. H., Lye, R. J., Kogoy, J. M., Soura, V., Hafezparast, M., & Pfister, K. K. (2007). Intermediate chain subunit as a probe for cytoplasmic dynein function: biochemical analyses and live cell imaging in PC12 cells. *Journal of neuroscience research*, *85*(12), 2640-2647.

Nabhan, J. F., Pan, H., & Lu, Q. (2010). Arrestin domain-containing protein 3 recruits the NEDD4 E3 ligase to mediate ubiquitination of the β2-adrenergic receptor. *EMBO reports*, *11*(8), 605-611.

Nagaoka, T., Ohashi, R., Inutsuka, A., Sakai, S., Fujisawa, N., Yokoyama, M., Huang, Y. H., Igarashi, M., & Kishi, M. (2014). The Wnt/planar cell polarity pathway component Vangl2 induces synapse formation through direct control of N-cadherin. *Cell reports*, *6*(5), 916-927.

Nair, R. R., Blankvoort, S., Lagartos, M. J., & Kentros, C. (2020). Enhancer-driven gene expression (EDGE) enables the generation of viral vectors specific to neuronal subtypes. *iScience*, *23*(3), 100888.

Nakagawa, Y., & O'Leary, D. D. (2003). Dynamic patterned expression of orphan nuclear receptor genes RORα and RORβ in developing mouse forebrain. *Developmental neuroscience*, *25*(2-4), 234-244.

Nakayama, Y., Kikuta, H., Kanai, M., Yoshikawa, K., Kawamura, A., Kobayashi, K., Wang, Z., Khan, A., Kawakami, K., & Yamasu, K. (2013). Gbx2 functions as a transcriptional repressor to regulate the specification and morphogenesis of the mid–hindbrain junction in a dosage-and stage-dependent manner. *Mechanisms of Development*, *130*(11-12), 532-552.

Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., & Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*, *393*(6683), 386-389. https://doi.org/10.1038/30764

Nastiuk, K. L., Mello, C. V., George, J. M., & Clayton, D. F. (1994). Immediate-early gene responses in the avian song control system: cloning and expression analysis of the canary c-jun cDNA. *Molecular Brain Research*, *27*(2), 299-309.

Natan, R. G., Rao, W., & Geffen, M. N. (2017). Cortical Interneurons Differentially Shape Frequency Tuning following Adaptation. *Cell Rep*, *21*(4), 878-890. https://doi.org/10.1016/j.celrep.2017.10.012

Nicholls, R. E., Zhang, X.-l., Bailey, C. P., Conklin, B. R., Kandel, E. R., & Stanton, P. K. (2006). mGluR2 acts through inhibitory Gα subunits to regulate transmission and long-term plasticity at hippocampal mossy fiber-CA3 synapses. *Proceedings of the National Academy of Sciences*, *103*(16), 6380-6385.

Nick, T. A., & Konishi, M. (2005). Neural song preference during vocal learning in the zebra finch depends on age and state. *Journal of neurobiology*, *62*(2), 231-242.

Nixdorf-Bergweiler, B. E. (1996). Divergent and parallel development in volume sizes of telencephalic song nuclei in and female zebra finches. *Journal of Comparative Neurology*, *375*(3), 445-456.

Nordeen, E., Grace, A., Burek, M., & Nordeen, K. (1992). Sex-dependent loss of projection neurons involved in avian song learning. *Journal of neurobiology*, *23*(6), 671-679.

Nordeen, E., & Nordeen, K. (1988a). Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. *Journal of Neuroscience*, *8*(8), 2869-2874.

Nordeen, K. W., & Nordeen, E. J. (1988b). Projection neurons within a vocal motor pathway are born during song learning in zebra finches. *Nature*, *334*(6178), 149-151.

Nottebohm, F., & Arnold, A. P. (1976). Sexual dimorphism in vocal control areas of the songbird brain. *Science*, *194*(4261), 211-213.

Novara, F., Beri, S., Giorda, R., Ortibus, E., Nageshappa, S., Darra, F., Dalla Bernardina, B., Zuffardi, O., & Van Esch, H. (2010). Refining the phenotype associated with MEF2C haploinsufficiency. *Clinical Genetics*, *78*(5), 471-477.

O'Connor, L. T., Lauterborn, J. C., Gall, C. M., & Smith, M. A. (1994). Localization and alternative splicing of agrin mRNA in adult rat brain: transcripts encoding isoforms that aggregate acetylcholine receptors are not restricted to cholinergic regions. *Journal of Neuroscience*, *14*(3), 1141-1152.

O'Donnell, D., Larocque, S., Seckl, J. R., & Meaney, M. J. (1994). Postnatal handling alters glucocorticoid, but not mineralocorticoid messenger RNA expression in the hippocampus of adult rats. *Brain Res Mol Brain Res*, *26*(1-2), 242-248. https://doi.org/10.1016/0169-328x(94)90096-5

Oishi, K., Aramaki, M., & Nakajima, K. (2016). Mutually repressive interaction between Brn1/2 and Rorb contributes to the establishment of neocortical layer 2/3 and layer 4. *Proceedings of the National Academy of Sciences*, *113*(12), 3371-3376.

Okuda, H., Miyata, S., Mori, Y., & Tohyama, M. (2007). Mouse Prickle1 and Prickle2 are expressed in postmitotic neurons and promote neurite outgrowth. *FEBS letters*, *581*(24), 4754-4760.

Olivares, A. M., Han, Y., Soto, D., Flattery, K., Marini, J., Mollema, N., Haider, A., Escher, P., DeAngelis, M. M., & Haider, N. B. (2017). The nuclear hormone receptor gene Nr2c1 (Tr2) is a critical regulator of early retina cell patterning. *Developmental biology*, *429*(1), 343-355.

Ölveczky, B. P., Otchy, T. M., Goldberg, J. H., Aronov, D., & Fee, M. S. (2011). Changes in the neural control of a complex motor sequence during learning. *Journal of neurophysiology*, *106*(1), 386-397.

Ortuno-Sahagun, D., Schliebs, R., & Pallas, M. (2019). Editorial: Epigenetic Mechanisms Regulating Neural Plasticity. *Front Cell Neurosci*, *13*, 118. https://doi.org/10.3389/fncel.2019.00118

Ozaki, M., Sasner, M., Yano, R., Lu, H. S., & Buonanno, A. (1997). Neuregulin-β induces expression of an NMDA-receptor subunit. *Nature*, *390*(6661), 691-694.

Pakkenberg, B., Pelvig, D., Marner, L., Bundgaard, M. J., Gundersen, H. J. G., Nyengaard, J. R., & Regeur, L. (2003). Aging and the human neocortex. *Experimental gerontology*, *38*(1-2), 95-99.

Palmer, E. E., Kumar, R., Gordon, C. T., Shaw, M., Hubert, L., Carroll, R., Rio, M., Murray, L., Leffler, M., & Dudding-Byth, T. (2017). A recurrent de novo nonsense variant in ZSWIM6 results in severe intellectual disability without frontonasal or limb malformations. *The American Journal of Human Genetics*, *101*(6), 995-1005.

Parlier, D., Moers, V., Van Campenhout, C., Preillon, J., Leclère, L., Saulnier, A., Sirakov, M., Busengdal, H., Kricha, S., & Marine, J.-C. (2013). The Xenopus doublesex-related gene Dmrt5 is required for olfactory placode neurogenesis. *Developmental biology*, *373*(1), 39-52.

Passantino, R., Antona, V., Barbieri, G., Rubino, P., Melchionna, R., Cossu, G., Feo, S., & Giallongo, A. (1998). Negative regulation of β enolase gene transcription in embryonic muscle is dependent upon a zinc finger factor that binds to the G-rich box within the muscle-specific enhancer. *Journal of Biological Chemistry*, *273*(1), 484-494.

Passini, M. A., Levine, E. M., Canger, A. K., Raymond, P. A., & Schechter, N. (1997). Vsx-1 and Vsx-2: differential expression of two paired-like homeobox genes during zebrafish and goldfish retinogenesis. *Journal of Comparative Neurology*, *388*(3), 495-505.

Payne, H. L. (2008). The role of transmembrane AMPA receptor regulatory proteins (TARPs) in neurotransmission and receptor trafficking. *Molecular membrane biology*, *25*(4), 353-362.

Pekhletski, R., Gerlai, R., Overstreet, L. S., Huang, X.-P., Agopyan, N., Slater, N. T., Abramow-Newerly, W., Roder, J. C., & Hampson, D. R. (1996). Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *Journal of Neuroscience*, *16*(20), 6364-6373.

Perrot, V., Vazquez-Prado, J., & Gutkind, J. S. (2002). Plexin B regulates Rho through the guanine nucleotide exchange factors leukemia-associated Rho GEF (LARG) and PDZ-RhoGEF. *Journal of Biological Chemistry*, *277*(45), 43115-43120.

Pers, T. H., Timshel, P., Ripke, S., Lent, S., Sullivan, P. F., O'Donovan, M. C., Franke, L., & Hirschhorn, J. N. (2016). Comprehensive analysis of schizophrenia-associated loci highlights ion channel pathways and biologically plausible candidate causal genes. *Human molecular genetics*, *25*(6), 1247-1254.

Peters, L. L., Birkenmeier, C. S., Bronson, R. T., White, R. A., Lux, S. E., Otto, E., Bennett, V., Higgins, A., & Barker, J. E. (1991). Purkinje cell degeneration associated with erythroid ankyrin deficiency in nb/nb mice. *The Journal of cell biology*, *114*(6), 1233-1241.

Petersohn, D., Schoch, S., Brinkmann, D. R., & Thiel, G. (1995). The human synapsin II gene promoter: possible role for the transcription factors zif268/EGR-1, polyoma enhancer activator 3, and AP2. *Journal of Biological Chemistry*, *270*(41), 24361-24369.

Peterson, C. L., & Laniel, M. A. (2004). Histones and histone modifications. *Curr Biol*, *14*(14), R546-551. https://doi.org/10.1016/j.cub.2004.07.007

Petschner, P., Balogh, N., Adori, C., Tamasi, V., Kumar, S., Juhasz, G., & Bagdy, G. (2018). Downregulation of the vitamin D receptor regulated gene set in the hippocampus after MDMA treatment. *Frontiers in pharmacology*, *9*, 1373.

Pfeiffer, B. E., Zang, T., Wilkerson, J. R., Taniguchi, M., Maksimova, M. A., Smith, L. N., Cowan, C. W., & Huber, K. M. (2010). Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. *Neuron*, *66*(2), 191-197.

Phan, M. L., Pytte, C. L., & Vicario, D. S. (2006). Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds. *Proceedings of the National Academy of Sciences*, *103*(4), 1088-1093.

Pieraccioli, M., Nicolai, S., Pitolli, C., Agostini, M., Antonov, A., Malewicz, M., Knight, R. A., Raschellà, G., & Melino, G. (2018). ZNF281 inhibits neuronal differentiation and is a prognostic marker for neuroblastoma. *Proceedings of the National Academy of Sciences*, *115*(28), 7356-7361.

Pinaud, R., Fortes, A. F., Lovell, P., & Mello, C. V. (2006). Calbindin-positive neurons reveal a sexual dimorphism within the songbird analogue of the mammalian auditory cortex. *Journal of neurobiology*, *66*(2), 182-195.

Pinaud, R., & Mello, C. V. (2007). GABA immunoreactivity in auditory and song control brain areas of zebra finches. *Journal of chemical neuroanatomy*, *34*(1-2), 1-21.

Pinaud, R., Terleph, T. A., Tremere, L. A., Phan, M. L., Dagostin, A. A., Leão, R. M., Mello, C. V., & Vicario, D. S. (2008). Inhibitory network interactions shape the auditory processing of natural communication signals in the songbird auditory forebrain. *Journal of neurophysiology*, *100*(1), 441-455.

Pinaud, R., Velho, T. A., Jeong, J. K., Tremere, L. A., Leao, R. M., Von Gersdorff, H., & Mello, C. V. (2004). GABAergic neurons participate in the brain's response to birdsong auditory stimulation. *European Journal of Neuroscience*, *20*(5), 1318-1330.

Price, P. H. (1979). Developmental determinants of structure in zebra finch song. *Journal of comparative and physiological psychology*, *93*(2), 260.

Pulipparacharuvil, S., Renthal, W., Hale, C. F., Taniguchi, M., Xiao, G., Kumar, A., Russo, S. J., Sikder, D., Dewey, C. M., & Davis, M. M. (2008). Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron*, *59*(4), 621-633.

Qi, X.-w., Zhang, F., Wu, H., Liu, J.-l., Zong, B.-g., Xu, C., & Jiang, J. (2015). Wilms' tumor 1 (WT1) expression and prognosis in solid cancer patients: a systematic review and meta-analysis. *Scientific Reports*, *5*(1), 1-9.

Qu, Y., Glasco, D. M., Zhou, L., Sawant, A., Ravni, A., Fritzsch, B., Damrau, C., Murdoch, J. N., Evans, S., & Pfaff, S. L. (2010). Atypical cadherins Celsr1-3 differentially regulate migration of facial branchiomotor neurons in mice. *Journal of Neuroscience*, *30*(28), 9392-9401.

Quinlan, A. R. (2014). BEDTools: The Swiss-Army Tool for Genome Feature Analysis. *Curr Protoc Bioinformatics*, *47*, 11 12 11-34. https://doi.org/10.1002/0471250953.bi1112s47

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841-842. https://doi.org/10.1093/bioinformatics/btq033

Raffaelli, G., Saviane, C., Mohajerani, M. H., Pedarzani, P., & Cherubini, E. (2004). BK potassium channels control transmitter release at CA3–CA3 synapses in the rat hippocampus. *The Journal of physiology*, *557*(1), 147-157.

Ramos, B., Gaudillière, B., Bonni, A., & Gill, G. (2007). Transcription factor Sp4 regulates dendritic patterning during cerebellar maturation. *Proceedings of the National Academy of Sciences*, *104*(23), 9882-9887.

Rao, A., Kim, E., Sheng, M., & Craig, A. M. (1998). Heterogeneity in the molecular composition of excitatory postsynaptic sites during development of hippocampal neurons in culture. *Journal of Neuroscience*, *18*(4), 1217-1229.

Rashid, A., Cole, C., & Josselyn, S. (2014). Emerging roles for MEF2 transcription factors in memory. *Genes, Brain and Behavior*, *13*(1), 118-125.

Rasika, S., Alvarez-Buylla, A., & Nottebohm, F. (1999). BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. *Neuron*, *22*(1), 53-62.

Remage-Healey, L., Coleman, M. J., Oyama, R. K., & Schlinger, B. A. (2010). Brain estrogens rapidly strengthen auditory encoding and guide song preference in a songbird. *Proceedings of the National Academy of Sciences*, *107*(8), 3852-3857.

Remage-Healey, L., Dong, S. M., Chao, A., & Schlinger, B. A. (2012). Sex-specific, rapid neuroestrogen fluctuations and neurophysiological actions in the songbird auditory forebrain. *Journal of neurophysiology*, *107*(6), 1621-1631.

Remage-Healey, L., Maidment, N. T., & Schlinger, B. A. (2008). Forebrain steroid levels fluctuate rapidly during social interactions. *Nature neuroscience*, *11*(11), 1327-1334.

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., Koren, S., Uliano-Silva, M., Chow, W., Fungtammasan, A., Kim, J., Lee, C., Ko, B. J., Chaisson, M., Gedman, G. L., Cantin, L. J., Thibaud-Nissen, F., Haggerty, L., Bista, I., Smith, M., . . . Jarvis, E. D. (2021). Towards complete and error-free genome assemblies of all vertebrate species. *Nature*, *592*(7856), 737-746. https://doi.org/10.1038/s41586-021-03451-0

Ribeiro, S., & Mello, C. (2000). Gene expression and synaptic plasticity in the auditory forebrain of songbirds. *Learning & memory*, *7*(5), 235-243.

Richardson, C., Tate, W., Mason, S., Lawlor, P., Dragunow, M., & Abraham, W. (1992). Correlation between the induction of an immediate early gene, zif/268, and long-term potentiation in the dentate gyrus. *Brain research*, *580*(1-2), 147-154.

Riebel, K. (2000). Early exposure leads to repeatable preferences for male song in female zebra finches. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1461), 2553-2558.

Riebel, K. (2003a). Developmental influences on auditory perception in female zebra finches-is there a sensitive phase for song preference learning? *Animal Biology*, *53*(2), 73-87.

Riebel, K. (2003b). The" mute" sex revisited: vocal production and perception learning in female songbirds.

Riebel, K., Naguib, M., & Gil, D. (2009). Experimental manipulation of the rearing environment influences adult female zebra finch song preferences. *Animal Behaviour*, *78*(6), 1397-1404.

Riebel, K., Smallegange, I. M., Terpstra, N. J., & Bolhuis, J. J. (2002). Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1492), 729-733.

Rieff, H. I., Raetzman, L. T., Sapp, D. W., Yeh, H. H., Siegel, R. E., & Corfas, G. (1999). Neuregulin induces GABAA receptor subunit expression and neurite outgrowth in cerebellar granule cells. *Journal of Neuroscience*, *19*(24), 10757-10766.

Rio, C., Rieff, H. I., Qi, P., & Corfas, G. (1997). Neuregulin and erbB receptors play a critical role in neuronal migration. *Neuron*, *19*(1), 39-50.

Ripke, S., O'dushlaine, C., Chambert, K., Moran, J. L., Kähler, A. K., Akterin, S., Bergen, S. E., Collins, A. L., Crowley, J. J., & Fromer, M. (2013). Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nature genetics*, *45*(10), 1150-1159.

Ritter-Makinson, S. L., Paquet, M., Bogenpohl, J. W., Rodin, R. E., Yun, C. C., Weinman, E. J., Smith, Y., & Hall, R. A. (2017). Group II metabotropic glutamate receptor interactions with NHERF scaffold proteins: Implications for receptor localization in brain. *Neuroscience*, *353*, 58-75.

Roberts, R. G., & Sheng, M. (2000). Association of dystrophin-related protein 2 (DRP2) with postsynaptic densities in rat brain. *Molecular and Cellular Neuroscience*, *16*(5), 674-685.

Robinson, J. T., Thorvaldsdottir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nat Biotechnol*, *29*(1), 24-26. https://doi.org/10.1038/nbt.1754

Rodenas-Ruano, A., Chávez, A. E., Cossio, M. J., Castillo, P. E., & Zukin, R. S. (2012). REST-dependent epigenetic remodeling promotes the developmental switch in synaptic NMDA receptors. *Nature neuroscience*, *15*(10), 1382-1390.

Romero, D. M., Poirier, K., Belvindrah, R., Moutkine, I., Houllier, A., LeMoing, A.-G., Petit, F., Boland, A., Collins, S. C., & Soiza-Reilly, M. (2022). Novel role of the synaptic scaffold protein Dlgap4 in ventricular surface integrity and neuronal migration during cortical development. *Nature communications*, *13*(1), 2746.

Roper, A., & Zann, R. (2006). The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology*, *112*(5), 458-470.

Ross-Innes, C. S., Stark, R., Teschendorff, A. E., Holmes, K. A., Ali, H. R., Dunning, M. J., Brown, G. D., Gojis, O., Ellis, I. O., Green, A. R., Ali, S., Chin, S. F., Palmieri, C., Caldas, C., & Carroll, J. S. (2012). Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature*, *481*(7381), 389-393. https://doi.org/10.1038/nature10730

Rowlands, D., Lensjø, K. K., Dinh, T., Yang, S., Andrews, M. R., Hafting, T., Fyhn, M., Fawcett, J. W., & Dick, G. (2018). Aggrecan directs extracellular matrix-mediated neuronal plasticity. *Journal of Neuroscience*, *38*(47), 10102-10113.

Rowson, S. A., Bekhbat, M., Kelly, S. D., Binder, E. B., Hyer, M. M., Shaw, G., Bent, M. A., Hodes, G., Tharp, G., & Weinshenker, D. (2019). Chronic adolescent stress sex-

specifically alters the hippocampal transcriptome in adulthood. *Neuropsychopharmacology*, *44*(7), 1207-1215.

Roysommuti, S., Carroll, S., & Wyss, J. (2003). Neuregulin-1β modulates in vivo entorhinal–hippocampal synaptic transmission in adult rats. *Neuroscience*, *121*(3), 779-785.

Rudenko, A., Dawlaty, M. M., Seo, J., Cheng, A. W., Meng, J., Le, T., Faull, K. F., Jaenisch, R., & Tsai, L. H. (2013). Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. *Neuron*, *79*(6), 1109-1122. https://doi.org/10.1016/j.neuron.2013.08.003

Rudolf, G., Lesca, G., Mehrjouy, M. M., Labalme, A., Salmi, M., Bache, I., Bruneau, N., Pendziwiat, M., Fluss, J., & De Bellescize, J. (2016). Loss of function of the retinoid-related nuclear receptor (RORB) gene and epilepsy. *European Journal of Human Genetics*, *24*(12), 1761-1770.

Saha, B., Ypsilanti, A. R., Boutin, C., Cremer, H., & Chédotal, A. (2012). Plexin-B2 regulates the proliferation and migration of neuroblasts in the postnatal and adult subventricular zone. *Journal of Neuroscience*, *32*(47), 16892-16905.

Salma, J., & McDermott, J. C. (2012). Suppression of a MEF2-KLF6 survival pathway by PKA signaling promotes apoptosis in embryonic hippocampal neurons. *Journal of Neuroscience*, *32*(8), 2790-2803.

Sara, S. J. (2015). Locus coeruleus in time with the making of memories. *Current opinion in neurobiology*, *35*, 87-94.

Sariñana, J., Kitamura, T., Künzler, P., Sultzman, L., & Tonegawa, S. (2014). Differential roles of the dopamine 1-class receptors, D1R and D5R, in hippocampal dependent memory. *Proceedings of the National Academy of Sciences*, *111*(22), 8245-8250.

Sarinana, J., & Tonegawa, S. (2016). Differentiation of forebrain and hippocampal dopamine 1-class receptors, D 1 R and D 5 R, in spatial learning and memory. *Hippocampus*, *26*(1), 76-86.

Sarmah, S., Hawkins, M. R., Manikandan, P., Farrell, M., & Marrs, J. A. (2022). Elf3 deficiency during zebrafish development alters extracellular matrix organization and disrupts tissue morphogenesis. *PloS one*, *17*(11), e0276255.

Sato, K., Otsu, W., Otsuka, Y., & Inaba, M. (2013). Modulatory roles of NHERF1 and NHERF2 in cell surface expression of the glutamate transporter GLAST. *Biochemical and biophysical research communications*, *430*(2), 839-845.

Saucedo-Cardenas, O., Quintana-Hau, J. D., Le, W.-D., Smidt, M. P., Cox, J. J., De Mayo, F., Burbach, J. P. H., & Conneely, O. M. (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proceedings of the National Academy of Sciences*, *95*(7), 4013-4018.

Saulnier, A., Keruzore, M., De Clercq, S., Bar, I., Moers, V., Magnani, D., Walcher, T., Filippis, C., Kricha, S., & Parlier, D. (2013). The doublesex homolog Dmrt5 is required for the development of the caudomedial cerebral cortex in mammals. *Cerebral cortex*, *23*(11), 2552-2567.

Scharff, C., & Nottebohm, F. (1991). A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *Journal of Neuroscience*, *11*(9), 2896-2913.

Scharnhorst, V., van der Eb, A. J., & Jochemsen, A. G. (2001). WT1 proteins: functions in growth and differentiation. *Gene*, *273*(2), 141-161.

Scheyltjens, I., Laramée, M. E., Van den Haute, C., Gijsbers, R., Debyser, Z., Baekelandt, V., Vreysen, S., & Arckens, L. (2015). Evaluation of the expression pattern of rAAV2/1, 2/5, 2/7, 2/8, and 2/9 serotypes with different promoters in the mouse visual cortex. *Journal of Comparative Neurology*, *523*(14), 2019-2042.

Schmid, R. S., McGrath, B., Berechid, B. E., Boyles, B., Marchionni, M., Šestan, N., & Anton, E. S. (2003). Neuregulin 1–erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. *Proceedings of the National Academy of Sciences*, *100*(7), 4251-4256.

Schmidt, M., Huber, L., Majdazari, A., Schütz, G., Williams, T., & Rohrer, H. (2011). The transcription factors AP-2β and AP-2α are required for survival of sympathetic progenitors and differentiated sympathetic neurons. *Developmental biology*, *355*(1), 89-100.

Schoenherr, C. J., & Anderson, D. J. (1995). Silencing is golden: negative regulation in the control of neuronal gene transcription. *Current opinion in neurobiology*, *5*(5), 566-571.

Schroeder, B. C., Waldegger, S., Fehr, S., Bleich, M., Warth, R., Greger, R., & Jentsch, T. J. (2000). A constitutively open potassium channel formed by KCNQ1 and KCNE3. *Nature*, *403*(6766), 196-199.

Schulz, S., Haesler, S., Scharff, C., & Rochefort, C. (2010). Knockdown of FoxP2 alters spine density in Area X of the zebra finch. *Genes, Brain and Behavior*, *9*(7), 732-740.

Scully, E. N., Hahn, A. H., Campbell, K. A., McMillan, N., Congdon, J. V., & Sturdy, C. B. (2017). ZENK expression following conspecific and heterospecific playback in the zebra finch auditory forebrain. *Behavioural brain research*, *331*, 151-158.

Semplici, F., Meggio, F., Pinna, L. A., & Oliviero, S. (2002). CK2-dependent phosphorylation of the E2 ubiquitin conjugating enzyme UBC3B induces its interaction with β-TrCP and enhances β-catenin degradation. *Oncogene*, *21*(25), 3978-3987.

Sgadò, P., Albéri, L., Gherbassi, D., Galasso, S. L., Ramakers, G. M., Alavian, K. N., Smidt, M. P., Dyck, R. H., & Simon, H. H. (2006). Slow progressive degeneration of nigral

dopaminergic neurons in postnatal Engrailed mutant mice. *Proceedings of the National Academy of Sciences*, *103*(41), 15242-15247.

Shen, Y., Ning, J., Zhao, L., Liu, W., Wang, T., Yu, J., & Wang, Y. (2023). Matrix remodeling associated 7 proteins promote cutaneous wound healing through vimentin in coordinating fibroblast functions. *Inflammation and Regeneration*, *43*(1), 5.

Shieh, P. B., & Ghosh, A. (1999). Molecular mechanisms underlying activity-dependent regulation of BDNF expression. *Journal of neurobiology*, *41*(1), 127-134.

Shimada, M., Konishi, Y., Ohkawa, N., Maruyama, C.-O., Hanaoka, F., Makino, Y., & Tamura, T.-a. (1999). Distribution of AP-2 subtypes in the adult mouse brain. *Neuroscience research*, *33*(4), 275-280.

Shivarama Shetty, M., Gopinadhan, S., & Sajikumar, S. (2016). Dopamine D1/D5 receptor signaling regulates synaptic cooperation and competition in hippocampal CA 1 pyramidal neurons via sustained ERK 1/2 activation. *Hippocampus*, *26*(2), 137-150.

Sík, A., Hájos, N., Gulácsi, A., Mody, I., & Freund, T. F. (1998). The absence of a major Ca2+ signaling pathway in GABAergic neurons of the hippocampus. *Proceedings of the National Academy of Sciences*, *95*(6), 3245-3250.

Simon, H. H., Thuret, S., & Alberi, L. (2004). Midbrain dopaminergic neurons: control of their cell fate by the engrailed transcription factors. *Cell and tissue research*, *318*(1), 53-61.

Simonet, J. C., Sunnen, C. N., Wu, J., Golden, J. A., & Marsh, E. D. (2015). Conditional loss of Arx from the developing dorsal telencephalon results in behavioral phenotypes resembling mild human ARX mutations. *Cerebral cortex*, *25*(9), 2939-2950.

Simonetti, M., Paldy, E., Njoo, C., Bali, K. K., Worzfeld, T., Pitzer, C., Kuner, T., Offermanns, S., Mauceri, D., & Kuner, R. (2021). The impact of Semaphorin 4C/Plexin-B2 signaling on fear memory via remodeling of neuronal and synaptic morphology. *Molecular psychiatry*, *26*(4), 1376-1398.

Skerjanc, I. S., & Wilton, S. (2000). Myocyte enhancer factor 2C upregulates MASH-1 expression and induces neurogenesis in P19 cells. *FEBS letters*, *472*(1), 53-56.

Slater, P., Richards, C., & Mann, N. (1991). Song learning in zebra finches exposed to a series of tutors during the sensitive phase. *Ethology*, *88*(2), 163-171.

Slater, P. J., Eales, L. A., & Clayton, N. (1988). Song learning in zebra finches (Taeniopygia guttata): progress and prospects. *Advances in the Study of Behavior*, *18*, 1-34.

Smith, C. C., & McMahon, L. L. (2006). Estradiol-induced increase in the magnitude of long-term potentiation is prevented by blocking NR2B-containing receptors. *Journal of Neuroscience*, *26*(33), 8517-8522.

Sohrabji, F., Nordeen, E. J., & Nordeen, K. W. (1990). Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. *Behavioral and neural biology*, *53*(1), 51-63.

Solis, M. M., Brainard, M. S., Hessler, N. A., & Doupe, A. J. (2000). Song selectivity and sensorimotor signals in vocal learning and production. *Proceedings of the National Academy of Sciences*, *97*(22), 11836-11842.

Sonnier, L., Le Pen, G., Hartmann, A., Bizot, J.-C., Trovero, F., Krebs, M.-O., & Prochiantz, A. (2007). Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1. *Journal of Neuroscience*, *27*(5), 1063-1071.

Sorg, B. A., Berretta, S., Blacktop, J. M., Fawcett, J. W., Kitagawa, H., Kwok, J. C., & Miquel, M. (2016). Casting a wide net: role of perineuronal nets in neural plasticity. *Journal of Neuroscience*, *36*(45), 11459-11468.

Soriano, O., Alcón-Pérez, M., Vicente-Manzanares, M., & Castellano, E. (2021). The crossroads between RAS and RHO signaling pathways in cellular transformation, motility and contraction. *Genes*, *12*(6), 819.

Sosa-Pineda, B. (2004). The gene Pax4 is an essential regulator of pancreatic β-cell development. *Molecules & Cells (Springer Science & Business Media BV)*, *18*(3).

Sousa, K. M., Mira, H., Hall, A. C., Jansson-Sjöstrand, L., Kusakabe, M., & Arenas, E. (2007). Microarray analyses support a role for Nurr1 in resistance to oxidative stress and neuronal differentiation in neural stem cells. *Stem Cells*, *25*(2), 511-519.

Sowers, L., Loo, L., Wu, Y., Campbell, E., Ulrich, J., Wu, S., Paemka, L., Wassink, T., Meyer, K., & Bing, X. (2013). Disruption of the non-canonical Wnt gene PRICKLE2 leads to autism-like behaviors with evidence for hippocampal synaptic dysfunction. *Molecular psychiatry*, *18*(10), 1077-1089.

Spool, J. A., Macedo-Lima, M., Scarpa, G., Morohashi, Y., Yazaki-Sugiyama, Y., & Remage-Healey, L. (2021). Genetically identified neurons in avian auditory pallium mirror core principles of their mammalian counterparts. *Current Biology*, *31*(13), 2831-2843. e2836.

Springer, S. J., Burkett, B. J., & Schrader, L. A. (2015). Modulation of BK channels contributes to activity-dependent increase of excitability through MTORC1 activity in CA1 pyramidal cells of mouse hippocampus. *Frontiers in cellular neuroscience*, *8*, 451.

Stark, R., & Brown, G. (2011). *DiffBind: Differential Binding Analysis of ChIP-Seq Peak Data. Bioconductor*. In http://bioconductor.org/packages/release/bioc/html/DiffBind.html

Stenman, J., Toresson, H., & Campbell, K. (2003). Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. *Journal of Neuroscience*, *23*(1), 167-174.

Stevens, S. J., van Essen, A. J., van Ravenswaaij, C. M., Elias, A. F., Haven, J. A., Lelieveld, S. H., Pfundt, R., Nillesen, W. M., Yntema, H. G., & van Roozendaal, K. (2016). Truncating de novo mutations in the Krüppel-type zinc-finger gene ZNF148 in patients with corpus callosum defects, developmental delay, short stature, and dysmorphisms. *Genome medicine*, *8*, 1-10.

Stevens, S. R., van der Heijden, M. E., Ogawa, Y., Lin, T., Sillitoe, R. V., & Rasband, M. N. (2022). Ankyrin-R links Kv3. 3 to the spectrin cytoskeleton and is required for Purkinje neuron survival. *Journal of Neuroscience*, *42*(1), 2-15.

Stone, D. M., & Nikolics, K. (1995). Tissue-and age-specific expression patterns of alternatively spliced agrin mRNA transcripts in embryonic rat suggest novel developmental roles. *Journal of Neuroscience*, *15*(10), 6767-6778.

Stripling, R., Kruse, A. A., & Clayton, D. F. (2001). Development of song responses in the zebra finch caudomedial neostriatum: role of genomic and electrophysiological activities. *Journal of neurobiology*, *48*(3), 163-180.

Stroud, M. J., Nazgiewicz, A., McKenzie, E. A., Wang, Y., Kammerer, R. A., & Ballestrem, C. (2014). GAS2-like proteins mediate communication between microtubules and actin through interactions with end-binding proteins. *Journal of Cell Science*, *127*(12), 2672-2682.

Stühmer, T., Anderson, S. A., Ekker, M., & Rubenstein, J. L. (2002). Ectopic expression of the Dlx genes induces glutamic acid decarboxylase and Dlx expression.

Sugiaman-Trapman, D., Vitezic, M., Jouhilahti, E.-M., Mathelier, A., Lauter, G., Misra, S., Daub, C. O., Kere, J., & Swoboda, P. (2018). Characterization of the human RFX transcription factor family by regulatory and target gene analysis. *BMC genomics*, *19*(1), 1-15.

Sumioka, A., Saito, Y., Sakuma, M., Araki, Y., Yamamoto, T., & Suzuki, T. (2008). The X11L/X11beta/MINT2 and X11L2/X11gamma/MINT3 scaffold proteins shuttle between the nucleus and cytoplasm. *Exp Cell Res*, *314*(5), 1155-1162. https://doi.org/10.1016/j.yexcr.2007.12.006

Sun, Y., Luo, Z.-M., Guo, X.-M., Su, D.-F., & Liu, X. (2015). An updated role of microRNA-124 in central nervous system disorders: a review. *Frontiers in cellular neuroscience*, *9*, 193.

Susalka, S. J., & Pfister, K. K. (2000). Cytoplasmic dynein subunit heterogeneity: implications for axonal transport. *Journal of neurocytology*, *29*(11-12), 819-829.

Svec, L. A., & Wade, J. (2009). Estradiol induces region-specific inhibition of ZENK but does not affect the behavioral preference for tutored song in adult female zebra finches. *Behavioural brain research*, *199*(2), 298-306.

Tada, T., Simonetta, A., Batterton, M., Kinoshita, M., Edbauer, D., & Sheng, M. (2007). Role of Septin cytoskeleton in spine morphogenesis and dendrite development in neurons. *Current Biology*, *17*(20), 1752-1758.

Takesian, A. E., & Hensch, T. K. (2013). Balancing plasticity/stability across brain development. *Progress in brain research*, *207*, 3-34.

Takeuchi, M., Hata, Y., Hirao, K., Toyoda, A., Irie, M., & Takai, Y. (1997). SAPAPs: a family of PSD-95/SAP90-associated proteins localized at postsynaptic density. *Journal of Biological Chemistry*, *272*(18), 11943-11951.

Tang, B. L. (2009). REST regulation of neural development: From outside-in? *Cell Adhesion & Migration*, *3*(2), 141-142.

Tang, Y. P., Peabody, C., Tomaszycki, M. L., & Wade, J. (2007). Sexually dimorphic SCAMP1 expression in the forebrain motor pathway for song production of juvenile zebra finches. *Developmental neurobiology*, *67*(4), 474-482.

Tang, Y. P., & Wade, J. (2013). Developmental changes in BDNF protein in the song control nuclei of zebra finches. *Neuroscience*, *250*, 578-587.

Tawarayama, H., Yoshida, Y., Suto, F., Mitchell, K. J., & Fujisawa, H. (2010). Roles of semaphorin-6B and plexin-A2 in lamina-restricted projection of hippocampal mossy fibers. *Journal of Neuroscience*, *30*(20), 7049-7060.

Terpstra, N. J., Bolhuis, J. J., & den Boer-Visser, A. M. (2004). An analysis of the neural representation of birdsong memory. *Journal of Neuroscience*, *24*(21), 4971-4977.

Terpstra, N. J., Bolhuis, J. J., Riebel, K., van der Burg, J. M., & den Boer-Visser, A. M. (2006). Localized brain activation specific to auditory memory in a female songbird. *Journal of Comparative Neurology*, *494*(5), 784-791.

Thakar, S., Wang, L., Yu, T., Ye, M., Onishi, K., Scott, J., Qi, J., Fernandes, C., Han, X., & Yates III, J. R. (2017). Evidence for opposing roles of Celsr3 and Vangl2 in glutamatergic synapse formation. *Proceedings of the National Academy of Sciences*, *114*(4), E610-E618.

Theunissen, F. E., Amin, N., Shaevitz, S. S., Woolley, S. M., Fremouw, T., & Hauber, M. E. (2004). Song selectivity in the song system and in the auditory forebrain. *Annals of the New York Academy of Sciences*, *1016*(1), 222-245.

Thiel, G., Schoch, S., & Petersohn, D. (1994). Regulation of synapsin I gene expression by the zinc finger transcription factor zif268/egr-1. *Journal of Biological Chemistry*, *269*(21), 15294-15301.

Thorpe, W. H. (1958). The learning of song patterns by birds, with especial reference to the song of the chaffinch Fringilla coelebs. *Ibis*, *100*(4), 535-570.

Thurman, R. E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M. T., Haugen, E., Sheffield, N. C., Stergachis, A. B., Wang, H., & Vernot, B. (2012). The accessible chromatin landscape of the human genome. *Nature*, *489*(7414), 75-82.

Tierney, A. L., & Nelson III, C. A. (2009). Brain development and the role of experience in the early years. *Zero to three*, *30*(2), 9.

Tischfield, D. J., Saraswat, D. K., Furash, A., Fowler, S. C., Fuccillo, M. V., & Anderson, S. A. (2017). Loss of the neurodevelopmental gene Zswim6 alters striatal morphology and motor regulation. *Neurobiology of disease*, *103*, 174-183.

Tissir, F., Bar, I., Jossin, Y., De Backer, O., & Goffinet, A. M. (2005). Protocadherin Celsr3 is crucial in axonal tract development. *Nature neuroscience*, *8*(4), 451-457.

Todd, S. C., Doctor, V. S., & Levy, S. (1998). Sequences and expression of six new members of the tetraspanin/TM4SF family. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, *1399*(1), 101-104.

Tomaszycki, M. L., & Adkins-Regan, E. (2005). Experimental alteration of male song quality and output affects female mate choice and pair bond formation in zebra finches. *Animal Behaviour*, *70*(4), 785-794.

Tomaszycki, M. L., Peabody, C., Replogle, K., Clayton, D. F., Tempelman, R. J., & Wade, J. (2009). Sexual differentiation of the zebra finch song system: potential roles for sex chromosome genes. *BMC neuroscience*, *10*, 1-14.

Tomaszycki, M. L., Sluzas, E. M., Sundberg, K. A., Newman, S. W., & DeVoogd, T. J. (2006). Immediate early gene (ZENK) responses to song in juvenile female and male zebra finches: effects of rearing environment. *Journal of neurobiology*, *66*(11), 1175-1182.

Treichel, D., Becker, M.-B., & Gruss, P. (2001). The novel transcription factor gene Sp5 exhibits a dynamic and highly restricted expression pattern during mouse embryogenesis. *Mechanisms of Development*, *101*(1-2), 175-179.

Tripathi, V., Ellis, J. D., Shen, Z., Song, D. Y., Pan, Q., Watt, A. T., Freier, S. M., Bennett, C. F., Sharma, A., & Bubulya, P. A. (2010). The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Molecular cell*, *39*(6), 925-938.

Trivisano, M., Santarone, M. E., Micalizzi, A., Ferretti, A., Dentici, M. L., Novelli, A., Vigevano, F., & Specchio, N. (2020). GRIA3 missense mutation is cause of an x-linked developmental and epileptic encephalopathy. *Seizure*, *82*, 1-6.

Typlt, M., Mirkowski, M., Azzopardi, E., Ruth, P., Pilz, P. K., & Schmid, S. (2013). Habituation of reflexive and motivated behavior in mice with deficient BK channel function. *Frontiers in integrative neuroscience*, *7*, 79.

Vallee, R. B., Shpetner, H. S., & Paschal, B. M. (1989). The role of dynein in retrograde axonal transport. *Trends in neurosciences*, *12*(2), 66-70.

Van Battum, E., Heitz-Marchaland, C., Zagar, Y., Fouquet, S., Kuner, R., & Chédotal, A. (2021). Plexin-B2 controls the timing of differentiation and the motility of cerebellar granule neurons. *Elife*, *10*, e60554.

van der Raadt, J., van Gestel, S. H., Nadif Kasri, N., & Albers, C. A. (2019). ONECUT transcription factors induce neuronal characteristics and remodel chromatin accessibility. *Nucleic acids research*, *47*(11), 5587-5602.

Van Lieshout, E., Van der Heijden, I., Hendriks, W., & Van der Zee, C. (2001). A decrease in size and number of basal forebrain cholinergic neurons is paralleled by diminished hippocampal cholinergic innervation in mice lacking leukocyte common antigen-related protein tyrosine phosphatase activity. *Neuroscience*, *102*(4), 833-841.

Vance, K. W., Sansom, S. N., Lee, S., Chalei, V., Kong, L., Cooper, S. E., Oliver, P. L., & Ponting, C. P. (2014). The long non-coding RNA P aupar regulates the expression of both local and distal genes. *The EMBO journal*, *33*(4), 296-311.

Vates, G. E., Broome, B. M., Mello, C. V., & Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (Taenopygia guttata). *Journal of Comparative Neurology*, *366*(4), 613-642.

Velho, T. A., Lu, K., Ribeiro, S., Pinaud, R., Vicario, D., & Mello, C. V. (2012). Noradrenergic control of gene expression and long-term neuronal adaptation evoked by learned vocalizations in songbirds. *PloS one*, *7*(5), e36276.

Velho, T. A., & Mello, C. V. (2008). Synapsins are late activity-induced genes regulated by birdsong. *Journal of Neuroscience*, *28*(46), 11871-11882.

Velho, T. A., Pinaud, R., Rodrigues, P. V., & Mello, C. V. (2005). Co-induction of activity-dependent genes in songbirds. *European Journal of Neuroscience*, *22*(7), 1667-1678.

Vetere, G., Restivo, L., Cole, C. J., Ross, P. J., Ammassari-Teule, M., Josselyn, S. A., & Frankland, P. W. (2011). Spine growth in the anterior cingulate cortex is necessary for the consolidation of contextual fear memory. *Proceedings of the National Academy of Sciences*, *108*(20), 8456-8460.

Vickers, E. D., Clark, C., Osypenko, D., Fratzl, A., Kochubey, O., Bettler, B., & Schneggenburger, R. (2018). Parvalbumin-Interneuron Output Synapses Show Spike-Timing-Dependent Plasticity that Contributes to Auditory Map Remodeling. *Neuron*, *99*(4), 720-735 e726. https://doi.org/10.1016/j.neuron.2018.07.018

Vigil, F. A., Mizuno, K., Lucchesi, W., Valls-Comamala, V., & Giese, K. P. (2017). Prevention of long-term memory loss after retrieval by an endogenous CaMKII inhibitor. *Scientific Reports*, *7*(1), 4040.

Visel, A., Blow, M. J., Li, Z., Zhang, T., Akiyama, J. A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., & Chen, F. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature*, *457*(7231), 854-858.

Von Bartheld, C. S., Bahney, J., & Herculano-Houzel, S. (2016). The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. *Journal of Comparative Neurology*, *524*(18), 3865-3895.

Wagnon, J. L., Briese, M., Sun, W., Mahaffey, C. L., Curk, T., Rot, G., Ule, J., & Frankel, W. N. (2012). CELF4 regulates translation and local abundance of a vast set of mRNAs, including genes associated with regulation of synaptic function. *PLoS genetics*, *8*(11), e1003067.

Wallner, M., Meera, P., & Toro, L. (1999). Molecular basis of fast inactivation in voltage and Ca2+-activated K+ channels: a transmembrane β-subunit homolog. *Proceedings of the National Academy of Sciences*, *96*(7), 4137-4142.

Wang, B., Guo, J., Feng, L., Suen, C.-w., Fu, W.-m., Zhang, J.-f., & Li, G. (2016). MiR124 suppresses collagen formation of human tendon derived stem cells through targeting egr1. *Experimental Cell Research*, *347*(2), 360-366.

Wang, F., Zhang, Y., Wang, L., Sun, P., Luo, X., Ishigaki, Y., Sugai, T., Yamamoto, R., & Kato, N. (2015a). Improvement of spatial learning by facilitating large-conductance calcium-activated potassium channel with transcranial magnetic stimulation in Alzheimer's disease model mice. *Neuropharmacology*, *97*, 210-219.

Wang, J., Wu, X., Dai, W., Li, J., Xiang, L., Tang, W., Lin, J., Zhang, W., Liu, G., & Yang, Q. (2020). The CCDC43-ADRM1 axis regulated by YY1, promotes proliferation and metastasis of gastric cancer. *Cancer letters*, *482*, 90-101.

Wang, K. C., Koprivica, V., Kim, J. A., Sivasankaran, R., Guo, Y., Neve, R. L., & He, Z. (2002). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature*, *417*(6892), 941-944.

Wang, L., Kang, H., Li, Y., Shui, Y., Yamamoto, R., Sugai, T., & Kato, N. (2015b). Cognitive recovery by chronic activation of the large-conductance calcium-activated potassium channel in a mouse model of Alzheimer's disease. *Neuropharmacology*, *92*, 8-15.

Wang, R.-M., Zhang, Q.-G., Li, J., Yang, L.-C., Yang, F., & Brann, D. W. (2009). The ERK5–MEF2C transcription factor pathway contributes to anti-apoptotic effect of cerebral ischemia preconditioning in the hippocampal CA1 region of rats. *Brain research*, *1255*, 32-41.

Wang, X., Fei, F., Qu, J., Li, C., Li, Y., & Zhang, S. (2018). The role of septin 7 in physiology and pathological disease: a systematic review of current status. *Journal of cellular and molecular medicine*, *22*(7), 3298-3307.

Wang, X., Zhang, C., Szábo, G., & Sun, Q.-Q. (2013). Distribution of CaMKIIα expression in the brain in vivo, studied by CaMKIIα-GFP mice. *Brain research*, *1518*, 9-25.

Wang, Y., Brzozowska-Prechtl, A., & Karten, H. J. (2010). Laminar and columnar auditory cortex in avian brain. *Proceedings of the National Academy of Sciences*, *107*(28), 12676-12681.

Wang, Z.-W. (2008). Regulation of synaptic transmission by presynaptic CaMKII and BK channels. *Molecular neurobiology*, *38*, 153-166.

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., Cui, K., Roh, T.-Y., Peng, W., & Zhang, M. Q. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature genetics*, *40*(7), 897-903.

Wassarman, K. M., Lewandoski, M., Campbell, K., Joyner, A. L., Rubenstein, J. L., Martinez, S., & Martin, G. R. (1997). Specification of the anterior hindbrain and establishment of a normal mid/hindbrain organizer is dependent on Gbx2 gene function. *Development*, *124*(15), 2923-2934.

Wei, F., Qiu, C.-S., Kim, S. J., Muglia, L., Maas, J. W., Pineda, V. V., Xu, H.-M., Chen, Z.-F., Storm, D. R., & Muglia, L. J. (2002). Genetic elimination of behavioral sensitization in mice lacking calmodulin-stimulated adenylyl cyclases. *Neuron*, *36*(4), 713-726.

Wei, J., Liu, Q., & Riebel, K. (2022). Generalisation of early learned tutor song preferences in female zebra finches (Taeniopygia guttata). *Behavioural Processes*, *201*, 104731.

Williams, H. (1990). Models for song learning in the zebra finch: fathers or others? *Animal Behaviour*, *39*(4), 745-757.

Williams, H., Kilander, K., & Sotanski, M. L. (1993). Untutored song, reproductive success and song learning. *Animal Behaviour*, *45*(4), 695-705.

Wingender, E., Schoeps, T., & Dönitz, J. (2013). TFClass: an expandable hierarchical classification of human transcription factors. *Nucleic acids research*, *41*(D1), D165-D170.

Wingender, E., Schoeps, T., Haubrock, M., & Dönitz, J. (2014). TFClass: a classification of human transcription factors and their rodent orthologs. *Nucleic acids research*, *43*(D1), D97-D102. https://doi.org/10.1093/nar/gku1064

Wlodarczyk, J., Mukhina, I., Kaczmarek, L., & Dityatev, A. (2011). Extracellular matrix molecules, their receptors, and secreted proteases in synaptic plasticity. *Developmental neurobiology*, *71*(11), 1040-1053.

Woo, A. J., Patry, C.-A. A., Ghamari, A., Pregernig, G., Yuan, D., Zheng, K., Piers, T., Hibbs, M., Li, J., & Fidalgo, M. (2019). Zfp281 (ZBP-99) plays a functionally redundant role with Zfp148 (ZBP-89) during erythroid development. *Blood advances*, *3*(16), 2499-2511.

Wood, K. C., Blackwell, J. M., & Geffen, M. N. (2017). Cortical inhibitory interneurons control sensory processing. *Curr Opin Neurobiol*, *46*, 200-207. https://doi.org/10.1016/j.conb.2017.08.018

Woodgate, J. L., Mariette, M. M., Bennett, A. T., Griffith, S. C., & Buchanan, K. L. (2012). Male song structure predicts reproductive success in a wild zebra finch population. *Animal Behaviour*, *83*(3), 773-781.

Woolley, C. S., Weiland, N. G., McEwen, B. S., & Schwartzkroin, P. A. (1997). Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. *Journal of Neuroscience*, *17*(5), 1848-1859.

Woolley, S. C., & Doupe, A. J. (2008). Social context–induced song variation affects female behavior and gene expression. *PLoS biology*, *6*(3), e62.

Wu, L.-G., Hamid, E., Shin, W., & Chiang, H.-C. (2014). Exocytosis and endocytosis: modes, functions, and coupling mechanisms. *Annual review of physiology*, *76*, 301-331.

Wu, X., & Zhang, Y. (2017). TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat Rev Genet*, *18*(9), 517-534. https://doi.org/10.1038/nrg.2017.33

Wu, Y., Arai, A. C., Rumbaugh, G., Srivastava, A. K., Turner, G., Hayashi, T., Suzuki, E., Jiang, Y., Zhang, L., & Rodriguez, J. (2007). Mutations in ionotropic AMPA receptor 3 alter channel properties and are associated with moderate cognitive impairment in humans. *Proceedings of the National Academy of Sciences*, *104*(46), 18163-18168.

Wurst, W., Auerbach, A. B., & Joyner, A. L. (1994). Multiple developmental defects in Engrailed-1 mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. *Development*, *120*(7), 2065-2075.

Xie, M.-J., Yagi, H., Kuroda, K., Wang, C.-C., Komada, M., Zhao, H., Sakakibara, A., Miyata, T., Nagata, K.-i., & Oka, Y. (2013). WAVE2–Abi2 Complex Controls Growth Cone Activity and Regulates the Multipolar–Bipolar Transition as well as the Initiation of Glia-Guided Migration. *Cerebral cortex*, *23*(6), 1410-1423.

Xie, Y., Vessey, J. P., Konecna, A., Dahm, R., Macchi, P., & Kiebler, M. A. (2007). The GTP-binding protein Septin 7 is critical for dendrite branching and dendritic-spine morphology. *Current Biology*, *17*(20), 1746-1751.

Yamada, J., & Jinno, S. (2017). Molecular heterogeneity of aggrecan-based perineuronal nets around five subclasses of parvalbumin-expressing neurons in the mouse hippocampus. *Journal of Comparative Neurology*, *525*(5), 1234-1249.

Yamagata, M., Weiner, J. A., & Sanes, J. R. (2002). Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. *Cell*, *110*(5), 649-660.

Yan, Y., Gnanapragasam, A., & Bailey, S. (2020). HisTrader: A Tool to Identify Nucleosome Free Regions from ChIP-Seq of Histone Post-Translational Modifications. *bioRxiv*, 2020.2003.2012.989228. https://doi.org/10.1101/2020.03.12.989228

Yanagihara, S., & Yazaki-Sugiyama, Y. (2016). Auditory experience-dependent cortical circuit shaping for memory formation in bird song learning. *Nature communications*, *7*(1), 11946.

Yang, X., Kuo, Y., Devay, P., Yu, C., & Role, L. (1998). A cysteine-rich isoform of neuregulin controls the level of expression of neuronal nicotinic receptor channels during synaptogenesis. *Neuron*, *20*(2), 255-270.

Yang, Y., Shu, X., Liu, D., Shang, Y., Wu, Y., Pei, L., Xu, X., Tian, Q., Zhang, J., & Qian, K. (2012). EPAC null mutation impairs learning and social interactions via aberrant regulation of miR-124 and Zif268 translation. *Neuron*, *73*(4), 774-788.

Yasuda, R., Hayashi, Y., & Hell, J. W. (2022). CaMKII: a central molecular organizer of synaptic plasticity, learning and memory. *Nature reviews neuroscience*, *23*(11), 666-682.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics*, *13*, 1-11.

Ye, X., & Carew, T. J. (2010). Small G protein signaling in neuronal plasticity and memory formation: the specific role of ras family proteins. *Neuron*, *68*(3), 340-361.

Yeo, T. T., Yang, T., Massa, S. M., Zhang, J. S., Honkaniemi, J., Butcher, L. L., & Longo, F. M. (1997). Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. *Journal of neuroscience research*, *47*(3), 348-360.

Yip, Z. C., Miller-Sims, V. C., & Bottjer, S. W. (2012). Morphology of axonal projections from the high vocal center to vocal motor cortex in songbirds. *Journal of Comparative Neurology*, *520*(12), 2742-2756.

Yoder, K. M., Phan, M. L., Lu, K., & Vicario, D. S. (2015). He hears, she hears: are there sex differences in auditory processing? *Developmental neurobiology*, *75*(3), 302-314.

Yokoi, M., Kobayashi, K., Manabe, T., Takahashi, T., Sakaguchi, I., Katsuura, G., Shigemoto, R., Ohishi, H., Nomura, S., & Nakamura, K. (1996). Impairment of hippocampal mossy fiber LTD in mice lacking mGluR2. *Science*, *273*(5275), 645-647.

Yoshizawa, A., Nakahara, Y., Izawa, T., Ishitani, T., Tsutsumi, M., Kuroiwa, A., Itoh, M., & Kikuchi, Y. (2011). Zebrafish Dmrta2 regulates neurogenesis in the telencephalon. *Genes to Cells*, *16*(11), 1097-1109.

Youn, Y. H., & Han, Y.-G. (2018). Primary cilia in brain development and diseases. *The American Journal of Pathology*, *188*(1), 11-22.

Yu, A. C., & Margoliash, D. (1996). Temporal hierarchical control of singing in birds. *Science*, *273*(5283), 1871-1875.

Yu, G., Wang, L. G., & He, Q. Y. (2015). ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics*, *31*(14), 2382-2383. https://doi.org/10.1093/bioinformatics/btv145

Zainolabidin, N., Kamath, S. P., Thanawalla, A. R., & Chen, A. I. (2017). Distinct activities of Tfap2A and Tfap2B in the specification of GABAergic interneurons in the developing cerebellum. *Frontiers in molecular neuroscience*, *10*, 281.

Zalcman, G., Federman, N., & Romano, A. (2018). CaMKII isoforms in learning and memory: localization and function. *Frontiers in molecular neuroscience*, *11*, 445.

Zann, R. A. (1996). *The zebra finch: a synthesis of field and laboratory studies* (Vol. 5). Oxford University Press.

Zemel, B. M., Nevue, A. A., Dagostin, A., Lovell, P. V., Mello, C. V., & von Gersdorff, H. (2021). Resurgent Na+ currents promote ultrafast spiking in projection neurons that drive fine motor control. *Nature communications*, *12*(1), 6762.

Zerucha, T., Stühmer, T., Hatch, G., Park, B. K., Long, Q., Yu, G., Gambarotta, A., Schultz, J. R., Rubenstein, J. L., & Ekker, M. (2000). A highly conserved enhancer in the Dlx5/Dlx6Intergenic region is the site of cross-regulatory interactions betweenDlx genes in the embryonic forebrain. *Journal of Neuroscience*, *20*(2), 709-721.

Zhang, D., Zeldin, D. C., & Blackshear, P. J. (2007). Regulatory factor X4 variant 3: a transcription factor involved in brain development and disease. *Journal of neuroscience research*, *85*(16), 3515-3522.

Zhang, L., Spratt, S. K., Liu, Q., Johnstone, B., Qi, H., Raschke, E. E., Jamieson, A. C., Rebar, E. J., Wolffe, A. P., & Case, C. C. (2000). Synthetic zinc finger transcription factor action at an endogenous chromosomal site. Activation of the human erythropoietin gene. *J Biol Chem*, *275*(43), 33850-33860. https://doi.org/10.1074/jbc.M005341200

Zhang, N., Liu, L., Fan, N., Zhang, Q., Wang, W., Zheng, M., Ma, L., Li, Y., & Shi, L. (2016). The requirement of SEPT2 and SEPT7 for migration and invasion in human breast cancer via MEK/ERK activation. *Oncotarget*, *7*(38), 61587.

Zhang, Y., Liu, T., Meyer, C. A., Eeckhoute, J., Johnson, D. S., Bernstein, B. E., Nusbaum, C., Myers, R. M., Brown, M., Li, W., & Liu, X. S. (2008). Model-based analysis of ChIP-Seq (MACS). *Genome Biol*, *9*(9), R137. https://doi.org/10.1186/gb-2008-9-9-r137

Zhao, X. W., & Kishino, H. (2020). Multiple Isolated Transcription Factors Act as Switches and Contribute to Species Uniqueness. *Genes (Basel)*, *11*(10). https://doi.org/10.3390/genes11101148

Zheng, X.-M., Moncollin, V., Egly, J.-M., & Chambon, P. (1987). A general transcription factor forms a stable complex with RNA polymerase B (II). *Cell*, *50*(3), 361-368.

Zhou, L., Bar, I., Achouri, Y., Campbell, K., De Backer, O., Hebert, J. M., Jones, K., Kessaris, N., De Rouvroit, C. L., & O'Leary, D. (2008a). Early forebrain wiring: genetic dissection using conditional Celsr3 mutant mice. *Science*, *320*(5878), 946-949.

Zhou, L., Tissir, F., & Goffinet, A. M. (2008b). The atypical cadherin Celsr3 regulates the development of the axonal blueprint. Cortical Development: Genes and Genetic Abnormalities: Novartis Foundation Symposium 288,

Zhou, S., & Yu, Y. (2018). Synaptic E-I Balance Underlies Efficient Neural Coding. *Front Neurosci*, *12*, 46. https://doi.org/10.3389/fnins.2018.00046

Zhou, X., Nie, Z., Roberts, A., Zhang, D., Sebat, J., Malhotra, D., Kelsoe, J. R., & Geyer, M. A. (2010). Reduced NMDAR1 expression in the Sp4 hypomorphic mouse may contribute to endophenotypes of human psychiatric disorders. *Human molecular genetics*, *19*(19), 3797-3805.

Zhou, Z., Shen, Y., Yin, J., Xi, F., Xu, R., Lin, D., Saijilafu, Chen, J., & Wang, Y. (2019). Matrix remodeling associated 7 promotes differentiation of bone marrow mesenchymal stem cells toward osteoblasts. *Journal of Cellular Physiology*, *234*(10), 18053-18064.

Zuo, H., Glaaser, I., Zhao, Y., Kurinov, I., Mosyak, L., Wang, H., Liu, J., Park, J., Frangaj, A., & Sturchler, E. (2019). Structural basis for auxiliary subunit KCTD16 regulation of the GABAB receptor. *Proceedings of the National Academy of Sciences*, *116*(17), 8370-8379.

Zwaka, T. P., Skowronska, M., Richman, R., & Dejosez, M. (2021). Ronin overexpression induces cerebellar degeneration in a mouse model of ataxia. *Disease Models & Mechanisms*, *14*(6), dmm044834.

Zweier, M., Gregor, A., Zweier, C., Engels, H., Sticht, H., Wohlleber, E., Bijlsma, E. K., Holder, S. E., Zenker, M., & Rossier, E. (2010). Mutations in MEF2C from the 5q14. 3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression. *Human mutation*, *31*(6), 722-733.

Zweier, M., & Rauch, A. (2012). The MEF2C-related and 5q14. 3q15 microdeletion syndrome. *Molecular syndromology*, *2*(3-5), 164-170.