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THE ROLE OF MELATONIN IN BIOLOGICAL RHYTHMS OF SONGBIRDS

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DISSERTATION
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requirements for the degree of Doctor of Philosophy in the
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ABSTRACT OF DISSERTATION

THE ROLE OF MELATONIN
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In vertebrates, melatonin is a hormone that is produced and secreted at night and inhibited by light. This unique "darkness-only" expression profile makes it an intellectually appealing candidate for a means of transmitting temporal information to an individual, both time of day and time of year.

In passerine birds, "time of day" information is certainly transmitted via melatonin secretion. The primary producer of systemic melatonin in this family of birds is the pineal gland, and surgical removal of it causes a bird to become arrhythmic in constant conditions. I find that as pinealectomized house sparrows (*Passer domesticus*) become behaviorally arrhythmic in extended constant darkness the molecular clock in their peripheral tissues largely continues to cycle absent its hypothesized synchronizing cues from the pineal gland. This suggests that the peripheral tissues are either autonomous or that there is a possible secondary circadian oscillator that synchronizes these tissues, such as the avian suprachiasmatic nucleus.

In passerine birds, "time of year" information is not transmitted via melatonin secretion to the primary gonads, unlike the case in seasonally breeding mammals. The duration of melatonin, longer in the winter and shorter in the spring as the photoperiod changes with the seasons, does affect secondary sexual characteristics, such as the vocal behavior of birds and the size of the associated nuclei in the brain. I find that long durations of melatonin are sufficient in preventing the photoperiodic expansion of vocal state in male house sparrows. This vocal state change in males consists of the development of a dawn and dusk chorus, as well as a switch from a vocal subtype associated with the wintertime birds flocking together to one of mate attraction and territory defense. This dynamic was independent of the size of the gonads, which were consistent those of photostimulated males. I also investigate this vocal state change in outdoor captive sparrows. This vocal state change is also present in female house

sparrows, although unlike in male birds, the presence of absence of the pineal gland does not affect the timing of their vocalizations.

Further, I investigate the ability for rhythmic presentations of vocalizations to influence the circadian clock, in zebra finches, *Taeniopygia guttata*. Aural cues have been shown to act as a weak external cue for entraining a passerine bird's circadian clock. I presented various permutations of zebra finch vocalization: a single song played repeatedly, the same song played reversed, random tones, and live monitoring of a breeding colony entrained to a light-dark cycle. The live monitoring was the strongest aural cue, and more generally it appears that novelty and context enhance the effect of audio cues on the circadian clock.

These experiments in sum suggest a role of melatonin in gating the seasonal expression of vocalization behavior in house sparrows. In male birds, a component of the seasonal dynamic is the development of a multimodal rhythm to when during the day the birds vocalize as the days lengthen consistent with spring, suggesting that the circadian clock may be involved with this diel variability. Additionally, this vocal behavior can also feedback onto the circadian clock. The pineal gland and its primary hormone melatonin function, in part, to regulate complex behavioral rhythms in passerine birds.

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DEDICATION

To Taylor Sterry, thank you for believing in me.

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CHAPTER 1.   INTRODUCTION

The earth rotates about its tilted axis, the moon around it, both altogether orbiting the sun. These astronomical phenomena create periodic events on earth on several time scales: the changing tides with a period of 12.4 hours, the rising and setting of the sun with a period of 24 hours, and the lengthening and shortening of the photoperiod associated with the changing seasons. To organisms, these dynamics mean the arrival and departure of resources and risks, thus creating an evolutionary advantage to predicting these cyclical occurrences. As such, marine organisms living near the shore have endogenous circatidal rhythms of physiology and behavior (Tessmar-Raible et al. 2011). Further, almost every studied phylum has evolved a circadian clock with a period of approximately 24 hours. Additionally, most free-living multicellular organisms exhibit seasonal rhythms such as plumage, breeding, or hibernation (Dardente et al. 2014). This dissertation will focus on two of these biological rhythms: circadian and seasonal rhythms.

1.1   Circadian rhythms

1.1.1   The discovery of circadian rhythms

Circadian rhythms were first documented in the 4th century BCE in the writings of Androsthenes, a ship captain in Alexander the Great's army, when he noted the turning of the leaves of the tamarind tree over the course of the day (Bretzl 1903; McClung 2006). Around 1230 AD, Chinese writings by Tou Han-Chhing on acupuncture noted their effectives might change over the course of a day or throughout the year (Lu and Needham 1980). Much later, a formal experiment was performed by Jean-Jacques d'Ortous de Marain in 1729, where he tracked the leaf movements of mimosa plants placed in his basement, demonstrating that this movement did not depend on the sun to persist, and he suggested that these endogenously generated rhythms were related to how humans would continue to express sleep-wake cycles even when bedridden (de Marain 1729). This leaf movement research was expanded on and further detailed, and was the

only known circadian rhythm for nearly a century (Bunning 1960; Cumming and Wagner 1968).

Experiments describing circadian rhythms in animals happened over a hundred years later. Charles Darwin described diurnal locomotor movements in earthworms in 1881, in his final scientific book published before his death (Darwin 1881). Viennese scientist Artur Kiesel noticed a diurnal rhythm of eye pigment movement in the retinae of a noctuid moth (Kiesel 1894). Soon after, Patrick and Gilbert noticed that a sleep deprived human would have sleep propensity cycle with a period of approximately 24 hours (Patrick and Gilbert 1896; Dijk and von Schantz 2005). In 1918, Szymanski found a 24 hour rhythm in European earthworms, the resulting earthworms upon cutting one in half would express similar circadian properties (Szymanski 1918; Harker 1958). In 1922, Curt Richter published his thesis on the behavior of rats, noticing diurnal rhythms using both cage rattling and wheel running activity as outputs that would free-run in constant environmental conditions (Richter 1922).

Richter's quantification of behavior set a precedent for the development of the modern circadian rhythms field. Jurgen Aschoff performed experiments on daily rhythms in humans and other vertebrates beginning in 1952. Among these, Aschoff bred several generations of birds and mice in constant dark conditions, proving that a prior exposure to a 24-hour cycle is not required for an individual's circadian clock to function (Aschoff and Meyer-Lohmann 1954; Aschoff 1955; Daan 2010). He coined the term *zeitgeber*, German for "time giver," for the external cues that are capable of entraining circadian clocks (Aschoff 1954). Aschoff went on to build a bunker where he could study circadian rhythms of humans in constant conditions, proving that humans are just as governed by these cycles as animals (Aschoff 1965). The term "circadian" was coined in 1959 by Franz Halberg, who contributed much to the field in regard to the biological rhythms in human health, including infradian and ultradian rhythms (Halberg 1969). "Circa" meaning about, and "dian" meaning a day, an organism's endogenous free-running period is rarely exactly 24 hours; this mismatch is thought to add lability to the clock. Colin Pittendrigh, inspired by a lecture he saw on the sun compasses of birds and preceding work on insect clocks, performed seminal experiment where he submerged

Drosophila pseudoobscura in an abandoned outhouse or in a pressure cooker in a mountain creek—a rudimentary means of controlling the temperature of the environment, discovering that with a decrease in ambient temperature, the speed of the clock did not slow down as much as if it relied simply on thermodynamic mechanisms (Pittendrigh 1993). This was then formalized in the laboratory (Pittendrigh 1954). This research from the mid-20th century produced the criteria for a rhythm to be considered "circadian" it must be 1) endogenously generated with a period of approximately 24 hours, 2) entrainable to external cues 3) persist in constant conditions and 4) temperature compensated, such that their Q_{10} temperature coefficient is close to one rather than the more normal two to three; that an organism's clock will be regulated such that it will still run at an appropriate 'speed' no matter if it gets abnormally hot or cold (Pittendrigh 1993).

1.1.2   The molecular clock

The next era of circadian research began with the generation of three clock mutant *Drosophila* by Seymour Benzer and Ronald Konopka in 1971. The circadian clock was a somewhat easy behavioral output to measure from fruit flies at the time. Thus, they generated three mutants: one arrhythmic, one with a free-running period of 19 hours, and one of 28 hours, all resulting from mutations in the same gene which they named *period* (Konopka and Benzer 1971). These were the first man-made behavioral mutant of any phenotype. Further characterization of the genetics of the *Drosophila* clock led to the Nobel Prize in Physiology in 2017 for Jeffrey Hall, Michael Rosbash, and Michael Young.

The first vertebrate clock mutant was discovered by Martin Ralph while in the laboratory of Michael Menaker, the *tau* mutant hamster (Ralph and Menaker 1988). This Syrian or golden hamster, *Mesocricetus auratus*, expressed an endogenous free-running period much shorter than the 24.1 hour period expressed by wild type hamsters, and was highly heritable, semidominant mutation at a single locus. Another landmark animal mutant was the *clockΔ19* mutant mouse produced by Joseph Takahashi in 1994, which was arrhythmic in constant conditions (Vitaterna et al. 1994). As the molecular era of

circadian research had begun, the gene responsible for the *tau* mutation in hamsters was revealed to be *ck1ε* via positional cloning (Lowrey et al. 2000).

Insights from these mutant animals and the explosion of molecular techniques led to the elucidation of the detailed innerworkings of the vertebrate molecular circadian clock. Briefly, this clock relies on transcription and translation of interlocking loops of genes that take about 24 hours to cycle (Figure 1.1). The positive elements, *bmal1* and *clock* (and *Npas2*) are translated and their proteins dimerize and translocate to the nucleus, where they bind to genes that contain e-box sequences in their promoter regions. Most important of these are the negative elements, the Cryptochromes and Period genes. Upregulated, the PERs and CRYs dimerize and translocate to the nucleus, where they interfere with the CLOCK/BMAL1 activation of transcription on e-box promoter elements. PERs and CRYs are targets for degradation via ubiquitination, relieving the repression on CLOCK:BMAL1, disinhibiting the cycle to begin anew. Another loop includes REV-ERBα, ROR-a, and DBP, which tune the transcriptional activity of Bmal1 and help link the molecular clock to many more clock-controlled genes: those containing the aforementioned e-box sequences, as well as d-box and Reverb-α/ROR response elements in their promoter regions (Partch et al. 2014; Cox and Takahashi 2019). Most of this knowledge of molecular clocks came from the study of mammals (nocturnal rodents, to be exact). Molecular clocks are presumed to be similar across all vertebrates, including birds, as homologs to these genes are present, and cycling (Yoshimura et al. 2000; Bell-Pedersen et al. 2005). Birds do not possess the Period1 gene.
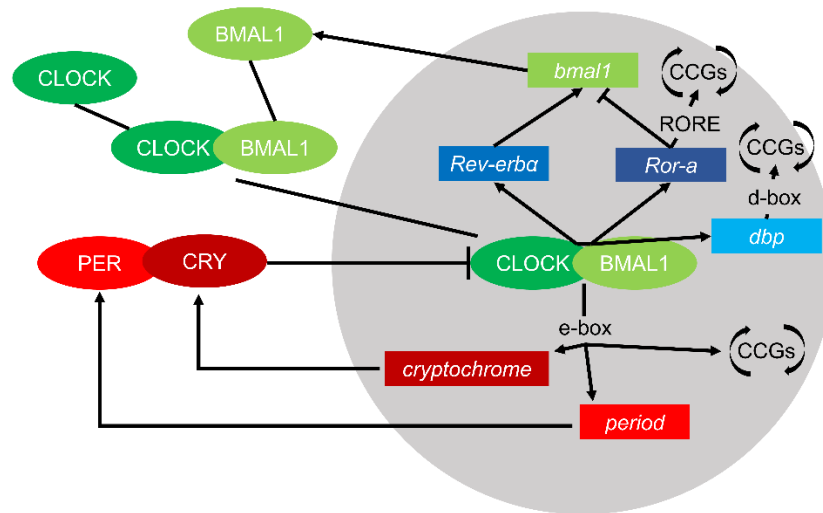
Figure 1.1 Diagram of vertebrate molecular clock
    Diagram of the molecular transcription-translation feedback look. Period genes can include Periods 1-3 (mammals) and 2 and 3 (birds), and cryptochromes 1 and 2. CCGs: core clock genes. Adapted from Cassone 2014 and Partch et al. 2014

1.1.3 Central control of circadian rhythms

    Vertebrates have their physiology and behavior under the control of this circadian clock. A well-studied physiology is body temperature. This output in true endotherm fits the criteria of a circadian rhythm, generally peaking at the time when the organism is most active and expressing their trough at times of inactivity, with species differences in the amplitude and time-of-peak (Refinetti 2010). Blood pressure is another physiology under clock control; in humans, for example, blood pressure falls during the day before rising again in the early morning, highest right after waking (Millar-Craig et al. 1978). Circadian rhythms also govern more systems-level physiology, such as immunity and metabolism (Rijo-Ferreira and Takahashi 2019). Behavior is a classically studied output for circadian clocks, as most of the properties of them were defined using wheel running or perch hopping activity beginning in the 1950s, as discussed previously. This extends to more complex behaviors, such as sleep (Franken and Dijk 2009) and feeding (Beldhuis et al. 1988; Pendergast and Yamazaki 2018). Pinealectomized zebra finches have their rhythms of song, call, and locomotor activity become arrhythmic at different rates when

individually housed in constant-dark conditions, and re-entrain to rhythmic presentation of melatonin at different rates, allowing the conclusion that these three behaviors are governed by separable circadian oscillators (Wang et al. 2012).

Mammalian circadian rhythms are integrated and broadcast via a single primary central pacemaker, the suprachiasmatic nucleus, or SCN (Weaver 1998). Curt Richter noted a single rat with a lesion in its anterior hypothalamus that had disrupted circadian rhythmicity (Richter 1965). In 1969, Robert Moore, curious about how light information might regulate the hypothalamic-pituitary axis, discovered that the retinohypothalamic tract was the structure through which light information reaches the SCN (Moore 1969; Moore and Lenn 1972; Moore 1973). Moore, Eichler and a second independent group lesioned the SCN, which produced arrhythmicity in adrenal corticosterone, and in locomotor and drinking behavior (Moore and Eichler 1972; Stephan and Zucker 1972). Transplantation of the SCN from an intact organism to one missing it confers rhythmicity at the phase of the donor (Lehman et al. 1987; Ralph et al. 1990). In sum, light information passes through the eyes and travels through the retinal hypothalamic tract to a collection of neurons above the optic chiasm and beneath the thirds ventricle, the SCN, which then projects to other brain centers (Hastings et al. 2018). The SCN is bipartite, with a retinorecipient "core" and a surrounding it, a "shell," with higher amplitude rhythms and more projections to elsewhere in the brain (Moore 1996a). In some mammals, this can be seen via a morphological delineation (Cassone et al. 1988). Even if no morphological difference is present, the mammalian SCN can be generally divided into a "core" population of cells that contain vasoactive intestinal peptide (VIP) and gastrin-releasing peptide (GRP), and a "shell" characterized via arginine vasopressin (AVP) expression (Moore 1996a).

The mammalian SCN innervates the pineal gland SCN by projecting to hypothalamic paraventricular nucleus, to the medial forebrain bundle, to the intermediolateral cell column of the upper thoracic spinal cord, to superior cervical ganglia (Moore 1996b). Outside of the brain, nearly every tissue in the body also contains its own circadian clock (Brown et al. 2019). The SCN affects the synchronization of peripheral tissues; despite being self-sustaining, as SCN lesion or brain-specific *Bmal1*

knockout causes the molecular clocks of tissues to fall out of phase with one another (Yoo et al. 2004; Tahara et al. 2012; Izumo et al. 2014). There are multiple suggested links between the SCN and the peripheral tissues., e.g. sympathetic tone is involved in the entrainment of circadian clocks in heart rate and liver function (Warren et al. 1994; Brusco et al. 1998; Shibata 2004; Cailotto et al. 2005; Cailotto et al. 2008), and a nonneural factor, as parabiosis between an SCN lesioned and intact mouse rescued the molecular rhythmicity of liver and kidney tissue (Guo et al. 2005).

     The central organization of avian circadian organism is more complicated, as there is more than one central pacemaker. The pineal gland is the primary pacemaker in oscine passerine birds, as surgical ablation causes the organism to become arrhythmic in constant environmental conditions over the course of several days (Gaston and Menaker 1968). Beyond the landmark study in house sparrow, pinealectomy abolishes or disturbs circadian rhythms in other oscine passeriformes: the Java sparrow, *Lonchura* (née *Padda*) *oryzivora* (Ebihara and Kawamura 1981); white-crowned sparrow, *Zonotrichia leucophrys* (Gaston 1971); white-throated sparrow, *Zonotrichia albicollis* (McMillan 1972); and house finch, *Haemorhous* (née *Carpodacus*) *mexicanus* (Fuchs 1983). Transplantation of the pineal gland from an entrained conspecific to the eye cup of an arrhythmic pinealectomized individual confers rhythmicity with the phase of the donor (Zimmerman and Menaker 1979). The pineal gland's entraining mechanism is likely due to the rhythmic secretion of its primary hormone, melatonin. Rhythmically administering melatonin to a pinealectomized songbird re-entrains the locomotor behavior (Lu and Cassone 1993a). The sympathetic superior cervical ganglia innervate the pineal gland, where the release of norepinephrine in correlation with the presence of light inhibits the pineal's production and thus release of melatonin (Cassone et al. 1986). The SCN may interface with this sympathetic input; the chicken vSCN has efferent and afferent projections and the mSCN afferent projections to the paraventricular nucleus (Cantwell and Cassone 2006a). Electrolytic lesion of the vSCN but not mSCN leads to the loss of norepinephrine turnover (Cassone et al. 1990). In addition to this noradrenergic input, the avian pineal gland itself is also directly photoreceptive (Binkley et al. 1978). Light passes through the skull and activates numerous photoreceptors, the types of which are species-specific but can include a pineal-specific opsin "pinopsin" as well as OPN4, or

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melanopsin, and OPN1, or iodopsin (Okano and Fukada 2001; Bailey and Cassone 2004; Bailey and Cassone 2005).

Intracranial photoreception is sufficient for entrainment of circadian clocks in birds, as house sparrows that have received bilateral enucleation can still entrain to light cycles, and the same for birds receiving both enucleation and pinealectomy (Menaker 1968; Menaker 1971). The sum of these extraretinal, extrapineal photoreceptors add an additional component to the central control of circadian rhythms in oscine passerine birds.

In taxonomic orders of birds beyond Passeriformes, the retinae also act as a pacemaker in this multi-organ system. In quail, the retinae themselves account for 13-50% of the serum melatonin content (Underwood et al. 1990). As such, pinealectomy does not abolish the locomotor activity rhythms in the Galliformes, the Japanese quail, *Coturnix japonica*, but enucleating the eyes does (Underwood and Siopes 1984). In the Columbiforme pigeon, *Columba livia*, neither pinealectomy nor enucleation alone abolishes overt rhythmicity of locomotor activity and body temperature, only the combination (Oshima et al. 1989). Further, the suboscine passerine bird the European starling also does not become fully arrhythmic upon pinealectomy (Gwinner 1978).

Birds also have a homolog to the mammalian SCN, that is split medial-lateral across the horizontal axis, and connected via an astrocyte bridge, the medial [m]SCN and visual [v]SCN (Cantwell and Cassone 2006a; Cantwell and Cassone 2006b). The vSCN, but not the mSCN, is retinorecipient (Cassone and Moore 1987), expresses rhythms in metabolic (Lu and Cassone 1993a; Lu and Cassone 1993b) and electrical outputs (Juss et al. 1994), and is sensitive to melatonin (Cassone and Brooks 1991; Lu and Cassone 1993b; Cassone et al. 1995; Cantwell and Cassone 2002). In house sparrow, both the mSCN and vSCN have rhythms in *per2* abundance (Abraham et al. 2002; Abraham et al. 2003). However in quail, only the mSCN has rhythms in clock gene abundance (Yoshimura et al. 2000; Yasuo et al. 2002). It is possible that in oscine passerine birds, this organ is a behaviorally relevant circadian pacemaker. There is evidence of a second pacemaker in oscine passerine birds since they do not become immediately arrhythmic upon release into constant conditions when pinealectomized, rather over the course of 3-7

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days or longer (Gaston and Menaker 1968). Also, pinealectomized oscine passerine birds are still able to entrain to light-dark cycles, as evinced by their anticipatory behavior before the simulated sunrise (Gaston and Menaker 1968). Electrolytic lesion aimed at the mSCN in Java sparrow and house sparrow caused the loss of overt rhythmicity in locomotor behavior (Ebihara and Kawamura 1981; Takahashi and Menaker 1982).

In sum, the central control of avian circadian rhythms is the sum of four pacemakers: the eyes, the pineal gland, some population of extraoptic photoreceptors, and a hypothalamic pacemaker (likely the bipartite SCN). The importance of these four oscillators may vary from order to order and even suborder to suborder; it is conceivable to consider the retinae of birds in which pinealectomy alone does not cause behavioral arrhythmicity as an additional, extrapineal source of the systemic melatonin, although they may have a further role (Cassone, Paulose, Harpole, et al. 2017). There are two theories for how these structures combine within the brain to serve this function of a central clock. In the "neuroendocrine loop model," the pineal and SCN are both damped oscillators. Damping can be caused by the cell autonomous clocks within these tissues going out of phase from one another. Melatonin secreted from the pineal gland at night inhibits many downstream structures, including the vSCN. The SCN has a similar action on the pineal during the day, where its norepinephrine secretion inhibits the pineal glands production and secretion of melatonin. This day-night switch, coupled with these two tissue's intrinsic rhythmicity, allows both tissues to synchronize and amplify the others' rhythms and function as a cohesive central clock (Cassone and Menaker 1984; Cassone, Paulose, Harpole, et al. 2017). The second model is the "internal resonance" model, in which each pacemaker synchronizes and amplifies the other through resonance, or strength gained from the coupling of oscillations, rather than inhibition (Gwinner 1989a). It is possible for both to be true (Woller and Gonze 2013). Through some deduction, it can be assumed that this central clock has a synchronizing effect on the peripheral organs and tissues, that have their own, separable circadian clocks, as is the case in mammals. This synchronizer could be the secretion of melatonin, some other circulating factor, sympathetic tone, and may vary from tissue-to-tissue. This synchronizer may function through phase shifts of the Period of Cryptochrome genes in response to light, or other factors, re-entraining daily to external cues.

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1.2 Photoperiodism and seasonal rhythms

1.2.1 The discovery of photoperiodism and seasonal rhythms

> *Once in each revolving year,*
> *Gentle Bird! we find thee here;*
> *When nature wears her summer vest,*
> *Thou com'st to weave thy simple nest.*

From "Ode XXV," Anacreon, 6th century BCE, (translated from Greek by Thomas Moore)

While the migrations and seasonal behaviors of birds have been noted for centuries, it was not until the late 1880s that the mechanism controlling the regulation of these behaviors began to be uncovered. A Finnish-Swedish poet Johan Ludvig Runeberg was bedridden for several years in Helsingfors in Finland, suggested that light-seeking behavior was the mechanism for avian migration. Runeberg…

> *"…occupied himself by close observation of the habits of birds, and specially with regard to the causes of migration, and he has at last put forward a singularly beautiful theory on the latter point. He believes, in fact, that it is the longing after light, and that alone, that draws the birds southwards. When the days shorten in the north, the birds go south, but as soon as ever the long northern nights set in, with all their luminous and long-drawn hours, the wanderers return to their old haunts. It is generally supposed that they move southward to get more abundant food; but why, asks Runeberg, do they leave their rich hunting-grounds to return to the north? The central regions of Europe are in every way more desirable than the wastes of Scandinavia. Only one thing is richer there, and that is light. The same instinct that makes plants firmly rooted in the ground strain towards the light, spreading upwards in search of it, works in the birds, who, on their free wings, fly after and follow it. This very suggestive and poetical notion is further carried out by reference to various analogies in natural history and the final sentence is quite epigrammatic: 'The bird of passage is of noble birth; he bears a motto, and his motto is Lux mea dux.'" ('The light is my guide') (Academy, 1874)*

Fourteen years later, Henry Seebohm is credited with first formally suggesting that the migrations of the family Charadriidae (e.g. plovers and sandpipers) were governed by photoperiod and not climate or abundance of resources (Seebohm 1888). Garner and Allard coined the term "photoperiodism" towards the regulation of seasonality in plants in 1920 (Garner and Allard 1920). In the mid-1920s, William Rowan formalized the effect of photoperiodism on migration in birds; by using artificial lights in an outdoor aviary during winter, he discovered that dark eyed juncos, *Junco hyemalis*, could experience photoperiodic stimulation of their testes, despite the temperature being cold (Rowan 1925; Rowan 1938). Rowan and the works of Jacques Benoit, to be discussed later, ushered in the modern field of avian seasonal research.

Seasonal vertebrates exhibit rhythms in changes in sexual physiology and behavior in preparation for climate events such as the cold and scarcity of winter (Gwinner 1989b; Gwinner 2003). Birds' annual life history events: breeding, plumage molt, migration and/or an overwintering state, need to be precisely timed for survival of the individual and any resulting young, as is theorized for all animals that experience photoperiodism (Bradshaw and Holzapfel 2007; Bradshaw and Holzapfel 2010). These large-scale rhythms are likely accomplished by a few interlocking mechanisms. First is photoperiodism, wherein the consistent changes in photoperiod correlating with the changes in season are anticipated and reacted to by the organism. Photoperiod is a consistent abiotic cue for the transmission of time-of-year information, more accurate than such cues as temperature, rainfall, resource abundance, or social cues from other animals. These additional cues are certainly involved in some processes and may tune the seasonal response, but photoperiodism is considered the primary cue in avian seasonal induction (Hahn et al. 2015). Most researched birds are long-day breeders, wherein the lengthening days of spring are stimulatory. The emu, *Dromaius novaehollandiae*, is an exception, which breeds in short days (Blache et al. 2001).

There is also evidence of free-running circannual rhythms in some species, that may gate the photoperiodic or other cue's state changes by creating an anticipatory permissive environment. This evidence is generally deduced from keeping wild-caught birds in an artificial 12:12 photoperiod for multiple calendar years. As an example, an

individual African stonechat that was kept in these equinoctial conditions for 12 calendar years expressed a circannual free-running period of testicular activity and molt of approximately 9 months (Gwinner et al. 1995; Gwinner 2003). This is the longest circannual study shown to date, and suggests that circannual rhythms persist throughout the life of an adult bird (Rani and Kumar 2013). These circannual rhythms are capable of synchronizing to external cues, as stonechats receiving a consistent photoperiod length of 12.5 hours with simulated seasonal increases and decreases in intensity could synchronize the period of their circannual rhythm in testes size and molt to these cues (Gwinner and Scheuerlein 1998).

1.2.2   Central control of seasonal induction

Melatonin's darkness-only expression profile makes it an intellectually appealing molecule to transmit time-of-year information to seasonally breeding organisms, as photoperiod is a reliable marker of season. This is the case in seasonally breeding mammals, such as temperate zone ferrets and rodents (Hazlerigg 2012). Pinealectomy prevents the decrease in gonad size associated with a decrease in photoperiod marking the end of the breeding season in these organisms (Reiter 1974; Herbert et al. 1975; Reiter 1975). This effect of the pineal is mediated by the nocturnal secretion of melatonin, which mirrors the scotoperiod, or the length of the night, as measured by blood melatonin titers in the long nights of the winter and the short days of the spring (Cassone 1990; Klein et al. 1997). Administration of a long, winter-like durations of melatonin is sufficient to cause mammalian gonadal regression as in a shortening photoperiod (Tamarkin et al. 1977; Goldman et al. 1979). These melatonin duration effects are mediated through high affinity melatonin receptors (Mel1a/MT1 and Mel1B/MT2) expressed in the mammalian *pars tuberalis* of the adenohypophysis (Morgan et al. 1994; Reppert 1997; Hanon et al. 2008; Ono et al. 2008; Yasuo et al. 2009).

In contrast with mammals, melatonin does not interface with the avian HPG axis. Pinealectomy and ocular enucleation have little effect on the size or activity of the gonads (Siopes 1983; Bentley 2001; Kumar et al. 2002). The *pars tuberalis* in passerine birds lacks melatonin receptor binding (Rivkees et al. 1989; Cassone et al. 1995) or receptors

(Reppert et al. 1995). Seasonal induction is instead transmitted through extra-optic and extra-pineal photoreceptors in the medial basal hypothalamus (Cassone and Yoshimura 2015). The role of extraoptic photoreceptors in avian photoperiodism has been known since the 1930s when Benoit showed that ducks can be photoinduced when enucleated or with denervation of the optic nerve (Benoit 1935a; Benoit 1935b), which he and colleagues later triangulated to the hypothalamus (Benoit 1964). Further, in house sparrows, the lack of photoinduction from decreasing the light intensity below the inducible threshold can be relieved by plucking the feathers from the skull, allowing more light to pass through the skull. Injecting India ink beneath the skull of these animals again prevents their photoinduction. Further, enucleated and pinealectomized passerine birds are still stimulated by increases in photoperiod (Menaker et al. 1970). The molecular pathway underlying this deep brain photoreceptor induction has been well-studied in Japanese quail. Stimulating the medial basal hypothalamus with a light pulse associated with long day induction of reproduction increases the expression of *Dio2*, which encodes the protein that converts inactive thyroxine (T4) to the much more active triiodothyronine (T3) (Yoshimura et al. 2003). In contrast, light pulses consistent with the short days of winter increase expression of *Dio3*, which inactivates T3 (Yasuo et al. 2005). The mechanical ability for this organ to measure photoperiod is theorized to being its circadian clock (Ikegami and Yoshimura 2012). These photoperiod-dependent local concentrations of thyroid hormone may alter the morphology of the median eminence and allow for the seasonal secretion of GnRH (Yamamura et al. 2004). It is possible that this mechanism does not extend into other orders of birds, as seasonally breeding European starlings do not share this same *Dio2* expression pattern underlying gonadal maturation (Bentley et al. 2013).

In addition to the stimulation of the HPG axis, seasonal rhythms in birds manifest in many aspects of physiology and behavior. Besides reproduction, molt, migration, and/or a wintering state are expressed in annual cycles. These states can be broken into various substates, e.g. the makeup of 'reproduction' includes behaviors of courtship, egg laying, incubation, care of the young, with physiological states underlying these behaviors, including physical maturation of the gonads (Wingfield 2008). Having high quality feathers is a requirement for the survival of birds, and mistiming it during the

year, such that it would coincide with a migration could be disastrous. Thus, all birds molt at least once a year. The timing of this is species specific, but generally molting requires long photoperiods, occurring after the summer solstice, and photoperiod can modulate that rate of molt (Dawson 2015). This is the case in house sparrows, which molt spontaneously after an exposure to long days, but a return to short days speeds its onset (Dawson 1991a; Dawson 1998). The body composition of migratory birds has a seasonal dynamic in preparation for their journey by increasing their fat and muscle stores, sometimes by as much as 50% (Driedzic et al. 1993; Piersma and Jukema 2002). It has been theorized that since birds are capable of flight, they have a pressure to reduce the size of their organs when not in use (Dawson et al. 2001). As such, the gonads of a house sparrow can increase 400 times when in the breeding season of springtime versus the winter (Witschi 1935; Whitfield-Rucker and Cassone 2000). Further, the notion of adult neurogenesis was popularized from the observation that canary (*Sirenus sirenus*) that parts of the brain that are involved with juvenile learning and adult production of song, the song control system (SCS)(Nottebohm and Arnold 1976). Song control nuclei recrudesce during the breeding season to 10-30% larger than their unstimulated regressed state before becoming photorefractory in many passerine birds (Brenowitz 2004). Potentially associated with these increases in brain size, breeding and courtship behaviors are concentrated to certain times of year.

As important to the induction of these seasonal events are the cessation of them as the breeding season subsides. This has been well studied, as birds become photorefractory, wherein an increase in photoperiod fails to induce the stimulation that would normally occur when the animal is photosensitive. This manifests in captive studies where birds that are maintained in long-day conditions for a long time, their reproductive systems will spontaneously regress. Photorefractoriness can be relieved by a return to short day conditions for a time, restoring the adult birds photosensitivity (Dawson and Sharp 2007). Temperature has been shown to affect breeding cessation, as low temperature speeds the testicular regression when Japanese quail are exposed to short days (Oishi and Konishi 1978; Wada 1993; Ikegami et al. 2015).

1.2.3   Avian photoperiodism

While melatonin is not a major component of seasonal activation of the HPG axis, it is involved in the control of seasonal vocal behavior. The SCS of male house sparrows (Whitfield-Rucker and Cassone 1996), zebra finches (Gahr and Kosar 1996), and European starlings (Bentley 2003) express sexually dimorphic, high-affinity melatonin receptor binding. The expression of mRNA of the three melatonin receptors, Mel_{1A}, Mel_{1B}, and Mel_{1C} are differentially expressed in the male song control system (Fusani and Gahr 2015). Further, expression of melatonin receptor mRNA is upregulated in long days of spring and summer in European starlings (Bentley et al. 2013). Continuous melatonin administration in European starlings decreases the size of Area X and prevented the photoperiodic expansion of the HVC (Bentley et al. 1999). Long durations of melatonin consistent with winter nights prevent the expansion of the HVC and RA as the birds transition from short days to long days in house sparrows (Cassone et al. 2008) and Indian weaver bird, *Ploceus philipinnus* (Surbhi et al. 2015), with no effect of melatonin on the size of the gonads. Pinealectomized house sparrows had an drastic earlier yet unsustained increase in vocalization rate and complexity in a simulated summer, and failed to respond to a reduction in photoperiod as rapidly as did sham operated males (Wang et al. 2014). This suggests that the pineal gland affects the seasonal timing and disbursement of seasonal vocal development.

Photoperiodism is most often studied in male birds. However, as the production of an egg is more energetically costly to a female bird, one would predict that seasonal timing would be even more important to this neglected sex (Ball and Ketterson 2008). There is evidence of female photoperiodism in vocalization behavior, often in tropical birds, but in temperate birds as well. While male house sparrows develop a 400 times increase in the size of their testes, females still see an impressive 50-fold recrudescence of their follicle (Witschi 1935; Anderson 2006a). This neglect of study likely comes from lack of inclusion of female organisms in laboratory research. Also, female birds may require additional nonphotic cues to stimulate their seasonal breeding, such as the presence of nest material, temperature, or changes in diet (Ball and Ketterson 2008). In European starlings, male gonad recrudescence happens a month before the female follicle

growth (Williams 2012). Full-maturation of the gonads in wild-caught passerine females is unlikely in captivity (King et al. 1966). However, captive female starlings show similar increases in GnRH, and higher increases of plasma LH and FSH levels than their male counterparts in response to increases in photoperiod, although the downstream gonadal development does not take place (Dawson et al. 1985). Additionally, vocal complexity is mostly appreciated in male passerine birds, since in most temperate zone birds there is a sexual dimorphism favoring the male production of song. The production of songs by female passerines is underappreciated, despite it potentially being standard in recent common ancestors (Odom et al. 2014). There are duetting temperate zone species such as the northern cardinal, *Cardinalis cardinalis* (Jawor and MacDougall-Shackleton 2008), and in tropical species, where intraspecific competition is high, female song is more conspicuous (Ball 2016). Even in cases where song behavior between the sexes is similar, male birds have larger nuclei in their SCS (Ball and Ketterson 2008).

Adult neurogenesis was discovered in the 1960s by Joseph Altman in a series of experiments on rats and other small mammals that were largely ignored at the time (Altman 1962; Altman 2011). It was not until Fernando Nottebohm noticed that parts of canary brains would be larger in the springtime than the winter for this long-day breeding bird, and that these seasonal increases in size were caused, in part, by the addition of new neurons (Nottebohm and Arnold 1976; Goldman and Nottebohm 1983). The SCS consists of that auditory sensory regions of the brain, including Area F projecting to the caudal mesopallium and the caudomedial nidopallium. The auditory regions then connect to the HVC (formerly the hyperstriatum ventral, pars caudalis, then the High Vocal Center, now a formal name), which connects the ascending forebrain pathway and the descending hind brain pathway. The ascending forebrain pathway includes Area X, which connects to the lateral magnocellular nucleus of the anterior nidopallium (LMAN) through the dorsolateral nucleus in the thalamus. The descending hindbrain pathway is more involved with the physical production of vocalization. The HVC and LMAN both project to the RA, the robust nucleus of the archipallium. The RA projects to the tracheosyringeal portion of the hypoglossal nucleus (nXIIts), the cranial nerve that innervates the musculature of the syrinx, the avian vocal organ (C. K. Catchpole and Slater 2008a; Cassone and Yoshimura 2015)(Figure 1.2).
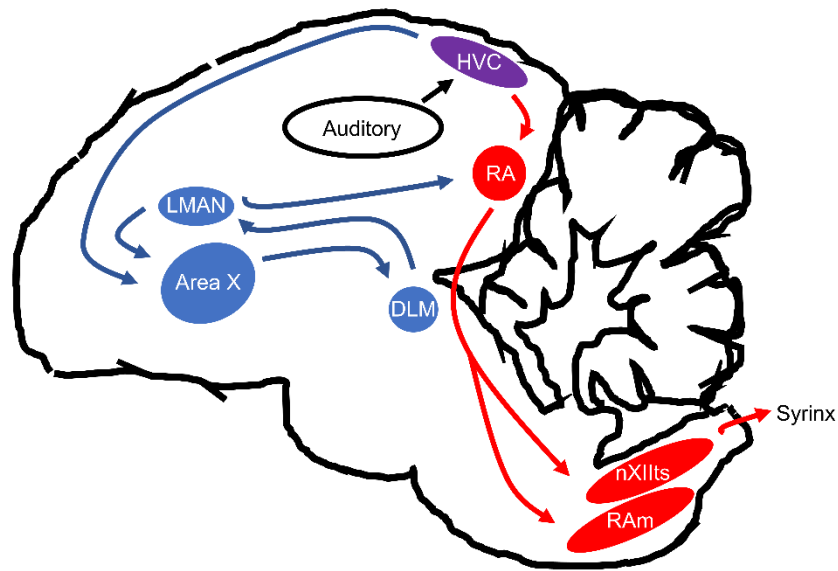
Figure 1.2 Diagram of song control system
Diagram of the major song control nuclei. Adapted from Brenowitz and Beecher 2005 and Nixdorf-Bergweiler and Bischof 2007.

The SCS is involved in the production of adult vocalizations. The functions of the SCS in the control of song behavior are best studied in juvenile birds, where these brain regions are involved with the acquisition of their species' song from a tutor (C. K. Catchpole and Slater 2008b), enhanced by their mother's reaction to their practicing (Carouso-Peck and Goldstein 2019). However, there have been several lesion studies in adult birds that point to a role for the SCS beyond learning. In canary, lesions of the HVC prevented the production of most sounds, although birds would still assume a singing posture, and lesions of RA or the hypoglossal nerve deteriorated the adult song (Nottebohm et al. 1976). Lesions of the HVC and RA in zebra finch, *Taeniopygia guttata*, abolished song behavior, but the lesioned birds still produced their unlearned calls (Simpson and Vicario 1990). Single unit recordings from the HVC and RA showed that specific motor neurons in the HVC were associated with particular syllables in the song of a mockingbird, *Mimus polyglottos*, followed by a subsequent firing of neurons in the RA (McCasland 1987) and in zebra finch (Yu and Margoliash 1996). There is a stereotyped temporal patterning of firing in the HVC of singing zebra finches (Hahnloser et al. 2002). In sum, these lesion and electrophysiological data form the working model

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that the descending motor pathway of the song control system includes the 'conductor' HVC to the 'muscians' of the RA (C. K. Catchpole and Slater 2008a). The role of the ascending forebrain pathway in the production of adult bird vocalization is less clear, even down to the mysteriously named "Area X" nuclei and how it only circuitously innervates the anatomically nearby LMAN. Neither lesion of the LMAN (Bottjer et al. 1984) nor Area X (Sohrabji et al. 1990) in adult zebra finches affected the song production, only song learning when lesioned in juveniles. However, singing behavior in adults activates early immediate gene expression (Jarvis and Nottebohm 1997) in the HVC and electrical signals (Hessler and Doupe 1999) in Area X and LMAN, suggesting that they are indeed involved in the adult singing process in some capacity. The search for functional changes one would expect to justify the energetic costs of increasing the size of the song control nuclei each year has had mixed results (Devoogd et al. 1993; Garamszegi and Eens 2004). Even zebra finches, which are not seasonal but rather opportunistic breeders and have a generally thought-of-as undynamic song, continue with neurogenesis in their SCS throughout adulthood (Ward et al. 2001). It is possible that the dynamics in song that are expressed because of SCS size increases are more subtle and/or species-specific than previously measured, and thus are underestimated (Hedley et al. 2017).

1.3   Interaction

Circadian rhythms and seasonal rhythms interact. The circadian clock is involved in proper photoperiodic time measurement. It is not the photoperiod, scotoperiod, nor the ratio between these that is the mechanism for photoperiodic induction, rather time measurement from the circadian clock (Cassone and Yoshimura 2015). This has been known since the 1960s, when William Hamner was investigating the mechanism underlying how an organism "knows" when a photoperiod if long enough to trigger their photostimulation. Hamner, and many subsequent studies by himself and others, showed that house finches had a "photoinducible phase," that photostimulation would occur only when light was present at a particular time of day set by the individual's circadian clock (Hamner 1963; Cassone et al. 2009). This proposed mechanism is known as the "external coincidence" model. Long-day breeding birds exposed to short days with 6 hours of light,

18 hours of darkness (6:18 LD) will have regressed testes. However, birds that receive 5 hours of light, and the 6th hour of light occurring as a 1 hr pulse in the middle of the night can have their gonads recrudesce if the pulse is timed correctly (e.g. 11 hours after initial lights-on), presumably during their photoinducible phase (Menaker and Eskin 1967; Follett et al. 1974; Sharp 2005). Yoshimura and colleagues' discovered that a clock in the Japanese quail medial basal hypothalamus that is entrained by its local extraoptic photoreceptors may induce the photoperiodic expression of *Dio2* via external coincidence (Ikegami and Yoshimura 2012).There is an alternative theory to the clock's involvement in photoperiodic induction: that the circadian clock has (at least) two clocks, one that entrains to dawn and one that entrains to dusk, and as the days lengthen, the phase relationship between these two oscillators change. This model, proposed by Pittendrigh, is known as the "internal coincidence" model (Pittendrigh and Minis 1964).

The converse is also true: changes in photoperiodic state also alter the expression of circadian outputs in complex behavior. When migratory birds are kept captive, the presumed drive for flight causes migratory restlessness, or *Zugunruhe*, wherein the birds perch and produce a "wing-whirring" behavior (Wagner 1930). In nocturnal migrants Zugunruhe occurs overnight during spring and fall, but the birds are purely diurnal during the summer and winter. Pinealectomy abolishes the free-running rhythm of Zugunruhe in white-throated sparrows, *Zonotrichia albicollis*, and redheaded buntings, *Emberiza bruniceps* (McMillan 1972; Trivedi et al. 2016). The daytime and Zungunruhe behaviors of garden warblers, *Sylvia borin*, may be under the control of separable oscillators, thus the phase relationships of these two may function as a calendar under the mechanism of an internal coincidence model (Bartell and Gwinner 2005). This will be discussed in more detail later in this dissertation, but when during the day birds vocalize can change depending on time-of-year; the dawn chorus is often absent at the beginning of a breeding season and then develops as the season progresses (Staicer et al. 1996).

Melatonin is an important molecule in the timing of biological rhythms at both time scales of interest, as has been discussed previously. Homogenized bull pineal glands were shown to lighten the skin coloration of tadpoles from extract of bull pineal glands (McCord and Allen 1917). In 1958, dermatologists Lerner and colleagues first isolated it

(Lerner et al. 1958). In an interesting reversal, mammalian pineal melatonin is a critical molecule in seasonal induction, but has a limited role in the regulation of circadian rhythms in melatonin-competent mammals (Cassone 1992; Arendt 1998). In birds, serum melatonin is of critical importance to circadian rhythms, but does not affect seasonal gonadal recrudescence (Cassone et al. 2009). However, the temporal profile of melatonin is not wasted on seasonally breeding birds, as it affects photoperiodic dynamics beyond the gonads.

This dissertation aims to investigate further the role of melatonin on biological rhythms in passerine birds. As the oscine passerine bird's pineal gland is required for overt circadian rhythmicity in locomotor behavior, it is still unclear if rhythmic clock gene expression in the central clock or peripheral clocks is dependent and/or entrained by pineal melatonin. To test this, we measured clock gene abundance at different days into constant dark conditions in pinealectomized or sham operated house sparrows (Chapter 2). Since long durations of melatonin prevented the photoperiodic expansion of song control nuclei in house sparrows, we investigated whether this result followed in any resulting vocalization behavior. To do so, we pinealectomized every subject, and presented daily rhythmic melatonin in the drinking water for long and short durations, simulating the winter and spring nights, respectively. We measured when during the day the birds vocalized, and the types of vocalizations produced at three times of day (Chapter 3). To continue the trend of including more female birds in laboratory research, we transitioned photosensitive birds that had received pinealectomy or a sham surgery from short day to long day conditions. We considered if female house sparrows had similar responses to photostimulation as male birds by analyzing their vocal and locomotor behavior (Chapter 4). Returning to circadian rhythms, this dissertation applied more modern audio technology to a long-studied phenomenon wherein vocalization behavior can act as a weak zeitgeber to the avian circadian clock. Using zebra finches, we rhythmically presented various audio cues of differing contexts and amounts of novelty to formalize these parameters' effect on the clock (Chapter 5). Chapter 6 will be a synthesis of the research presented here and a discussion on how this fits into the literature. This dissertation explores the effect of melatonin on complex behavior in biological rhythms of seasonal and circadian rhythms, the interaction between these two rhythmic

timescales, and the interaction of these complex behaviors themselves when presented as a cue to the circadian clock.

CHAPTER 2.   EFFECT OF PINEALECTOMY ON SONGBIRD PERIPHERAL
RHYTHMS OF CLOCK GENES AND METABOLISM

2.1   Introduction

Circadian rhythms are cyclic events in physiology and behavior with a period of approximately 24 hours, that entrain to external cues, and persist in constant environmental conditions of constant darkness or constant dim light. The fundamental properties of these rhythms were first formalized in the 1950s and 60s, using clever behavioral experiments on diverse model organisms and mathematical modeling (Aschoff 1960; Pittendrigh 1960; Halberg 1969).

These rhythms are at least in part genetically determined. The first behavioral phenotype from genetic mutation was a clock mutant *Drosophila* in the 1970s, wherein mutations to the *period* gene would make the flies arrhythmic or express an abnormal free-running period (Konopka and Benzer 1971). Later, a clock phenotype was discovered in the Syrian hamster, *Mesocricetus auratus*, where a short free-running period locomotor activity clock phenotype was shown to be heritable (Ralph and Menaker 1988), which was later discovered to be the gene casein kinase 1 epsilon (Lowrey et al. 2000). In the 90s, a clock mutant mouse was intentionally engineered, which opened the molecular revolution in mammalian circadian genetics (Vitaterna et al. 1994), and leading to the first cloned clock gene, *Clock* (King et al. 1997).

The circadian field has since advanced with an explosion of molecular biological research to where the broad strokes of the core circadian clock are well known in mammals, through the cloning of genes, and forward and reverse genetics (Partch et al. 2014). Briefly, two main transcription factors act as positive elements of this mechanism, CLOCK and BMAL1, which increase the transcription of many genes that express an E-box in their promoters. Among these genes are the negative elements, clock, the cryptochromes and period genes. Once translated, these genes' proteins then translocate to the nucleus and interfere with the transcription of *clock* and *bmal1*, such that the positive elements of the clock essentially inhibit their own transcription. This process takes approximately 24hr to complete. CLOCK and BMAL1 also control a third transcriptional loop, which includes expression of REV-ERB-α and RORα, which

activate and inhibit *bmal1* transcription, respectively, and DBP, which links to many downstream clock-controlled genes. These three transcriptional and translation feedback loops are then able to alter the expression of many downstream clock-controlled genes that contain e- and d-box binding sites and ROREs.

In mammals, these molecular clocks cycle in almost every tissue, and these many oscillators are synchronized and coordinated by a single primary pacemaker, the suprachiasmatic nucleus (SCN) (Hastings et al. 2019). The mammalian SCN receives light information from the retino-hypothalamic tract (RHT) (Moore and Lenn 1972), which entrains this central pacemaker. Lesion of the SCN results in loss of overt rhythmicity in constant conditions, and the ability to entrain to a light:dark cycle (Moore and Eichler 1972; Stephan and Zucker 1972). Grafting of SCN tissue to an arrhythmic conspecific confers rhythmicity, with the donor's phase (Ralph et al. 1990). Regardless of whether an animal's activity patterns are diurnal or nocturnal, their SCN is more active during the day in both metabolism as measured by 2DG absorption (Schwartz et al. 1980; Schwartz et al. 1983; Jay et al. 1985; Cassone 1988; Rivkees et al. 1988) and electrical activity (Schaap et al. 2003; Kuhlman and McMahon 2006; Ko et al. 2009; Colwell 2011), circadian rhythms in firing action potentials peaking at around 6-10Hz in the middle of the day. Beyond this central organization, circadian rhythms cycle in nearly every tissue studied (Yoo et al. 2004; Brown et al. 2019). The best case for a behaviorally relevant pacemaker comes from a population of VIP-ergic cells in the SCN; in mice, firing of these neurons correlates with inactivity, and stimulation of them decreases locomotor activity (van Oosterhout et al. 2012; Mazuski et al. 2018). The SCN functioning in this manner and the molecular clock's links with metabolism at large are the working theory as to how circadian rhythms in behavior are regulated in mammals.

The molecular mechanisms of avian circadian clock appear to function similarly to that in mammals but have been studied less extensively, and some of their functions are assumed rather than demonstrated (Bell-Pedersen et al. 2005; Cassone 2014). Many homologs of the clock genes have been cloned and characterized in birds (Larkin et al. 1999; Noakes et al. 2000; Yoshimura et al. 2000; Brandstätter et al. 2001). The Aves class is, however, missing the *period1* gene (Yoshimura et al. 2000; Bailey et al. 2003;

Bailey et al. 2004; Yasuo et al. 2004; Yasuo and Yoshimura 2009). The avian clock genes also appear to function at least largely in the same way as in mammals, as shown by how they are rhythmically expressed in several tissues that have been studied (Bailey et al. 2002; Yasuo et al. 2002; Brandstätter and Abraham 2003; Singh et al. 2013).

The central control of circadian clocks is more complicated in oscine passerine birds than that in mammals, as there are several pacemakers. The pineal gland is required for overt rhythmicity in locomotor activity, as surgical removal of it results in birds becoming arrhythmic in constant conditions (Gaston and Menaker 1968). Transplantation of an entrained birds' pineal gland to the eye cup of a pinealectomized bird re-entrains the recipient, and with the phase of the donor (Zimmerman and Menaker 1979). This transplantation experiment, and others, provides evidence that some secreted factor, likely melatonin from the pineal gland is responsible for its pace-making abilities, through melatonin binding and the three known melatonin receptors throughout the body (Cassone 2014). As such, rhythmic presentation of melatonin can reinstate behavioral rhythms in song birds (Lu and Cassone 1993a; Wang et al. 2012). The pineal gland itself also demonstrates rhythmic expression of clock genes (Bailey et al. 2003). Avian clock genes have been shown to cycle in central and peripheral rhythms in passerine birds (Singh et al. 2013). These genes are also differentially regulated across different life history events in birds, showing that the clocks can be regulated across the seasons (Trivedi et al. 2014). Additionally, rhythms in 2-deoxy[^{14}C]glucose (2DG) absorption, a correlate to glucose metabolism, is entrained by melatonin in many avian tissues (Lu and Cassone 1993c; Adachi et al. 2002; Cantwell and Cassone 2002).

In contrast to mammals and their sole central pacemaker, there is evidence of a secondary or multiple behaviorally relevant circadian pacemakers in birds. In addition to the pineal gland, birds have a hypothalamic pacemaker, likely their homolog to the SCN (Ebihara and Kawamura 1981; Takahashi and Menaker 1982; Cassone and Menaker 1984). In non-passerine birds, the retinae also contribute to the systemic melatonin rhythms, thus pinealectomy alone is insufficient to abolish overt rhythmicity in locomotor behavior (Underwood and Siopes 1984; Underwood et al. 1990). When pinealectomized, oscine passerine birds take 5-15 days to become arrhythmic when introduced to constant

environmental conditions. Additionally, birds still express anticipatory locomotor activity when in a light-dark cycle, showing evidence that birds are fully capable of entraining to these cycles and that behavior is not solely masked (Gaston and Menaker 1968). This current experiment asks the question whether molecular rhythms in clock genes and metabolism in several tissues (muscle, liver, intestine, heart, and eye) follow a similar pattern to the deterioration of behavioral circadian amplitude.

2.2 Methods

2.2.1 Housing conditions and surgery

Adult house sparrows were caught locally using mist nets and allowed to acclimate in outdoor aviaries for at least 2 weeks prior moving to the individual cages (42cm L × 48cm W × 40cm H) in an indoor activity recording facility. The activity cage was made up of wire mesh and an infrared sensor was fitted on top of individual box, connected with the data acquisition system (Mini-Mitter Co., Sunriver, OR). The birds were visually isolated in the activity chambers, and white noise (average power ~ 21 dB, frequency range of 0 to 10kHz, inside the cabinets) was played as a background noise to increase acoustic isolation. The birds received a photoperiod of 12 hours of light, 12 hours of darkness (12:12 LD, 40 $\mu W/cm^2$/dim light (less than 1E-4 $\mu W/cm^2$; lights on at 06:00 h and lights off at 18:00 h). Food (2:1 white millet and chick starter) and water were provided *ad libitum.*

Surgeries were performed after 7-10 days in LD conditions. Pinealectomy (PINX) was performed as previously described (Lu and Cassone 1993a). Sham (SHAM) birds underwent identical surgery except pineal was not removed. Birds were allowed to recover from surgeries in LD for 7-10 days before being placed in constant darkness (DD).

All animals were treated in accordance with National Institutes of Health guidelines; procedures have been approved by the University of Kentucky Laboratory Animal Care Committee.

2.2.2 Experimental procedure

After recovery, birds were released into constant dark conditions (DD) and tissues were collected either on the day of exposure to DD (Day 0), 3 days of exposure to DD (Day 3), 10 days of exposure to DD (Day 10) or 17 days of exposure to DD (Day 17)(Figure 2.1 A-D). Birds were sacrificed and tissues collected every 6 hr at four time points, 5 or 6 birds per timepoint (and 7, once). We used the locomotor activity data to calculate our phase reference, where circadian time 0 (CT0) corresponded to activity onset, CT6 corresponded to mid-subjective day (middle of the active phase), CT12 corresponded to activity offset, and CT18 corresponded to mid-subjective night (middle of the inactive phase). For estimation of CT 0, we used the recording of previous days as a reference point. The number of animals for each these tissue collections were: Day 0: sham 24, PINX 24; Day 3: sham 22, PINX 21; Day 10: sham 21, PINX 21; Day 17: sham 21, PINX 25.
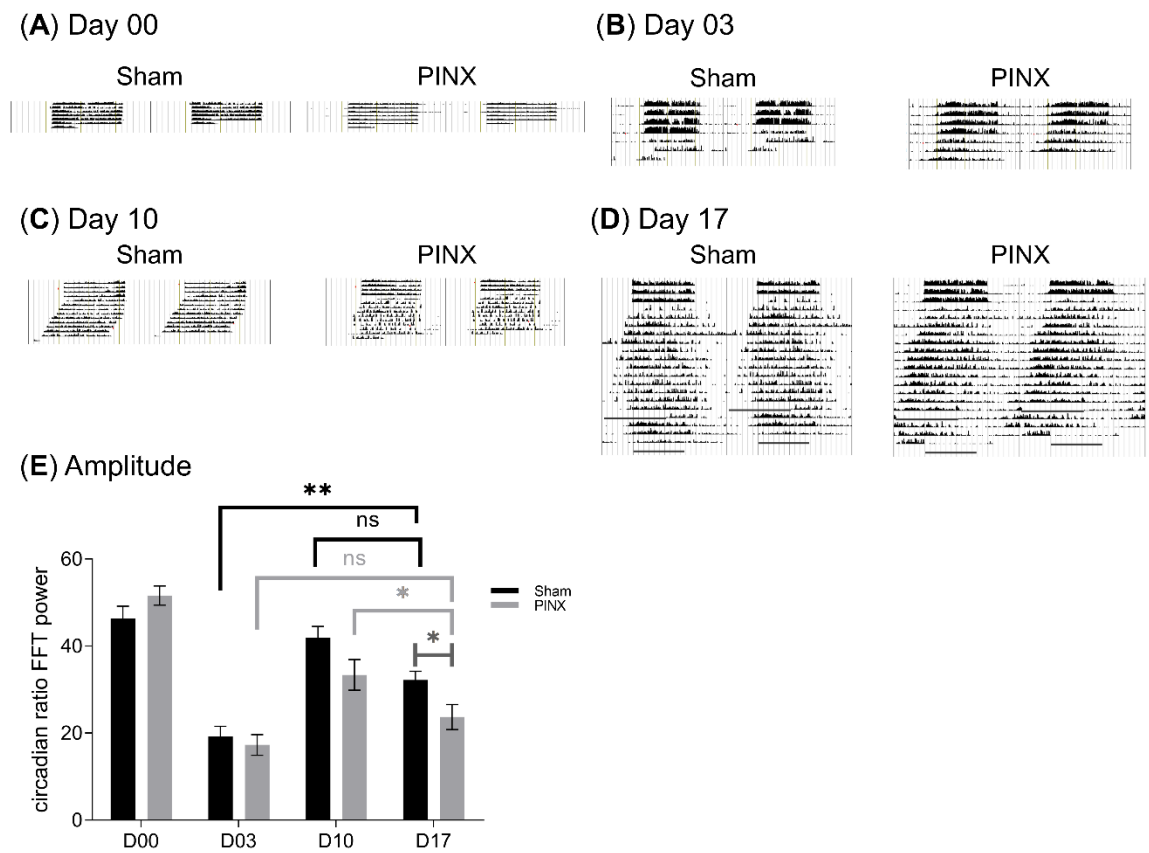
Figure 2.1 Experimental procedure and activity amplitude

*(previous page) **A.**–**D.** Representative actograms from sham operated and pinealectomized birds at the indicated timepoints. Actograms were chosen by having a locomotor activity amplitude near the mean amplitude for the indicated surgical group. **E.** Mean amplitude of locomotor activity per time point, as ratio of fast Fourier transform (FFT) of circadian periods (18 to 30 hours) to every period. Asterisks (*) above lines with ticks indicate a relevant statistically significant relationship in a 2-way ANOVA with a Tukey post-hoc multiple comparison test and p-value of 0.0465, 2 asterisks (**) above lines with ticks indicate a p-value of 0.0046. The letters "ns" above a ticked line indicates relevant non-significant result. An asterisks (*) above a capped line (as in 'H') indicates a p-value of 0.0252 in an ordinary paired t-test.*

2-Deoxy-d-glucose is a modified glucose molecule that competes with endogenous glucose-6-phosphate at the phosphoglucosisomerase step of glycolysis, where it is phosphorylated but unable to move forward through glycolysis. Accumulation of carbon-14 labelled 2DG in a given tissue is an analog of glucose absorption, which we refer to in short as metabolism. One hour prior to tissue collection, birds were injected with an intramuscular injection of 2-deoxy[^{14}C]glucose (2DG; 200µCi/kg; 300 mCi/mmol; American Radiolabeled Chemicals, St. Louis, MO). Exactly one hour after injection, animals were sacrificed by decapitation and the brain, eyes, breast muscle, liver, heart, and intestinal tissues were excised and frozen in dry ice

2.2.3   Tissue processing and 2DG uptake

Frozen tissue samples (100 mg) were placed in 1 mL TRIzol reagent (Invitrogen) and homogenized using a Fast Prep (FP 120; Thermo Electron) homogenizer. For the eye tissue, the vitreus humor was not included. An aliquot of tissue homogenate (100 µL) was used for beta emission using a liquid scintillation counter (Beckman Instruments LS 6500; Atlanta GA). 2DG uptake levels were calculated based on specific activity of the isotope (300mCi/mmol).

2.2.4   Real-time qPCR analysis

Total RNA was extracted from lysed homogenate using TRIzol reagent (Invitrogen). 1 µg of RNA was DNase (Promega) treated and then qScript™ cDNA SuperMix (Quanta Biosciences) was used to prepare cDNA. Quantitative real-time PCR

(qPCR) amplification and detection was performed on StepOnePlus™ real-time PCR instrument (Applied Biosystems, Foster City, CA) using Fast SYBR Green Master Mix (ABI). mRNA levels of targeted genes were determined according to Karaganis et al. (2009). We used the standard curve method of relative quantification and *β-actin* as an endogenous control gene. Each PCR plate included non-template control samples and samples which lacked reverse transcriptase during cDNA synthesis reaction. The primer sequences were as follows: for *per2* [GenBank: AY007259], forward: 5' - TGA AGC ACC CAG AAT TCC TC - 3' and reverse: 5' - TGC AAA ACC TGA TTG GTA AA - 3; for *per3* [GenBank: AF410957], forward: 5' - CCC AGA AGA TCG TCC TTT GA - 3' and reverse: 5' - TTC CTG CTC CAA GGA TTC AC - 3; for *cry1* [GenBank: AF410956], forward: 5' - ACA GCC AGC AGA TGT TTC CC - 3' and reverse: 5' - ATC CGA ACA ATG ACC TCC AC - 3'; for *clock* [GenBank: AF416453], forward: 5' - GTC CGA AAA GAA ACG TCG AG - 3' and reverse: 5' - TCC ATC TTC CGA GCA TTA CC - 3'; for *bmal1* [GenBank: AF410958], forward: 5' - TCT GCA GGA TGA AGT GCA AC - 3' and reverse: 5' - GCG GTC TGC TTT CTT CTT TG - 3'; for *β-actin* [GenBank: AF416454], forward: 5' - GAT GAA GCC CAG AGC AAA AG - 3' and reverse: 5' - TCT CCA TGT CAT CCC AGT TG - 3'; and for *mel1c* receptor [GenBank: AY743658], forward: 5' - GTC CTT TTT GCT GTG TGC TG - 3' and reverse: 5' - AGC CAT TCT GGA ATG TGT GG - 3'. The relative expression levels of mRNA were normalized from the maximum expression level of mRNA from the same group.

2.2.5   Locomotor activity analysis

To determine the amplitude of the locomotor activity, we used a measure that minimizes differences between the level of activity: we used the ratio of fast fourier transform (FFT) along circadian periods (18 to 30 hrs) compared with the ratio of the FFT for every possible period (Cassone 1992). We loaded the actogram data into ClockLab Analysis software (Actimetrics, Wilmette, IL), and calculated the two FFTs using three days of activity.

2.2.6 Statistical analyses

Two-way ANOVAs were calculated using GraphPad Prism 8 software (GraphPad Software, San Diego, CA), using a Tukey's post-hoc test for multiple comparisons. As we only had 4 timepoints per day in DD, ANOVA was the most sophisticated measure of rhythmicity we could appropriately utilize. For brevity in the results section, we use the word "rhythmic" for when circadian hour contributed to the variance of the data, but we acknowledge the weaknesses of this and that cosinor, periodogram, or auto-correlation would be preferred if the experimental design allowed it. We discuss "phase" as the time of the peak of a given timepoint and "amplitude" as the difference of the lowest of the four means subtracted from the highest.

An ordinary t-test was used to differentiate the day 17 locomotor activity amplitude, as a 2-way ANOVA of the total locomotor activity dataset does not allow for the assumption that one experimental group would change state over time.

Cohen's d values were calculated manually in Microsoft Excel from the difference in means between two experimental groups or timepoints of interest divided by the same two groups' pooled standard deviation. Results are presented as mean ± standard error.

2.3 Results

2.3.1 Locomotor behavior amplitude

By Day 17 in constant darkness, the pinealectomized birds had become arrhythmic in locomotor activity by our measures (Figure 2.1E). The ratio of FFT power for circadian periods to every other period for pinealectomized birds at day 17 in constant darkness was 23.7 ± 14.5, which was significantly less than they were at day 10 (33.4 ± 16.2, $p = 0.0465$, Cohen's $d = 0.631$), and not statistically different from Day 03 ($p = 0.3107$), where transients and low levels of activity over a small amount of relevant days falsely decrease birds' circadian FFT power ratio. There was no statistical difference in sham operated birds' circadian FFT power ratio between Day 10 and Day 17 ($p = 0.0595$).

However, the PINX birds had a significantly lower circadian FFT power ratio at Day 17 than the sham operated animals (32.2 ± 9.3, $p = 0.0252$, Cohen's $d = 0.699$) via an unpaired ordinary t-test.
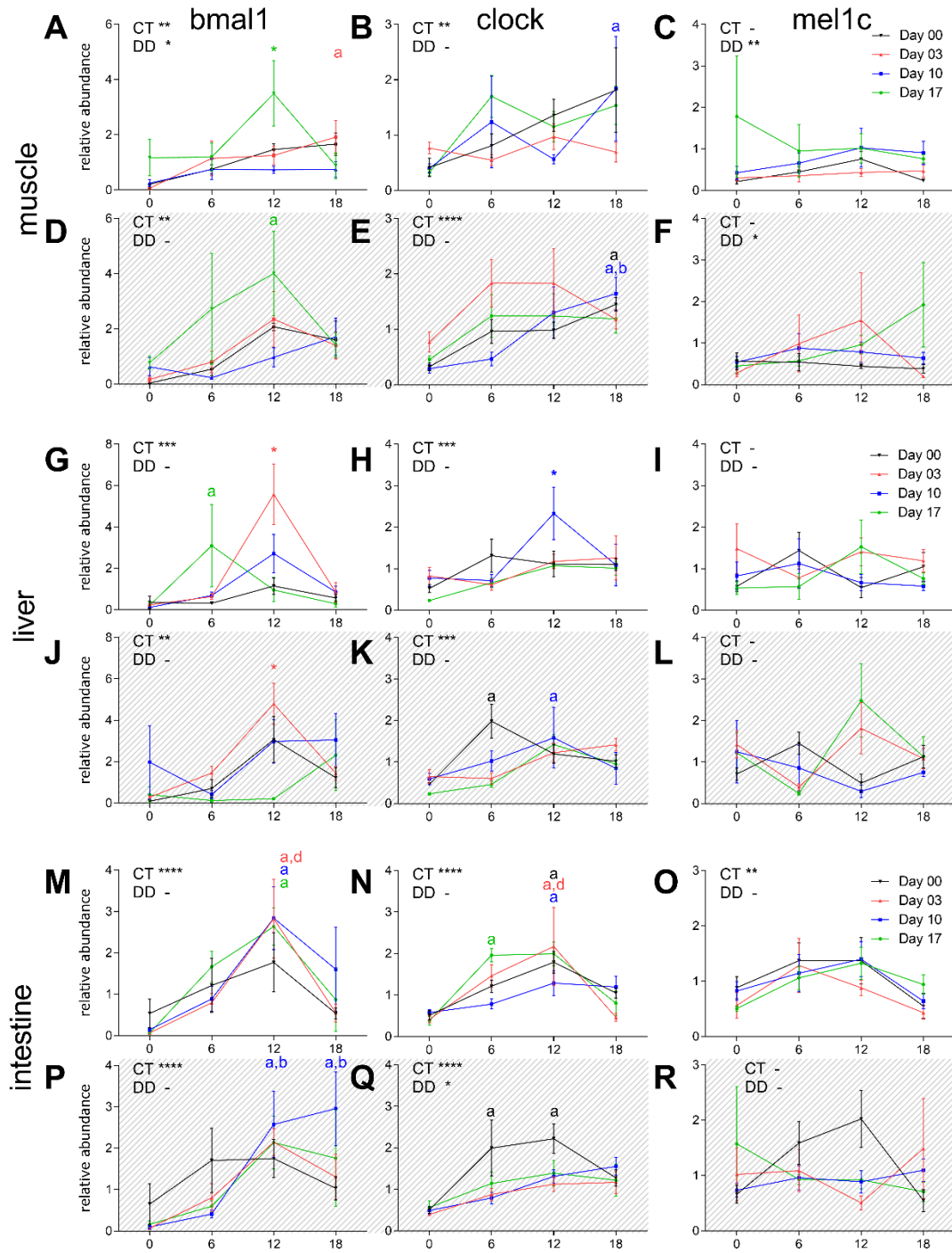
Figure 2.2 Positive elements for muscle, liver, and intestine

　　　mRNA abundance for the indicated gene and tissue via qPCR. Mel1C included here for space, it is not a canonical member of the core circadian clock. In the upper left, CT indicates that time of day contributed to the dataset's variance, DD indicates that days in constant darkness significantly contributed to the data (number of asterisks =

number of 0s in significance, '-' is non-significant). Over a particular data point, lowercase letters indicate a pairwise difference from a particular timepoint via a Tukey's post-hoc test, color-coded as the days in DD ('a' indicates 'different from CT00, 'b' = CT06, 'c' = CT12, 'd' = CT18, '*' = all other data points).

Figure 2.3 Positive elements for heart and eye

mRNA abundance for the indicated gene and tissue via qPCR. Mel1C included here for space, it is not a canonical member of the core circadian clock. In the upper left, CT indicates that time of day contributed to the dataset's variance, DD indicates that days in constant darkness significantly contributed to the data (number of asterisks = number of 0s in significance, '-' is non-significant). Over a particular data point, lowercase letters indicate a pairwise difference from a particular timepoint via a Tukey's post-hoc test, color-coded as the days in DD ('a' indicates 'different from CT00, 'b' = CT06, 'c' = CT12, 'd' = CT18, '*' = all other data points).

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2.3.2 Clock gene abundance

2.3.2.1 By tissue

Clock genes in muscle tissue were consistently rhythmic in both surgical conditions (Figures 2.2 and 2.4, A-F), with the exception of *mel1C*. Circadian time (hr) contributed to the variance in both surgical conditions, in every day measured in constant dark conditions (p-values ranging from <0.0001 to 0.016).

As in muscle, genes in liver tissue were also expressed consistently rhythmically in both surgical conditions, with the exception of *mel1c* (p-values ranging from <0.0001 to 0.0014)(Figures 2.2 and 2.4, G-L). Days spent in DD contributed to the variance of the expression of the period genes in liver tissue, as the amplitude of the rhythm dropped by day 17 in constant dark conditions (Figure 2.4 H, I, K, L).

Intestines expressed the most consistently rhythmic molecular clock, across all genes and the days in constant darkness (Figures 2.2 and 2.4, M-R). Circadian time of day contributed to the variance of the data in every gene and surgical condition (p values varying from <0.0001 to 0.0018) except the PINX birds' *mel1C* abundance. As these tissues remained rhythmic, one could conclude that intestinal tissue was also relatively resilient to surgery, as well. Intestine's molecular clock was resilient to days spent in DD, days in DD only contributed to the variance for *per3* (Figure 2.4 O,R) and PINX's *clock* expression (Figure 2.2Q).

Heart tissues' molecular clock was the least rhythmic across tissues (Figures 2.3 and 2.5, A-F). Clock genes were largely rhythmic in both surgical conditions for the negative elements (*cry1*, *per2*, *per3*). *Bmal1* was only rhythmically abundant in PINX birds and *Clock* was only rhythmically abundant in sham operated birds in this tissue.
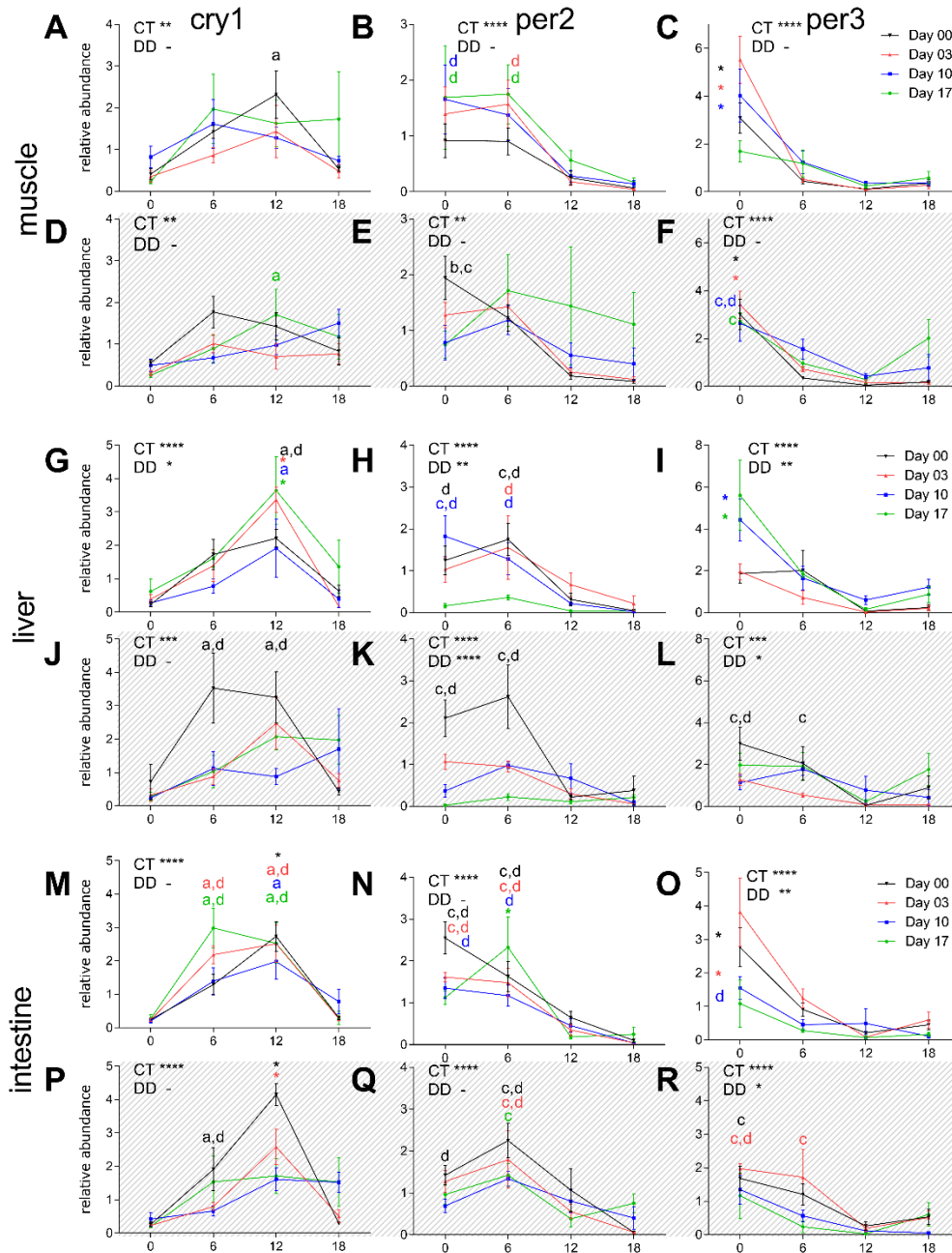
Figure 2.4 Negative elements for muscle, liver, and intestine

mRNA abundance for the indicated gene and tissue via qPCR. In the upper left, CT indicates that time of day contributed to the dataset's variance, DD indicates that days in constant darkness significantly contributed to the data (number of asterisks = number of 0s in significance, '-' is non-significant). Over a particular data point, lowercase letters indicate a pairwise difference from a particular timepoint via a Tukey's post-hoc test, color-coded as the days in DD ('a' indicates 'different from CT00, 'b' = CT06, 'd' = CT18, '' = all other data points).*

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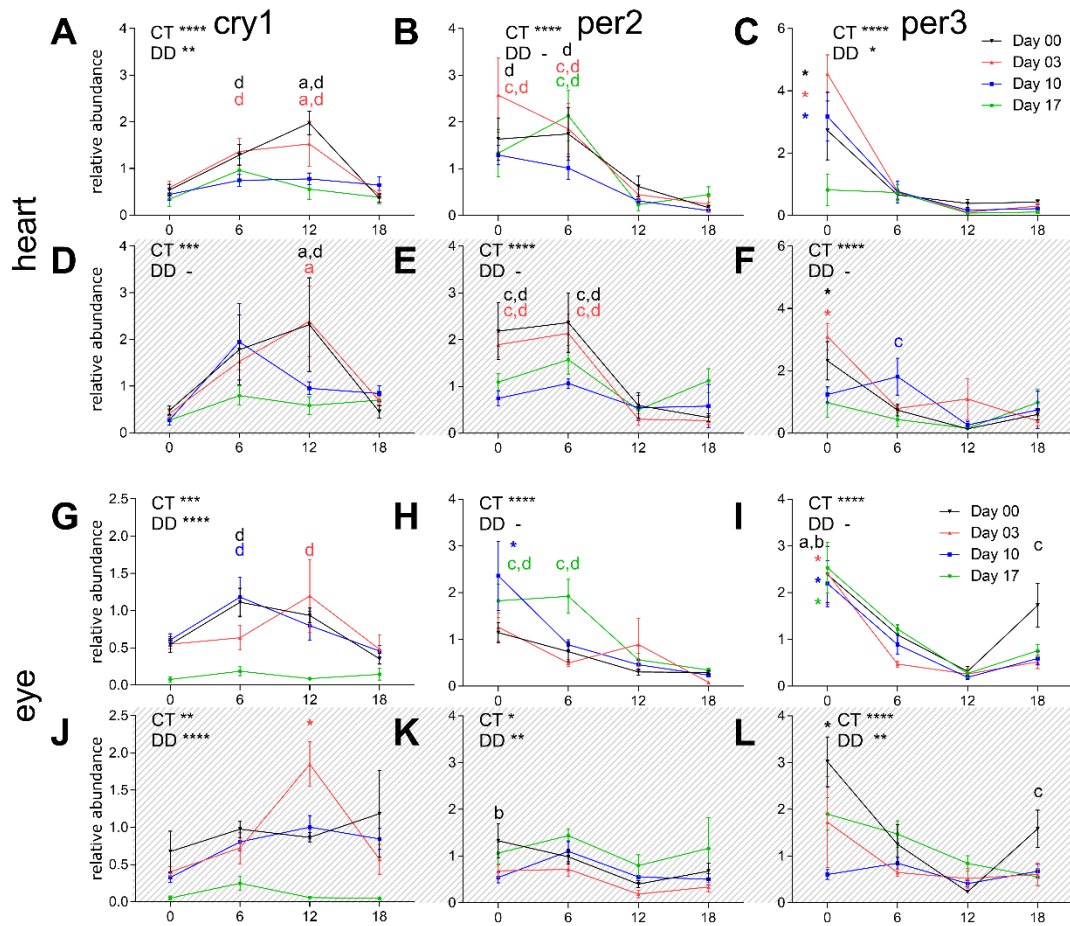
Figure 2.5 Negative elements for heart and eye

(previous page) - mRNA abundance for the indicated gene and tissue via qPCR. In the upper left, CT indicates that time of day contributed to the dataset's variance, DD indicates that days in constant darkness significantly contributed to the data (number of asterisks = number of 0s in significance, '-' is non-significant). Over a particular data point, lowercase letters indicate a pairwise difference from a particular timepoint via a Tukey's post-hoc test, color-coded as the days in DD ('a' indicates 'different from CT00, 'b' = CT06, 'c' = CT12, 'd' = CT18, '*' = all other data points).

Eye tissue's molecular clock was the quickest to deteriorate as the animals remained in constant dark conditions in pinealectomized tissue (Figures 2.3 and 2.5, G-L). Days in DD contributed to the variance to *bmal1*, *mel1c*, *per2*, and *per3* in pinealectomized birds (p-values ranging from 0.006 to 0.011), but also in *clock* and *mel1C* in Sham operated animals (p= 0.03 and 0.0045, respectively). For the molecular

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clock, if CT time contributed to the variance in the data, it occurred in both tissues. However, eyes were the only tissue in sham operated birds that did not have CT time contribute to the variance of *clock* gene expression.

2.3.2.2   By gene

Examining the qPCR results by gene also reveals some possible insights into the regulation of the molecular clock in house sparrows.

Bmal1 was consistently rhythmic across both surgical conditions (p-values ranging from <0.0001 to 0.016) (Figures 2.2, 2.3), and lost amplitude as the pinealectomized birds remained in DD for heart and eye tissue (p-values 0.0025 and 0.008, respectively).

Clock mRNA abundance was less rhythmic than its fellow positive element, *bmal1* (Figures 2.3). *Clock* was rhythmic in most measured tissues for sham animals, but PINX birds lost some *clock* rhythmicity in heart tissue. Eye tissue's clock abundance was not rhythmic in either surgical group. The positive elements mRNA abundance was roughly antiphase to the negative element *per3*, and possibly *per2* as well.

Mel1c mRNA was rarely rhythmic (Figures 2.3). Days in DD did contribute to the variability of some surgical groups and tissues.

Cry1 was rhythmic in all tissues and surgical groups (Figures 2.3, 2.4). However, it appears to be largely abundant in phase with Bmal1, both commonly peaking at CT=12.

Per2 and *per3* were also rhythmic in all tissues and surgical groups (Figures 2.3, 2.4). *Per3* consistently peaked at CT=0 across both surgical groups, but this consistent phase deteriorated in PINX birds' heart and eye tissue (Figure 2.7 U-Y).

Our measures of amplitude did not deteriorate in PINX birds' tissues while remaining elevated in sham operated birds' tissues (Figures 2.8 and 2.9).

2.3.3   2DG absorption

2DG absorption was not consistently rhythmic across tissues (Figure 2.6). It was rhythmic in both surgical groups in heart tissue (Sham and PINX, p <0.0001 and 0.0005, respectively), and rhythmicity was lost in pinealectomized birds in muscle and eye tissue (Sham p-values 0.0009 and 0.0088 to PINX p-values 0.4092 and 0.4868). Days spent in DD contributed to the variance in pinealectomized birds for every measured tissue (p-values ranging from <0.0001 to 0.0255), while muscle and liver were not affected by DD in sham operated animals.

(following page) - 2DG abundance for the indicated gene and tissue. In the upper left, CT indicates that time of day contributed to the dataset's variance, DD indicates that days in constant darkness significantly contributed to the data (number of asterisks = number of 0s in significance, '-' is non-significant). Over a particular data point, lowercase letters indicate a pairwise difference from a particular timepoint via a Tukey's post-hoc test, color-coded as the days in DD ('b' indicates 'different from CT06, 'd' = CT18, '*' = all other data points).
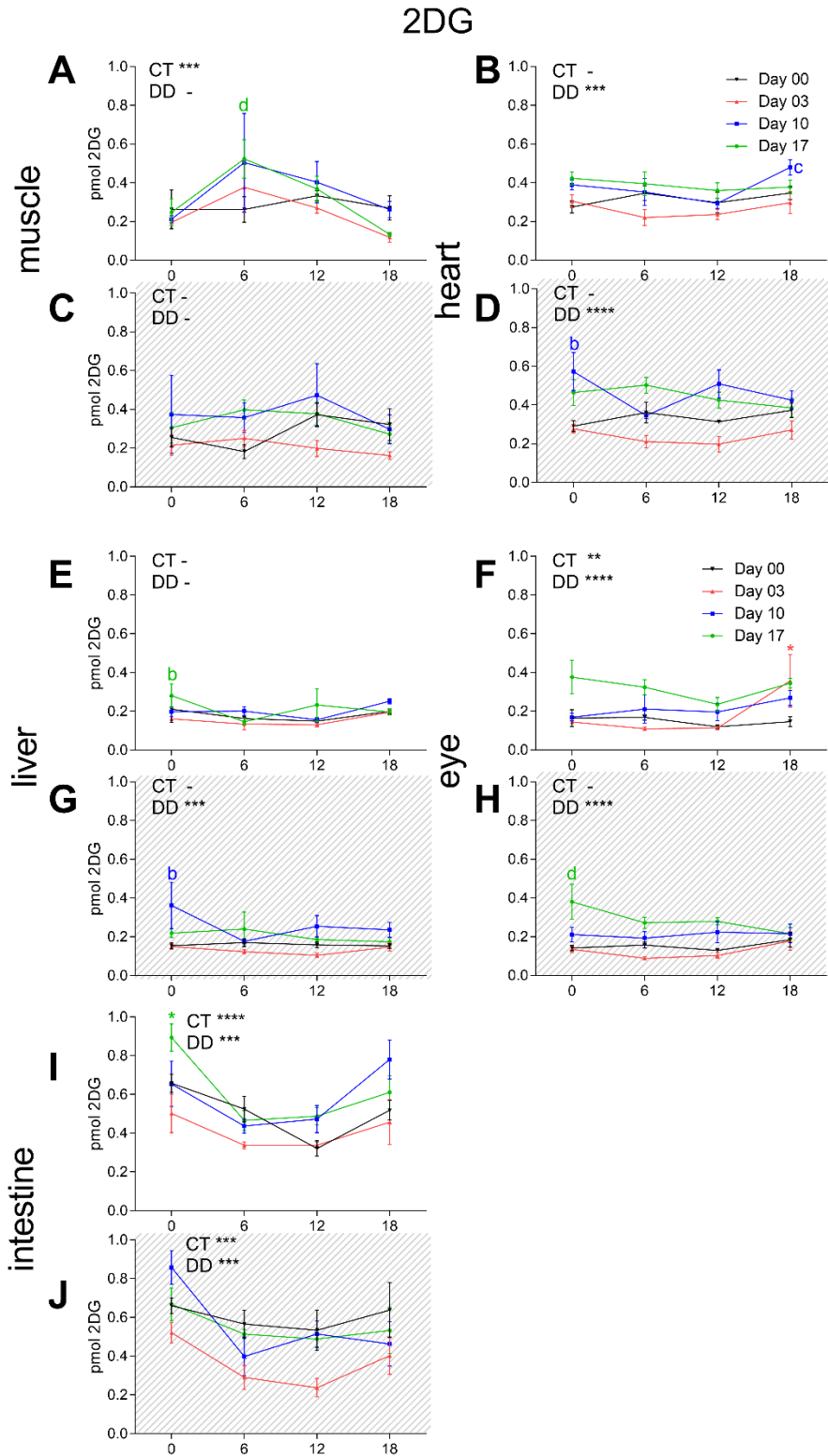
Figure 2.6 2DG Absorption
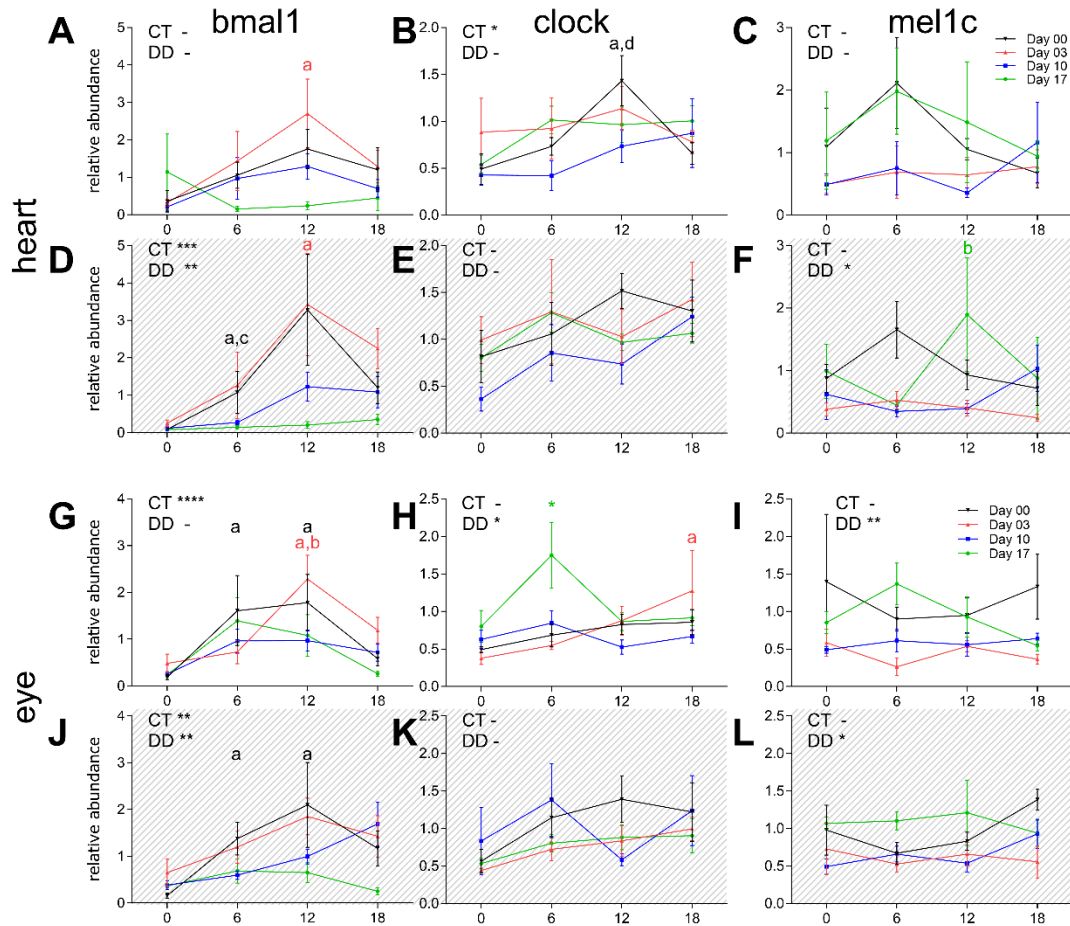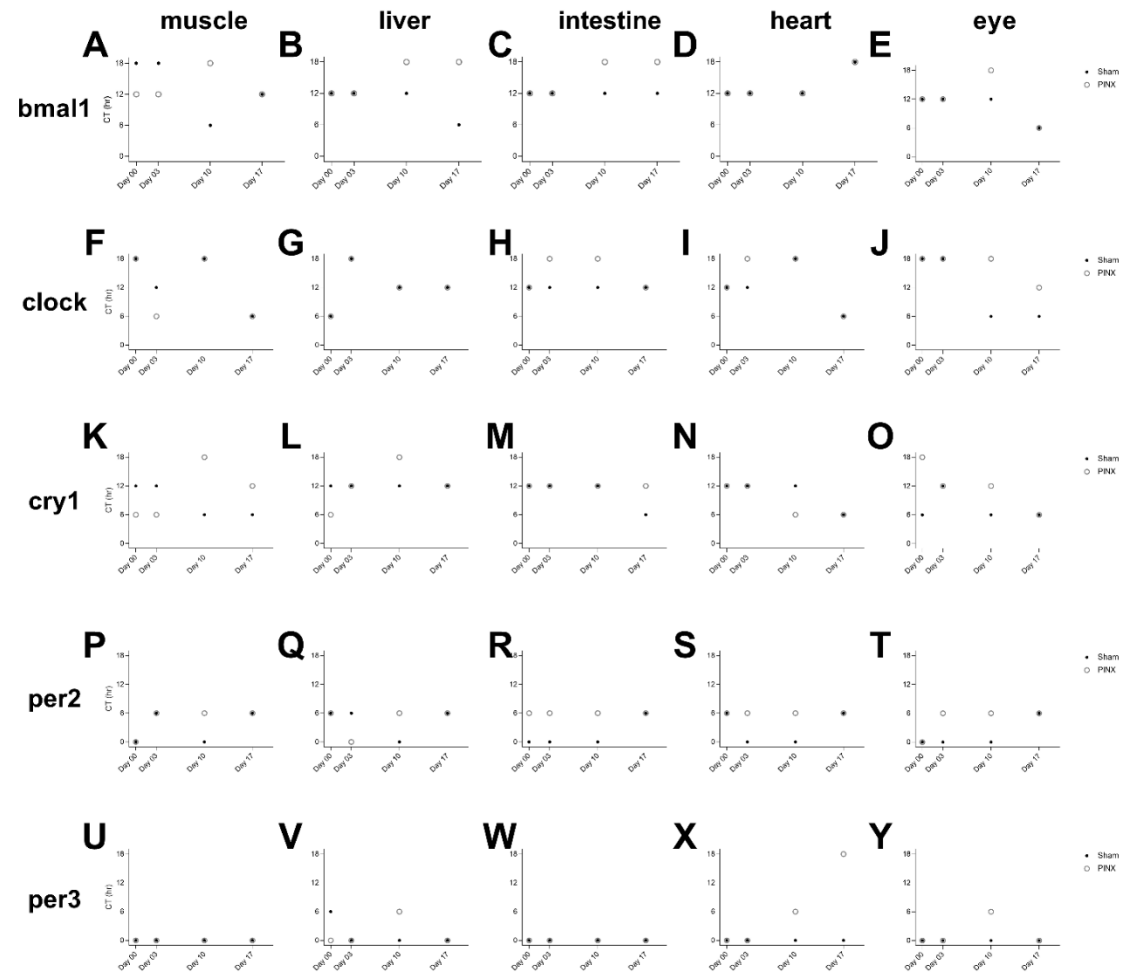
Figure 2.7 Phase
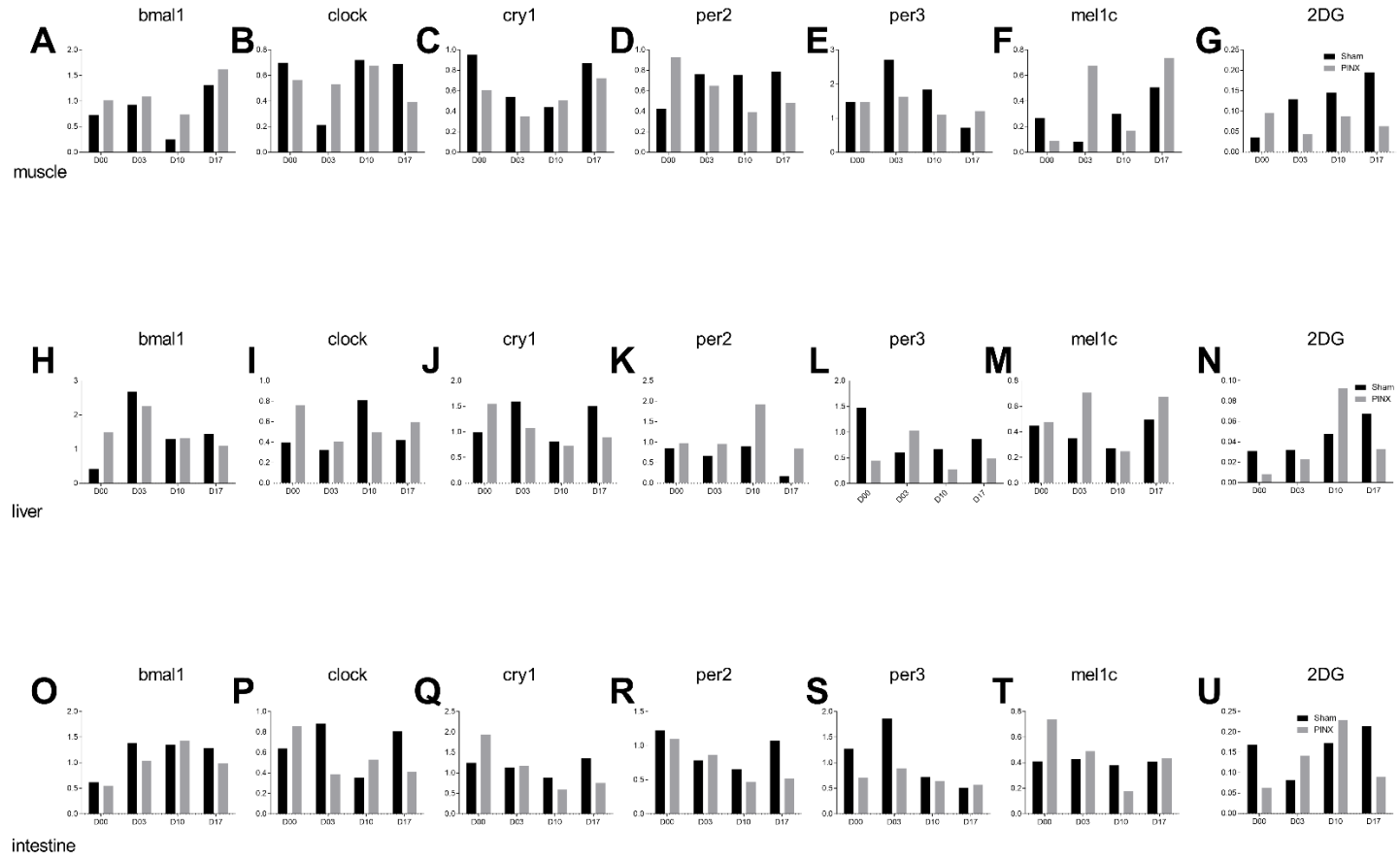
Figure 2.8 Amplitude: muscle, liver, intestine

A bmal1　B clock　C cry1　D per2　E per3　F mel1c　G 2DG

heart

H bmal1　I clock　J cry1　K per2　L per3　M mel1c　N 2DG

eye

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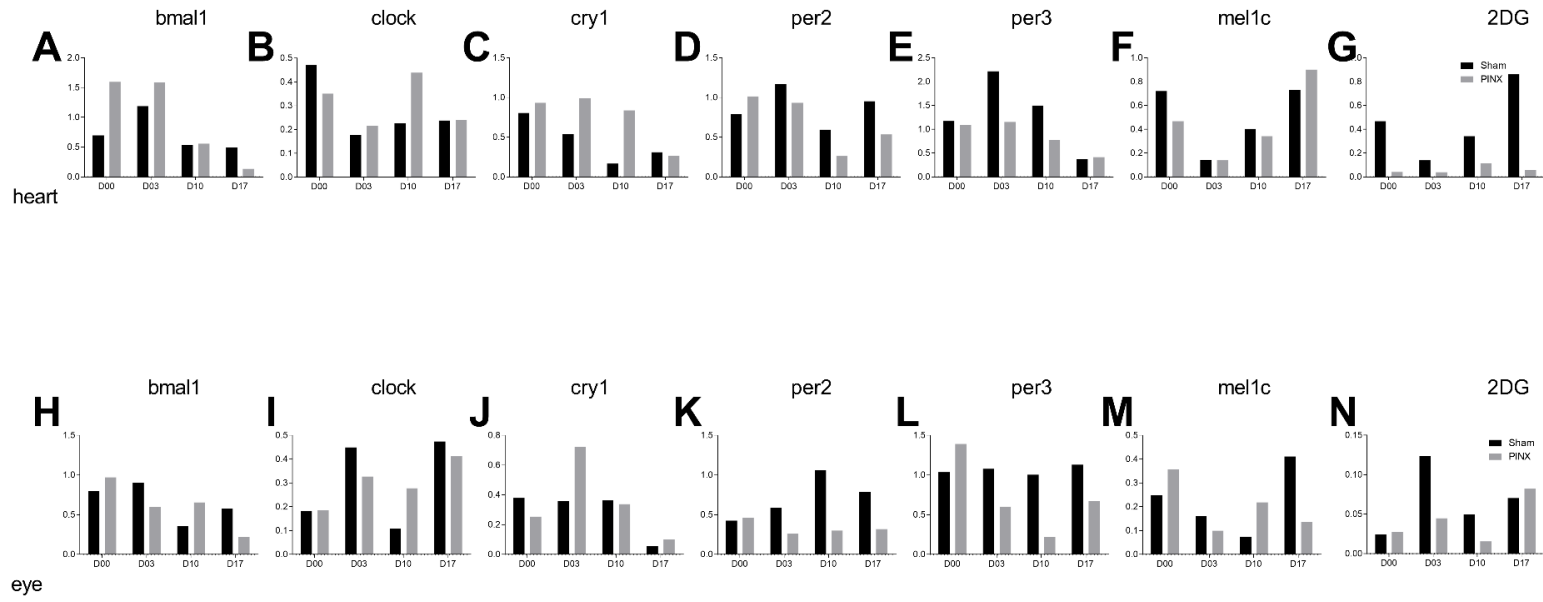
Figure 2.8 Amplitude: heart and eye

(previous three pages) "Phase" and "Amplitude" for the indicated genes and tissue, as time of peak (phase) and lowest mean timepoint subtracted from the highest mean timepoint (amplitude).

2.4   Discussion

Pinealectomy strongly affects the overt rhythmicity of locomotor behavior but has a limited effect on the daily clock gene mRNA abundance of birds' peripheral tissues as they remained in constant dark conditions. By day 17 in constant dark conditions, pinealectomized house sparrows were significantly less rhythmic than sham operated birds in their locomotor activity rhythms. Muscle, liver, and intestine tissue remained rhythmic for all measured clock genes (but not *mel1c*) (Figures 2.2 and 2.4). Heart tissue had the smallest total of clock genes which were classified as rhythmic (Figures 2.3 and 2-5). As days spent in constant dark conditions continued, eye tissue had clock genes that were previously classified as rhythmic lose this designation, and occasionally was rhythmic in sham but not PINX birds (Figures 2-3 and 2-5). 2DG rhythms deteriorated in constant dark conditions in more tissues in pinealectomized birds than in sham-operated birds (Figure 2.2 thru 2.6).

Muscle, liver, and intestine were relatively resistant to any effect of pinealectomy (Figures 2.2 and 2.4). This result for intestine and liver is surprising based on our predictions, as time of feeding can make these peripheral tissues go out of phase with the rest of the body in mammals that have food available at only certain times of day (Cassone, Paulose, and Harpole 2017; Pendergast and Yamazaki 2018). Muscle circadian rhythms can also be entrained to exercise (Wolff and Esser 2012). Presumably with the rhythmic locomotor activity would, in effect, coincide with rhythmicity of feeding and exercise in the form of locomotion, that could potentially strengthen and synchronize these tissues. This was not the case, as relative loss of locomotor behavior rhythmicity in PINX house sparrows did not coincide with a meaningful loss of rhythmicity or synchrony in these tissues.

The mRNA abundance of *Per3* behaved most in accordance with our predictions. In our study, *Per3* had the most consistent phase of any gene product, but when this phase deteriorated, it happened most often in pinealectomized birds. *Per3* also had a large

circadian amplitude in a microarray and qPCR study in chick pineal gland (Bailey et al. 2003; Karaganis et al. 2008).

These results are in accordance with those shown in other species. A pinealectomy experiment with qPCR from several tissues for clock gene expression has been performed previously in another songbird, the redheaded bunting, *Emberiza bruniceps*, and showed that there were only small effects in the effect of pinealectomy on the peripheral tissues (Trivedi et al. 2016). Five of the seven studied clock genes (*clock*, *per2*, *cry1*, *rorα*, *reverbα*, but not *bmal1* and *npas2*) in the liver were affected by pinealectomy and 10 days in constant dim light. The retina tissue of these birds was mostly unaffected by pinealectomy (none except *per2* although its variability statistically interacted with time of day in the two-way ANOVA). In chickens pinealectomy or enucleation decreased the amplitude of circadian rhythms in most tissues, and altered the phase in some tissues of the measured mRNA abundances of *bmal1*, *per3*, and *cry1* (Karaganis et al. 2009); days spent in DD contributed to the variance of the data in a number of tissues and genes measured in this study as well.

While the SCN is necessary and sufficient for mammalian overt circadian rhythmicity in locomotor behavior, the molecular clock of peripheral tissues continues to cycle when the mammal has received an SCN lesion or is otherwise rendered arrhythmic from a brain-specific clock gene knockout (Brown et al. 2019). The molecular rhythms in the measured tissues persisted in these studies but their phase relationships drifted apart. In Yoo and colleagues landmark study, tissue explants from arrhythmic SCN lesion mice were capable of self-sustained rhythms in PER2::luciferase similar to those from intact mice for more than a week without media change, but had their phases dispersed in all examined peripheral tissues but the eye (Yoo et al. 2004). Imaging of arrhythmic SCN lesioned PER2::Luc mice showed that peripheral rhythms persist *in vivo* in addition to Yoo et al's *ex vivo* finding, although with a decreased amplitude when compared with intact controls. (Tahara et al. 2012), with similar results *in vivo* imaging of BMAL1 luciferase in free-moving mice (Saini et al. 2013). As in the lesion studies, a neuron-specific forebrain enriched tissue knockout of *bmal1* in mice from constant dark conditions caused the phases of explanted tissues to disperse relative to age-matched

controls (Izumo et al. 2014). In contrast to these luciferase results in mice, SCN-lesioned hamsters do lose peripheral rhythms in clock gene mRNA abundance in addition to their behavioral arrhythmicity, and behaviorally successful SCN transplant restored the rhythms in some but not all tissues (Guo et al. 2006).

In oscine passerine birds, there are likely other pacemakers that help coordinate the phase in other tissues; the pineal gland is not the whole story of avian circadian organization. The effects of pinealectomy on oscine passerine bird peripheral tissue molecular clocks are not what one would predict if it were the sole synchronizer of clocks in these tissues, as the SCN is in mammals. Loss of peripheral tissue synchrony would reveal itself with these measures via "phase dispersal" as with the mammals, wherein the molecular clocks continue to function at the tissue level but become out of phase with other tissues. Another artifact of desynchrony could be a loss of amplitude in molecular clock rhythms, wherein the cell autonomous clocks making up the tissues become out of phase from one another, rendering the tissue level amplitude lower. Neither of these expected results for the hypothesis were supported. This negative data adds to evidence that there are functional pacemakers in oscine passerine birds beyond pineal gland; that pinealectomized birds are still capable of entraining their locomotor behavior to a light-dark cycle, as evinced by the expression of anticipatory behavior before lights-on, and that locomotor activity does not become immediately arrhythmic upon release into constant environmental conditions from a light-dark cycle. Additionally, these data could support that the various organs themselves can function as semi-independent oscillators.

Candidates for these extra-pineal pacemakers are the avian mSCN and vSCN, likely homologs to the mammalian SCN (Cassone and Menaker 1984; Gwinner and Brandstätter 2001; Underwood et al. 2001). In avian families beyond the oscine passerines, the retinae contribute to the central circadian organization, as the melatonin produced there gets into the systemic circulation; in Japanese quail, 13-50% of circulating melatonin originated from the eyes (Underwood et al. 1990). Additionally, ocular enucleation but not pinealectomy abolishes activity rhythms in the Galliformes chickens and quail (Nyce and Binkley 1977; Underwood 1994). In mammals, there are also extra-SCN food entrainable oscillators, which if they exist in birds, could be a

candidate for how the liver and intestine peripheral tissues maintained their rhythms (Pendergast and Yamazaki 2018). There were several studies in a sub-oscine passeriform, the European starling, *Sturnus vulgaris*, and house sparrow where pinealectomy abolished rhythmicity in perch-hopping behavior, but not feeding behavior (Gwinner et al. 1987; Beldhuis et al. 1988; Janik et al. 1992; Hau and Gwinner 1996).

The data presented here and in other literature (Karaganis et al. 2009; Trivedi et al. 2016) suggest a re-evaluation of the pineal gland in the hierarchy of the multiple circadian oscillators that comprise the central clock in birds (Cassone, Paulose, Harpole, et al. 2017). In mammals melatonin administration has little effect on clock genes in the SCN (Poirel et al. 2003), but does inhibit metabolism and electrical activity of the SCN (Cassone et al. 1988; Shibata et al. 1989). This is the case in birds as well, as pinealectomy in sparrows abolishes rhythms of metabolism in the SCN (Lu and Cassone 1993a). Administering melatonin inhibits SCN metabolic activity in house sparrows and chickens (Lu and Cassone 1993b; Cantwell and Cassone 2002), and daily administration can re-entrain formerly arrhythmic house sparrows and zebra finches (Lu and Cassone 1993a; Gwinner et al. 1997; Wang et al. 2012). However, melatonin has little effect on clock gene expression in the Japanese quail mSCN (Yasuo et al. 2002); neither continuous administration of melatonin nor acute injection affected the circadian patterns of *per2*, *per3*, or *clock* in the mSCN. Sympathetic input disrupts patterns of melatonin production and release from the pineal gland (Cassone and Menaker 1983), and administration of norepinephrine to arrhythmic pinealocytes *in vivo* re-entrains their rhythms of melatonin release (Zatz and Mullen 1988; Li and Cassone 2015). However, this effect of norepinephrine is brought about without altering the clock genes of the pinealocytes (Li and Cassone 2015). This evidence suggests that pineal melatonin affects the central clock of birds, but perhaps more weakly than previously thought; it may take several cycles of melatonin to do so. In cultured chick astrocytes, a single dose of melatonin does not affect clock gene expression, however 7 days of rhythmic presentation synchronizes a rhythm of metabolism and period genes (Paulose et al. 2009). Similarly, daily administration of melatonin is required for the re-entrainment of locomotor activity rhythms in oscine Passeriformes rendered previously arrhythmic from pinealectomy or constant light conditions (Lu and Cassone 1993a; Gwinner et al. 1997;

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Wang et al. 2012). The loss of these daily melatonin cycles were detrimental to locomotor behavior rhythms, but had a limited effect on clock gene expression in the measured peripheral rhythms.

2.5　Acknowledgements

CHAPTER 3.   MELATONIN DURATION GATES PHOTOPERIODIC VOCAL
STATE CHANGE IN A SONGBIRD.

3.1   Introduction

Many organisms anticipate changes in season in order reproduce at an appropriate time of year, exhibiting dramatic seasonal changes in primary and secondary sexual characteristics. For example, house sparrows' (*Passer domesticus*) testis weight increases 400-fold during the breeding season compared to winter (Witschi 1935; Whitfield-Rucker and Cassone 2000). The song control nuclei, brain structures involved with song learning in juvenile passerine birds and vocal production in adults, exhibit 10-30% increases in size and neuropil density annually before their subsequent seasonal regression and apoptosis (Ball 2016). With these increases in mass, passerine birds transition behaviors from a non-breeding to a breeding vocal state (Nottebohm et al. 1986; Nottebohm et al. 1987; Voigt and Leitner 2008; Van Hout et al. 2009; Van Hout et al. 2012). The species-specific changes in vocal state can vary from repertoire size (Van Hout et al. 2009; Van Hout et al. 2012), acoustic parameters such as stereotypy, rate, and bout length, (Nottebohm et al. 1986; G Troy Smith et al. 1997), or proportion of "mate-desired" syllables (Voigt et al. 2001; Leitner and Catchpole 2004; Hill et al. 2015).

The pineal gland is involved in two biological rhythms on different time scales: the daily circadian clock and the circannual "calendar" (Cassone et al. 2009). The pineal is the dominant circadian pacemaker in the central clock of passerine birds (Cassone and Kumar 2015). It is required for overt rhythmicity in locomotor behavior, as pinealectomy (PINX) causes birds to become arrhythmic in constant lighting conditions (Gaston and Menaker 1968). Rhythmic administration of the pineal hormone melatonin in drinking water can reinstate rhythmicity in arrhythmic PINX birds in constant dark conditions and intact arrhythmic birds in constant light (Lu and Cassone 1993a; Heigl and Gwinner 1994).

In addition to circadian patterns of perch hopping, feeding behavior, and body temperature (Gaston and Menaker 1968; Binkley et al. 1971; Gaston 1971; Chabot and Menaker 1992), vocalization behavior is also under circadian and pineal control in songbirds (Wang et al. 2012). Wang and colleagues (2012) found that circadian patterns

of locomotor behavior, calling, and song persist in constant darkness in the zebra finch, *Taeniopygia guttata.* These rhythms are abolished by PINX in constant darkness (DD) and in intact birds in constant light (LL). They can be reestablished by daily administration of melatonin in the drinking water.

The pineal gland is also involved with avian seasonality in song control but not in gonadal cycles. Melatonin is produced at night and is inhibited by light (Ralph et al. 1975). Long durations of melatonin, as an organism would experience in winter, suppress the seasonal recrudescence of song control nuclei in male house sparrows (Cassone et al. 2008) and Indian weaver birds, *Ploceus philipinnus* (Surbhi et al. 2015). PINX of house sparrows alters the responses in vocalization rate and complexity to changing photoperiod (Wang et al. 2014). However, no effect of melatonin or PINX on testes was observed in any of these studies.

Since long durations of melatonin suppress the growth of brain structures underlying song, it would be important to know if this intervention affected vocal behavior. To address this question, we pinealectomized male house sparrows and administered different durations of melatonin in their drinking water to simulate long winter nights, short nights, and a vehicle control. We examined the vocalization behavior produced and some analogs of reproductive state. The results show that long durations of melatonin are sufficient to prevent an expanded spring-like vocalization state while leaving the gonads unaffected.

3.2   Methods

3.2.1   Animal housing and surgeries

Adult male house sparrows (n=24) were captured via mist net in central Kentucky and moved to an outdoor aviary, with food (2:1 white millet seed and chick starter) and water *ad libitum* at the University of Kentucky Ecological Research and Education Center field station. Captures occurred in the winter months up to two months preceding the experiment. In the short day on January 30th (9.92 hrs of light, 14.08 hrs of darkness at 38° N), birds were transferred indoors (constant 22.2°C) to isolation cabinets, each

with timer-controlled white LED lights (40 µW/cm^2, approximating the phase and photoperiod outdoors at the time with 10 hours of light, 14 hours of darkness, LD 10:14, lights on at approximate sunrise), a microphone (either a Røde NT3 (n=12) or an AKG Perception 170 (n=12)), and an infrared detector to measure locomotor activity. Beginning in this short winter photoperiod ensured that the birds commenced the study in a photosensitive state.

Constant background white noise was played from speakers outside the cabinets to improve acoustic isolation among birds (average power = 21 dB, frequency range from 0 to 10 KHz, measured within the cabinets). In total, birds remained in the isolation cabinets for 105 days.

All birds were subjected to PINX (n=24) over the course of three days and at days 19 and 27, as previously described (Lu and Cassone 1993a; Wang et al. 2014), after at least a week indoors. Birds received acetaminophen in their drinking water for three days following the surgery.

All animal care and procedures were approved by University of Kentucky's Institutional Animal Care and Use Committee and comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

3.2.2   Experimental timeline and melatonin treatments

Four days after surgery, the following procedures commenced: the short duration group (n=8) received 200 µg/mL melatonin in 0.5% ethanol daily for 6 hours, beginning 10 hours after lights on (Zeitgeber time ZT=10) and replaced with water at ZT=16. The long duration group (n=8) received 200 µg/mL melatonin daily for 14 hours, added at ZT=10 and removed before lights on the following day at ZT=24. This melatonin concentration (200 µg/mL = 0.86mM) is sufficient to restore behavioral rhythmicity in melatonin: water cycles and result in an plasma melatonin amplitude similar to intact physiology (Heigl and Gwinner 1994). The control group (n=8) had their drinking water replaced daily with 0.5% ethanol, added at ZT=10 and removed at ZT=24. Throughout these treatments (62 days), each dish was refreshed weekly and when necessary as consumed, and stock solutions replaced weekly (Figure 3.1).

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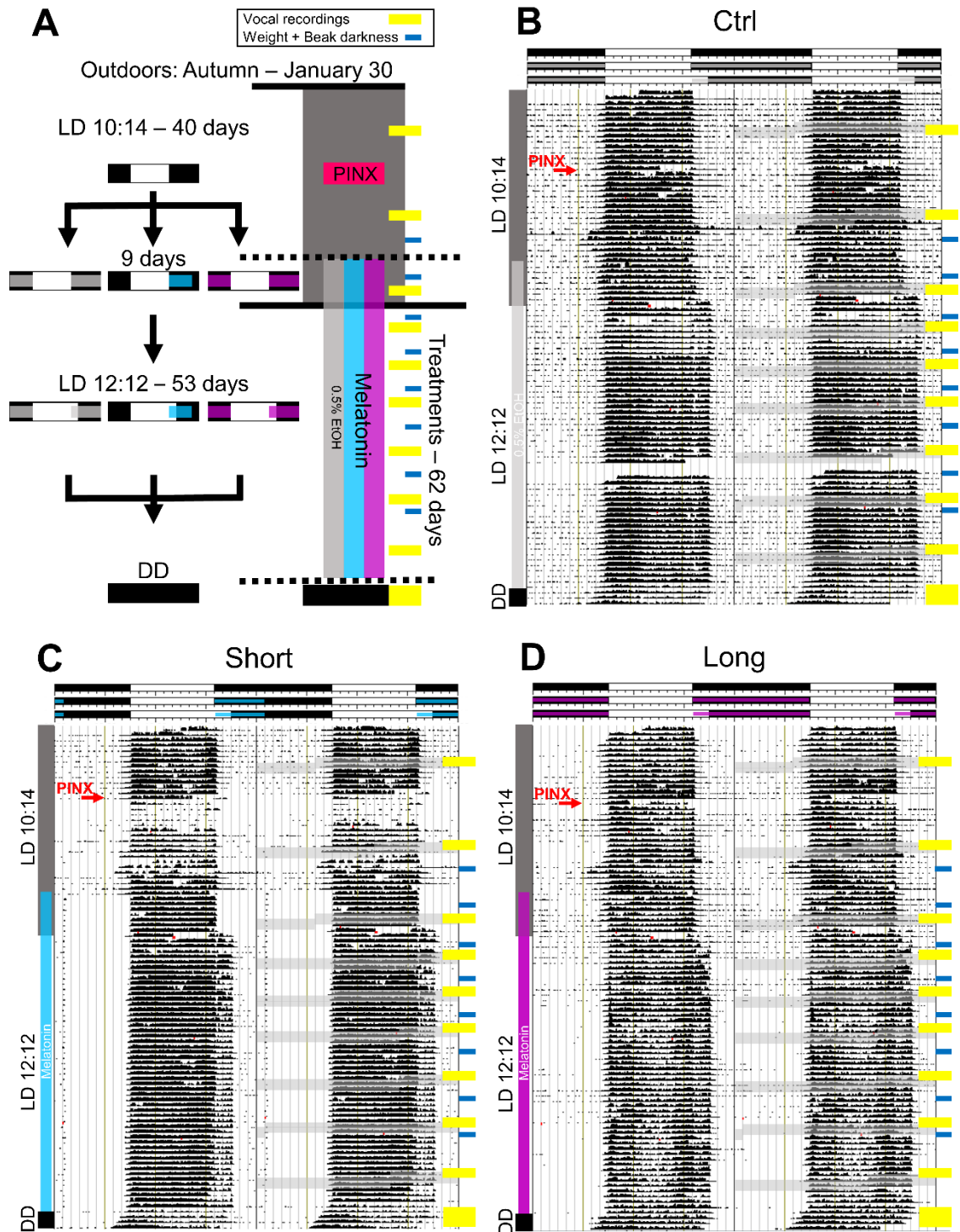
Figure 3.1 Experimental timeline diagram and double-plotted actograms

Experimental timeline diagram (A) & Double-plotted actograms of a representative Ctrl (B), Short (C), and Long (D) treatment-receiving male house sparrow. The **left** border of each actogram labels the experimental manipulations. PINX = pinealectomy surgery. The **right** border of each actogram labels with color blocks when ~weekly measures were taken.

After 40 days of LD 10:14, the last 9 days with treatments, the photoperiod was then extended two hours from previous lights off, LD 10:14 to LD 12:12 for the remaining 53 days. This was approximately the maximum photoperiod the birds would have experienced if they had remained outdoors at our latitude (38° N). The treatments were maintained throughout. To check if the birds were entrained and if there were any differences in their endogenous circadian clocks, the birds were placed in constant darkness (DD) without treatments for 7 days until sacrifice, taking place over the course of 3 days. The birds were transcardially perfused with 4% paraformaldehyde, the testes collected and weighed.

3.2.3 Data collection and recordings

Locomotor activity was continuously recorded from each cage's IR detector with VitalView data acquisition system in 5 minute bins (STARR Life Sciences Corp., Oakmont, PA). At ten stages, 48 hours of continuous audio was recorded using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY): before surgery (beginning day 01), after surgery (day 17), a week into treatments in LD 10:14 (day 31), 6 weekly recordings in LD 12:12 (days 38, 45, 52, 61, 70, 80), and DD (day 85). The LD 12:12 recordings are referred to by Week number despite being taken up to 10 days apart.

3.2.4 Activity analyses

Vocalizations were extracted using Raven Pro 1.4, generating timestamps for each vocalization. The locomotor activity deflections coincident with the vocal recordings were plotted as mean by hour of each bird's daily activity.

3.2.4.1 Repertoire

We examined the repertoire for the first 100 morning vocalizations (starting at ZT=0), first 100 vocalizations at ZT=4, and the available evening vocalizations for 4 of the recordings (10:14 treatment, LD 12:12 Weeks 2, 4, and 6). Sonograms were produced in Raven Pro 1.4, visually and aurally inspected, and manually placed into categories based on criteria described in Wang et al. (2014) (Figure 3.2).
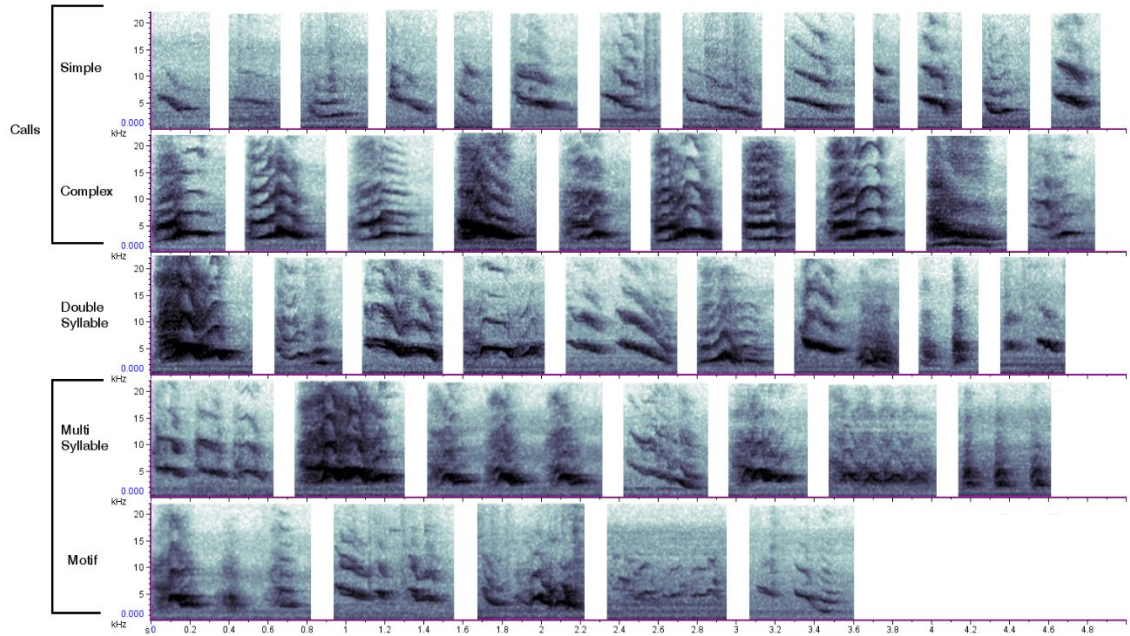
Figure 3.2 Representative house sparrow sonograms

The vocalizations were categorized as calls, which were further split into the categories of 'simple' and 'complex;' double-syllable vocalizations (DS); and multiple-syllable vocalizations, which also include motifs. Axes are approximate as this composite figure was sourced from multiple birds and instances of our sonogram software.

These categories originally did not consider social context. The single syllable call category included vocalizations classified as the "quiet, chirping noises ("social song")" generally produced by birds in flocks for contact, absent any sexual component. We created a division in our classifications and designated such vocalizations "simple calls" by being mid-toned (having most dominant frequencies above 5,000 Hz), having few harmonics, and aurally a squeaky or chirp quality.

The single syllable call category also included the most common vocalization of the house sparrow, the basic form of the "chirrup." Chirrups are described as being used by males to attract a mate and establish a territory (Anderson 2006b; Summers-Smith 2009). We formally classified these "complex calls" (Clive K. Catchpole and Slater 2008) as having a powerful lower-frequency component below 5,000 Hz, many harmonic frequencies, and aurally a coarser "wheeze" quality (Supplemental Video 3.1).

3.2.5   Constant darkness circadian parameters

We analyzed circadian parameters of the behaviors produced in constant dark conditions. Activity onsets and offsets were determined using ClockLab Analysis 5 software (Actimetrics, Willmette, IL). Length of active period, α (Figure 3.8A) was determined as the length of time between onset and offset, as a mean of the first 3 days in DD. Free running period, τ (Figure 3.8B), was determined using ClockLab's least-squares fit function to the corrected activity onsets for the first three days in DD. The activity distribution for both vocalization and locomotor behavior were plotted by group, by hour, for the first two subjective days in constant dark conditions (Figure 3.8C-F).

3.2.6   Body state estimates

Birds were weighed every 7-9 days: after the surgeries, and then 2-5 days before each vocal recording, to limit the potential effects of stress on the vocal output sampling(days 20, 28, 35, 42, 49, 56, 65, and 72).

At those times and at day 80, we quantified photographs to track darkening of the birds' beaks along a range from yellow (value of 0) to black (value of 1), modified slightly from Laucht, Kempenaers, & Dale. Beak darkness is a noninvasive correlate to increasing plasma testosterone (Laucht et al. 2010).

3.2.7   Analytical statistics

Statistical analyses were calculated in Graph Pad Prism 6 (GraphPad Software, San Diego, CA), two-way repeated measures ANOVA for comparing the three experimental groups (either weeks or the hours of a day), with a Tukey's multiple comparison test, except for Figure 3.3 A&E (Sidak's) and Figure 3.8A&B (Bonferroni). Testes weights were compared with a one-way ANOVA. Morning and afternoon repertoire measures were compared with paired two-tailed t-tests (Figure 3.7A).

Cohen's d values were calculated manually in Microsoft Excel from the difference in means between two groups of interest divided by the same two groups' pooled standard deviation.

3.3 Results

3.3.1 Vocalization and activity totals

All experimental groups exhibited increased vocalizations as they were transferred from short to equinoctial days (repeated measures two-way ANOVA: Interaction $F_{14, 147} = 2.031$, P= 0.191; Time $F_{7, 147} = 33.69$, P < 0.0001, Treatment $F_{2, 21} = 0.1355$, P = 0.8741) (Figure 3.6A). In contrast, total locomotor activity did not vary week-to-week with just one pairwise difference at a single timepoint (repeated measures two-way ANOVA: Interaction $F_{14, 147} = 0.6756$, P = 0.7953; Time $F_{7, 147} = 0.4910$, P = 0.8400, Treatment $F_{2, 21} = 1.770$, P = 0.1948) (Figure 3.6B).

3.3.2 Post-surgery and LD 10:14

After surgery, the birds' vocalization patterns temporarily changed (Figure 3.3A), but these changes did not persist into the next recording (Figure 3.3B). Surgery had little effect on the distribution of locomotor activity (Figure 3.3E). In LD 10:14, there were more pair-wise differences between groups in vocal behavior (Figure 3.3B) than locomotor behavior (Figure 3.3F).

3.3.3 LD 12:12

Once in LD 12:12, there were differences between groups in vocalization distribution (Figure 3.3C, D, I-L), at dawn, dusk, and most consistently at ZT=4 in vocalization. There were few changes in locomotor activity (Figure 3.3 G, H, M-P).

Figure 3.3 Daily distributions
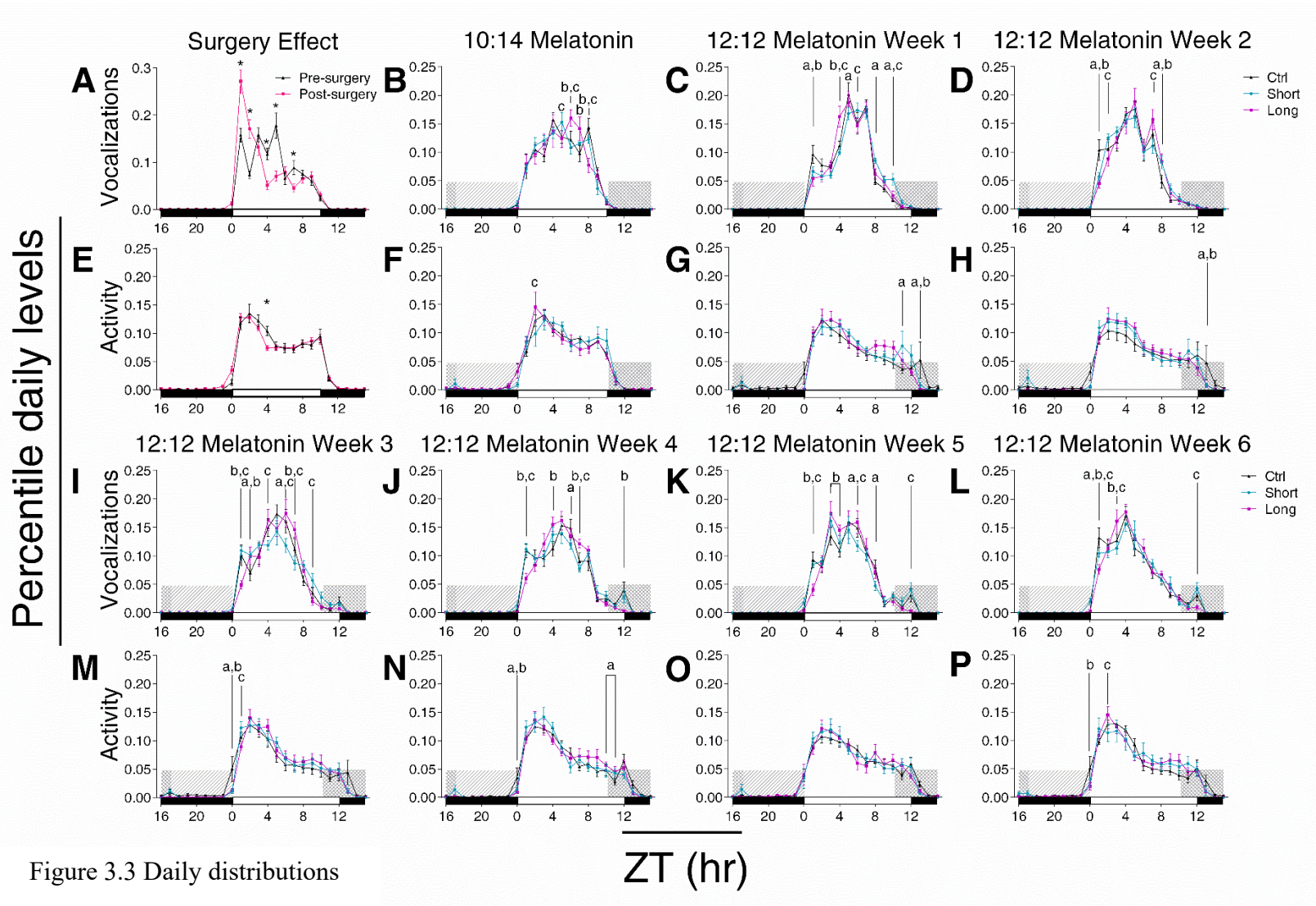
(previous page) ***Daily distributions*** *of vocalization and locomotor activity under different photoperiod (graph titles) and treatment conditions (down hashing short duration, upward hashing long and control). Each graph represents 48 hours of continuous recording (when available, see Materials and Methods), as mean ± standard error, by hour, in treatment groups. Both activities are presented as percentile of each individual bird and day's daily total, e.g. a value of 0.1 would indicated 10% of that bird's daily activity occurred during the corresponding hour. The top graph of each pair is vocalization (A-D, I-L), the bottom is locomotor activity (E-H, M-P).*

3.3.4   Dawn chorus in LD 12:12

Birds receiving the long duration of melatonin consistently vocalized less after lights on (ZT=1) than did the other experimental groups in LD 12:12. An increase in the dawn chorus was observed in the control group in 12:12 LD treatments week 1 (Figure 3.3C, control higher than Short, p=0.0107, Cohen's d= 0.22; control higher than long p= 0.0002, Cohen's d= 0.51) and week 2 (Figure 3.3D, control higher than short p= 0.0007, Cohen's d= 0,007; control higher than long p<0.0001, Cohen's d= 0.75). In weeks 3, 4, and 5 of LD 12:12 (Figure 3.3I-J), the dawn chorus was significantly higher in both the short (three p-values≤0.0014. Cohen's d = 0.88-1.49) and control (three p-values p≤0.0001, Cohen's d= 0.83-1.22) group than in the long group. In week 6 (Figure 3.3L), all three groups were differentiated from one another by percentile vocalization at ZT=1 (three p-values≤0.0388, Cohen's d= 0.52-1.62).

To better illustrate the week-to-week changes in the dawn choruses, we separated out those values (ZT=1) and plotted them on their own (Figure 3.4A). Time and treatment both significantly contributed to the variation in the data for the dawn chorus (Figure 3.4A, Two-way repeated measures ANOVA: Interaction $F_{10, 105} = 2.350$, p = 0.0151; Time, $F_{5, 105} = 9.160$, p<0.0001; Treatment $F_{2, 21} = 8.530$, p= 0.0019).
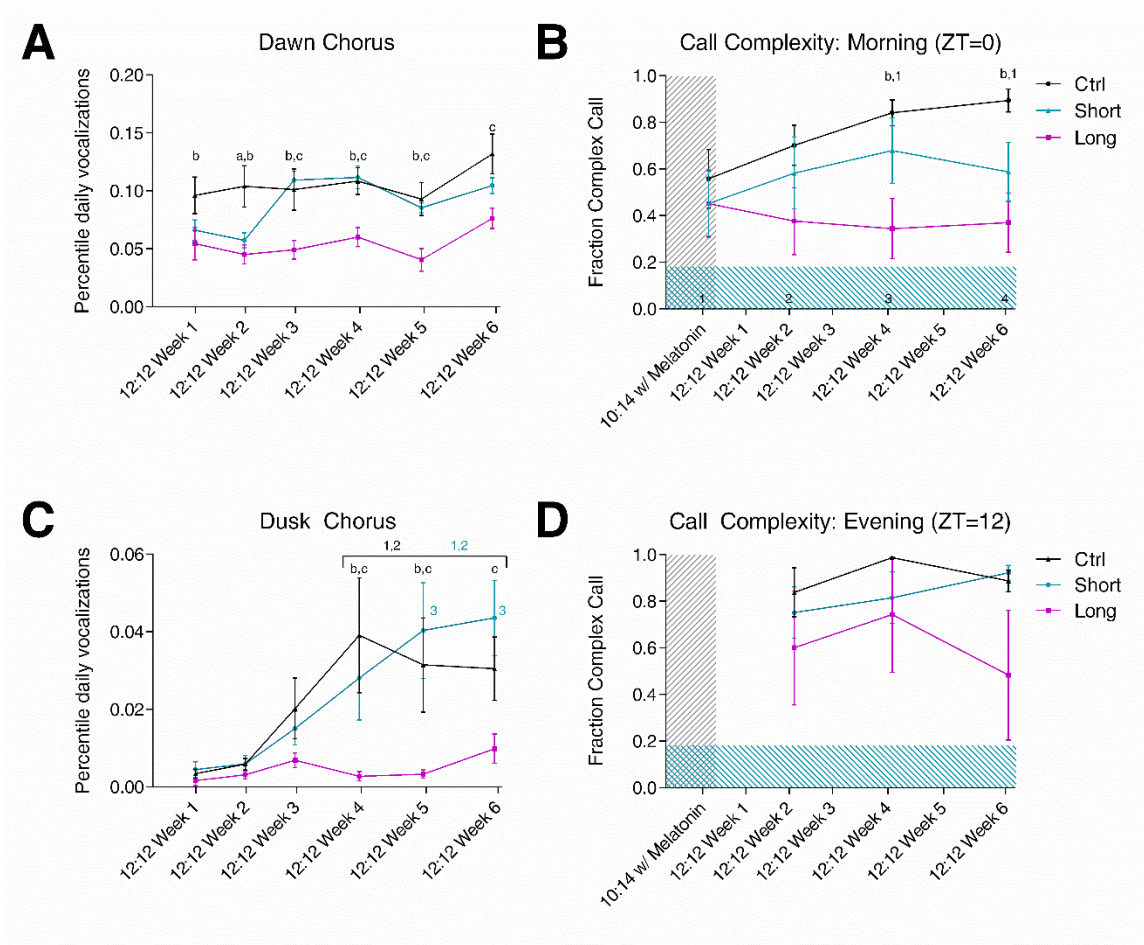
Figure 3.4 Seasonal dawn and dusk differences

*The vocalization distribution values, as percentile of each individual bird and day's daily total (as in Figure 3.3), of the morning's **Dawn Chorus**, first hour of daylight, ZT= 1, (A) and the evening's **Dusk Chorus**, hour after lights off at ZT=12 (C).*

*Of the vocalizations classified as **calls**, the **complexity** at each week, wherein a value of 1.0 indicates all calls sampled are classified as the 'complex' type and 0.0 indicates all calls sampled are of the 'simple' type. The complexity of calls within the 100 vocalizations after lights on at ZT=0 (Morning, B), and the available vocalizations for ZT=12-14 (Evening, D)*

Full length, grey background hatching indicates short days of 10:14 LD. Shorter, blue background hatching indicates the presence of treatments of melatonin or control in the drinking water (Panels B&D).

Numerals above the data point indicate a significant difference from the indicated timepoint, as week number in LD 12:12 (C) or labeled in the text above the tick marks from the x-axis (B), at the same color code as the plotted data (p<0.05, repeated measures Two-way ANOVA, Tukey's multiple comparison test). Lowercase letters indicate a difference between experimental groups (remaining panels, repeated measures Two-way ANOVA Tukey's multiple comparison test, p<0.05): a = significant difference

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In panel D the color-coded numerals indicate the mean vocalizations per bird in each experimental group for the evening peak. Statistical comparisons were not performed for this panel as the sample size was small and uneven.

3.3.5   Dusk chorus in LD 12:12

Later in the experiment, birds receiving the long duration of melatonin did not produce the dusk chorus present in the other groups at ZT=12. By 12:12 LD Week 4 (Figure 3.3J), this ZT=12 peak was higher in the control group (0.039 ± 0.015) versus Long (0.003 ± 0.001) (p=0.0035, Cohen's d= 1.22), and in weeks 5 and 6, in the short group (0.04 ± 0.012 and 0.044 ± 0.0097) over long (0.003 ± 0.001 and 0.010 ± 0.004) (p=0.0110 and p=0.0077, Cohen's d= 1.50 and d= 1.62 respectively).

We plotted the percentile vocalization values on their own to better see this development (Figure 3.4B; Two-way repeated measures ANOVA: Interaction $F_{10, 105}$ = 3.454, p = 0.0006; Time, $F_{5, 105}$=13.69, p<0.0001; Treatment $F_{2, 21}$= 3.500, p= 0.0488).

The long duration birds were active at ZT=12, coincident with the timing of the other groups' evening peak in vocalization distribution (Figure 3.3M-P). However, they vocalized significantly less than did the short or control groups.

3.3.6   Repertoire

As control birds were photostimulated, the complexity of their calls increased during the first hour following lights-on (0.558 ± 0.126 increasing to 0.894 ± 0.0049 fraction complexity, p=0.0021 from 10:14 to the last week in LD 12:12; Interaction $F_{6, 63}$ = 2.691, p = 0.0217; Time $F_{3, 63}$ = 3.001, p=0.0370; Treatment $F_{2, 21}$= 2.702, p= 0.0903)). The long group remained in a simple vocal state (0.3853 ± 0.02398 fraction complexity, p=0.4321 from LD 10:14 to 12:12). The control group expressed more complex calls than did the long group for LD 12:12 Weeks 4 (p= 0.015, Cohen's d= 1.77) and 6 (p=0.0098, Cohen's d= 1.92) (Supplemental Video 3.2). When calls were produced in the developing evening peak (Figure 3.4D), they were complex: short 0.8096 ± 0.04031, long 0.6438 ± 0.06387, control 0.8657 ± 0.04945 fraction complexity.

3.3.7   Constant darkness behavior

Release into DD and cessation of treatments revealed that the birds had entrained to their former exposure to the LD cycle (Figure 3.8). Prior exposure to the various durations of melatonin of a control had some pairwise effects on when during the subjective days the birds vocalized, but not consistently between the two days of DD analyzed (Figure 3.8C & D).

3.3.8   Body weight, beak darkness, testis weight

No statistical differences in body weight were observed among experimental groups (Figure 3.5A). Birds gained weight over time as the experiment continued, starting at 24.795 ± 0.366 g to 25.527 ± 0.562 g at the final weighing (Two-way repeated measures ANOVA: Interaction $F_{14, 147}= 1.310$, p=0.2081, Time $F_{7, 147}= 17.98$, p<0.0001, Treatment $F_{2, 21}= 1.150$, p=0.3358).

 Beaks became darker, on average, over time, with no effect among groups (Figure 3.5B, Two-way repeated measures ANOVA: Interaction $F_{16, 152}=1.170$, p=0.2983; Time $F_{8, 152}=15.41$, p<0.0001; Treatment $F_{2, 10}=0.3199$, p=0.7300). As wild-caught birds, the individuals began the experiment at various stages of beak darkness (1 totally black, 0 totally white: 0.784 ± 0.011). Beak darkness increased in LD 12:12, peaking four weeks into these conditions (0.808 ± 0.008 , p<0.0001 to p= 0.0158).

No statistical difference in testis weight was observed among experimental groups (Figure 3.5C, ANOVA: Treatment $F_{2, 21}= 2.721$, p= 0.0889). The average weight for each groups was short 276.5 ± 25.66, long 196.1 ± 20.94, and control 229.0 ± 26.52 mg.
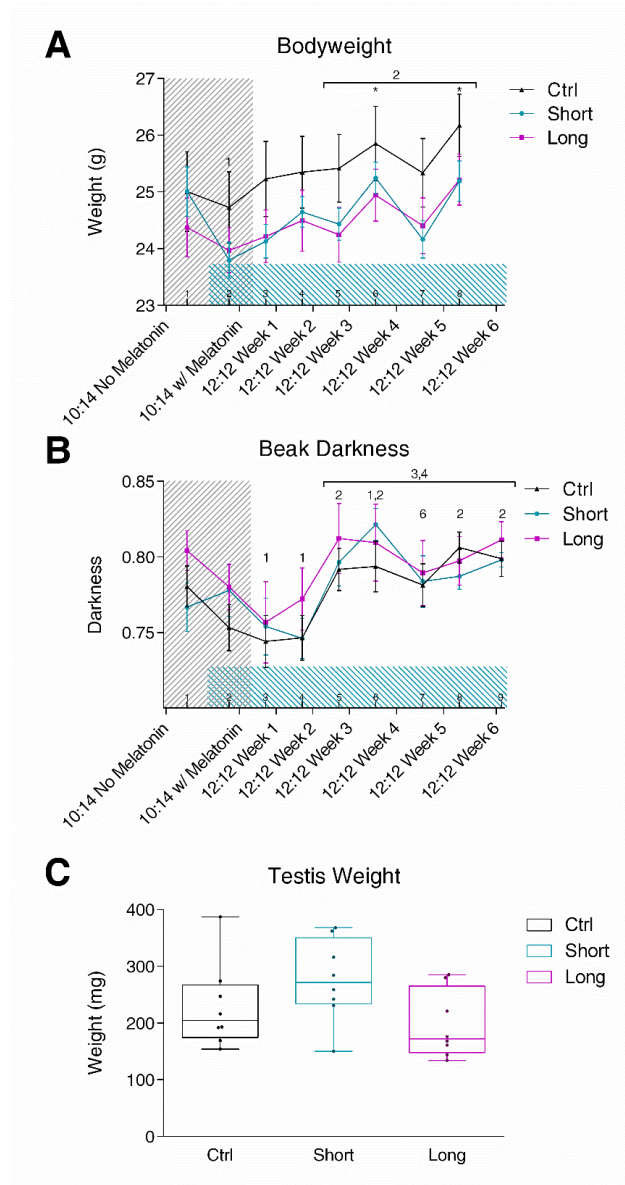
Figure 3.5 Estimates of body and reproductive state

Bodyweight (A) and Beak darkness (B), as measured every 7-10 days throughout the experiment via weighing or quantifying a photograph, respectively. Beak darkness is a reasonable correlate for plasma testosterone, such that darker beaks indicate higher plasma testosterone. Full length hatched shading indicates 10:14 photoperiod, the shorter hatched shading indicates the rhythmic presentation of treatments in the drinking water.

Numerals above the data point indicate a significant difference from the indicated previous timepoint, as labeled above the tick marks on the x-axis (p<0.05, repeated measures Two-way ANOVA Tukey's multiple comparison test). Asterisks (*) represents a significant difference from all other timepoints that lack asterisks, replacing in panel A what could have been printed as "1-4, 7."

Weights of the testes upon sacrifice (C), no difference among groups via ordinary one-way ANOVA.

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Box is 25th and 75th percentile, line is mean testis weight, circles represent all collected testes weights, and whiskers represent the range of the data for each experimental group.
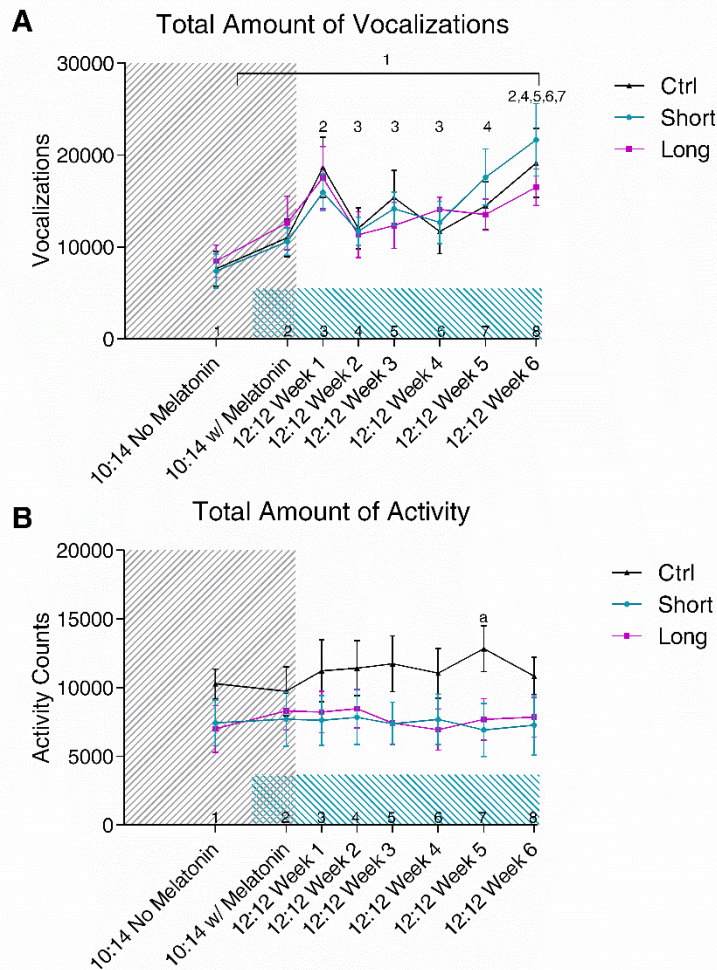
Figure 3.6 Behavior totals

Mean ± standard error of the totals of the measured behaviors, when sampled, as amount for vocalizations per 48 hour recording (A), and amount of locomotor activity (B), as IR detector deflections per correlated 48 hour recording (when available, sometimes reduced to 24 hrs, see Materials and Methods). Full length grey hatched shading indicates 10:14 photoperiod, the shorter, blue hatched shading indicates the rhythmic presentation of treatments in the drinking water. Numerals above the data point indicate a significant difference from the indicated timepoint, as labeled above the tick marks on the x-axis (p<0.05, repeated measures Two-way ANOVA with Tukey's multiple comparison test). Lowercase letters indicate a difference between experimental groups (p<0.05): a = significant difference between Short and Control
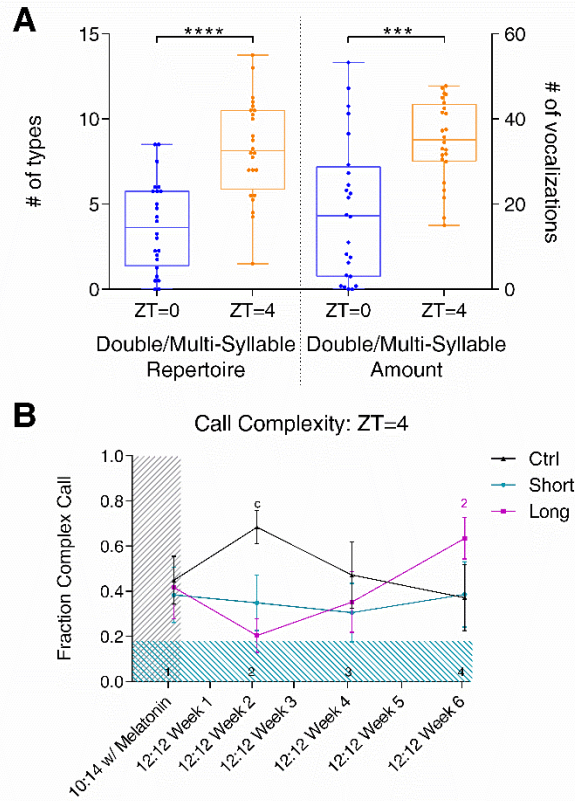
Figure 3.7 ZT=4 quantifications

A: Across the entire experiment in LD, per bird, mean size of double- and multiple-syllable vocalization repertoire (left two plots), and mean amount of vocalizations produced within those categories (right two plots). Blue plots represent analysis of the morning samples (ZT= 1) and orange plots the midday samples (ZT=4). Box is 25th and 75th percentile, line within the box is mean repertoire or amount, circles represent all values, and whiskers represent the range of the data for each time-of-day. *** is a p-value = 0.0003 and **** is a p-value <0.0001, from paired two-tailed t-tests.

Panel B: The complexity of calls within the 100 vocalizations after lights on at ZT=4 (Midday).

Full length, grey background hatching indicates short days of 10:14 LD. Partial length, blue background hatching indicates the presence of treatments of melatonin or control in the drinking water.

Numerals above the data point indicate a significant difference from the indicated timepoint, labeled in the text above the tick marks from the x-axis, at the same color code as the plotted data (p<0.05, repeated measures Two-way ANOVA, Tukey's multiple comparison test). Lowercase letters indicate a difference between experimental groups (remaining panels, repeated measures Two-way ANOVA Tukey's multiple comparison test, p<0.05): c = significant difference between Short and Long.

3.4   Discussion

In both control birds and birds that received a short, summer-like melatonin duration, morning and evening peaks of vocalization emerged as they were transitioned from short days of LD 10:14 to equinoctial photoperiods of LD 12:12. In contrast, birds receiving a long durations of melatonin continue to vocalize in a winter state despite having large testes and dark beaks, consistent with the sexual maturity seen annually in spring. The duration of melatonin determined the distribution of vocalization during the course of the day. The long, winter-like durations of melatonin suppressed these morning and evening "choruses." In contrast to the pattern of vocalization, locomotor activity distributions were largely unaffected by melatonin.

Long durations of melatonin also prevented the expansion to a more complex, spring-like dawn chorus repertoire (Figure 3.4B, Supplemental Video 3.2). Control birds in long days developed close to 90% complex calls for the last half of the LD 12:12 recordings, higher than birds receiving long durations of melatonin that produced a mean call complexity of less than 45% throughout. The lack of developed call complexity among these birds suggests that the vocal control pathways remained in their winter, inhibited state.

While long duration birds did not vocalize during the dusk chorus as did control and short duration birds, they continued to be active, albeit not vocalizing. Thus, the melatonin treatment did not merely decrease activity or induce sleep; it selectively affected vocalization. The calls produced during this dusk chorus were complex, suggesting that they are regulated in a similar manner to the state change observed during the dawn chorus. Dawn and dusk choruses are considered systematically different in passerine bird behavior from vocalizations occurring during the bulk of the day (Staicer et al. 1996; Lein 2007).

The changes in vocalization state we observed were independent of any changes in the body state. Birds gained weight as the experiment progressed, with no differences among groups. The size of the testes at sacrifice were consistent with that of a photostimulated house sparrow, although not quite as high as mean weights taken from the middle of a long photoperiod (LD 16:8; 400-500 mg) rather than the DD conditions

here (Witschi 1935; Whitfield-Rucker and Cassone 2000; Cassone et al. 2008). However, there were no differences in testis size among groups. The darkening of the beaks was consistent with high levels of plasma testosterone in male house sparrows (Laucht et al. 2010). There were no differences among treatment groups in these measures, suggesting that all birds had plasma testosterone at consistent levels throughout the experiment.

The data presented here are consistent with the following scenario. During the short days of winter, melatonin duration in male house sparrows is long, congruent with the duration of the night, or scotoperiod. The testes are regressed, song control nuclei are small, and birdsong and call are low in quantity, distributed in a single bout during the day, calls relatively low in complexity. As photoperiod increases, the duration of melatonin decreases, the testes recrudesce and increase secretion of testosterone, beaks darken in response, song control structures increase in size and complexity, and bird vocalization amount, daily distribution, and complexity change. Morning and evening choruses emerge with complex calls. However, if the melatonin duration is maintained in the winter-like duration, the emergence of the morning and evening choruses, and the increased call complexity, is blocked, even though all birds increase in body weight, increase in testis size, and increase in beak darkness in response to the increase in photoperiod. This is consistent with previously published data showing long durations of melatonin prevent the increase in the size of song control nuclei HVC and RA (Cassone et al. 2008; Surbhi et al. 2015). Thus, the melatonin duration signal specifically affects the song control system, independently of the effects of photoperiod on the gonadal reproductive axis.

In mammals, seasonal melatonin has a marked effect on gonad size (Hazlerigg 2012), as pinealectomy prevents gonadal regression associated with a decrease in photoperiod marking the end of the breeding season (Reiter 1974; Herbert et al. 1975; Reiter 1975). Administration of long, winter-like durations of melatonin to long-day breeders such as hamsters are sufficient to cause mammalian gonadal regression, as does a short photoperiod (Tamarkin et al. 1977; Goldman et al. 1979). These melatonin duration effects are mediated through high affinity melatonin receptors (Mel_{1a}/MT1 and

Mel$_{1B}$/MT2) expressed in the mammalian *pars tuberalis* of the adenohypophysis (Reppert 1997).

The *pars tuberalis* in passerine birds, in contrast, lacks melatonin receptor binding (Rivkees et al. 1989; Cassone et al. 1995) or receptor mRNA (Reppert et al. 1995). Removal of the pineal gland from seasonally breeding birds has little or no effect on photoperiodic induction of primary sexual characteristics. Seasonal induction is instead transmitted through extra-optic and extra-pineal photoreceptors in the medial basal hypothalamus (Cassone and Yoshimura 2015). There is, however, high-affinity melatonin binding (Gahr and Kosar 1996; Whitfield-Rucker and Cassone 1996; Bentley 2003) and mRNA expression (Bentley et al. 2013; Fusani and Gahr 2015) in the song control systems of several passerine species, and receptor binding (Whitfield-Rucker and Cassone 1996) and mRNA expression (Bentley et al. 2013) are increased by long days.

The valuable calendar information provided by the scotoperiod proxy in melatonin is not wasted in songbirds. The dawn choruses we associate with spring mornings is in part produced by the shortening of melatonin duration in seasonally breeding birds. Rather than gating the size of the gonads as in mammals, shorter durations of melatonin disinhibits the development of a dawn and dusk chorus, and the increased complexity of vocalization in house sparrows.

3.5   Acknowledgements

Additional files:

*Supplemental Video 3.1 – **Demonstration of Call Types** a dawn chorus sample from a bird receiving a Control treatment in 10:14 LD. Then, a sample from the same bird at the final dawn chorus recording, 6 weeks into 12:12 LD conditions.*

*Supplemental Video 3.2 – **Dawn Chorus Complexity Comparison between a Control and Long Duration Bird** a dawn chorus sample from a bird receiving a Control treatment in 10:14LD. Then, the same bird at the final dawn chorus recording, 6 weeks into 12:12 LD conditions. Followed by a single long duration-receiving bird's samples of a short day and 12:12 week 6 dawn chorus recording.*

CHAPTER 4.   PHOTOPERIODIC CONTROL OF VOCALIZATION AND
            COMPLEXITY IN FEMALE HOUSE SPARROWS: LACK OF A ROLE FOR
            THE PINEAL GLAND

4.1   Introduction

        As days grow longer in the early spring in temperate latitudes, we may awaken to
the sounds of the dawn choruses of local songbirds. Among the sounds of this chorus in
the northern hemisphere, at least, are the *cheeps* and *chirrups* of the house sparrow,
Passer domesticus. This vocal behavior accompanies a life history stage, as male house
sparrows break away from the winter flocks to establish territories in which to attract
mates, while females are thought to simply await these solicitations. Gonads increase in
size during this time; the testes of house sparrows recrudesce up to 400 times larger in the
breeding season than during the winter (Witschi 1935; Dawson 1991b; Whitfield-Rucker
and Cassone 2000), and ovary size in females increase 50-fold (Witschi 1935; Anderson
2006a). Coincident with gonadal recrudescence, the brain structures associated with the
production of song, the so-called "song control nuclei," increase in size and complexity
through a combination of adult neurogenesis, cell growth, and differentiation, resulting in
a 10-30% increase in size of the parts of the brain involved with song learning in juvenile
passerine birds and production in adults (Ball 2016).

        Seasonal changes in vocalization are most often studied in male passerines, as
their seasonal dynamics are readily revealed in laboratory settings (Nottebohm et al.
1986; Nottebohm et al. 1987; Voigt and Leitner 2008; Van Hout et al. 2009; Van Hout et
al. 2012). Male songbirds vocalize more often than female birds, and the song control
nuclei are sexually dimorphic in most species, such that the HVC, *nucleus robustus
archipallium* (RA) and Area X are larger and more complex than in female conspecifics.
Further, the amplitude of seasonal changes is greater in male songbirds.

        Melatonin is an intellectually appealing molecule to transduce photoperiodic
information to seasonally breeding vertebrates, as it is only expressed at night and
inhibited by light (Klein et al. 1997). This expression profile results in blood melatonin
titers that are short in duration during the long days of summer and long in duration
during the short days of winter (Cassone 1990; Klein et al. 1997). Pineal melatonin

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indeed mediates mammalian annual reproductive cycles via high affinity melatonin receptors (Mel_{1A} /MT1 and Mel_{1B}/MT2) within the pars tuberalis of the adenohypophysis (Reppert 1997).

In contrast, melatonin duration has no effect on the primary sexual characteristics of birds. Unlike mammals, the *pars tuberalis* of the adenohypophysis expresses almost no melatonin receptor binding (Rivkees et al. 1989; Cassone et al. 1995) or receptor mRNA (Reppert et al. 1995), and neither pinealectomy nor exogenous melatonin affects annual growth or regression of the gonads (Cassone and Yoshimura 2015). However, there is growing evidence that melatonin affects the brains and behavior of passerine birds' vocal production. The song control systems of male house sparrows (Whitfield-Rucker and Cassone 1996), zebra finches, *Taeniopygia guttata* (Gahr and Kosar 1996), and European starlings, *Sturnus vulgaris* (Bentley 2003) express sexually dimorphic, high-affinity melatonin receptor binding, and expression of mRNA of the three melatonin receptors, Mel_{1A}, Mel_{1B}, and Mel_{1C} are differentially expressed in the male song control system (Fusani and Gahr 2015). Further, expression of melatonin receptor mRNA is upregulated in long days of spring and summer in European starlings (Bentley et al. 2013).

Melatonin functions in photoperiodic control of vocalization behavior in male passerines, such that the disinhibition of winter's long durations of melatonin allows for the expansion vocal behavior and the song control nuclei. Long durations of melatonin consistent with winter nights prevent the expansion of the HVC and RA as they go from short days to long days in house sparrows (Cassone et al. 2008) and Indian weaver bird, *Ploceus philipinnus* (Surbhi et al. 2015). In parallel, long durations of melatonin prevent the spring-like expansion of vocal state in male house sparrows (Harpole et al. 2020). Pinealectomy also affects the timing of vocal expansion and complexity upon photostimulation (Wang et al. 2014).

This study had two objectives: (1) to provide a detailed analysis of photoperiodic dynamics in female house sparrow; the quantity and quality of female vocalizations in short and long days, as well as when during the day these behaviors are produced. In parallel, (2) we examined the effect of pinealectomy surgery on seasonal vocal behavior, as there is evidence of pineal control in males. The results show that female house

sparrows have a richer vocal repertoire than has been previously thought, that photoperiod affects the quantity and quality of female vocalizations, and that, in contrast to the situation in males, the pineal gland plays little role in seasonal control of female vocalizations.

4.2 Methods

4.2.1 Animal housing, surgeries, and experimental timeline

Adult female house sparrows (n=12) were captured via mist net in central Kentucky (38° N) and moved to an outdoor aviary, with food (2:1 white millet seed and chick starter) and water ad libitum at the University of Kentucky Ecological Research and Education Center field station. As the captures occurred in the summer and we wished the birds to begin the experiment in a photosensitive state, we moved them indoors (constant 22.2°C) to isolation cabinets, each with timer-controlled white LED lights (40 µW/cm2, approximating the phase and photoperiod outdoors at the time with 6 hours of light, 18 hours of darkness, LD 6:18, lights on at approximate sunrise), a microphone (either a Røde NT3 (n=6) or an AKG Perception 170 (n=6)), and an infrared detector to measure locomotor activity. Constant background white noise was played from speakers outside the cabinets to improve acoustic isolation among birds (average power = 21 dB, frequency range from 0 to 10 KHz, measured within the cabinets). In total, birds remained in the isolation cabinets for 183 days.

After a week of adjusting to the isolation cabinets, birds were subjected to either a PINX (n=6) or Sham (n=6) surgery over the course of four days, as previously described (Lu and Cassone 1993a; Wang et al. 2014) . Birds received acetaminophen in their drinking water for three days following the surgery.

As the birds were moved indoors during the long days of summer, they were kept in the simulated short days (LD 6:18) for 88 days before beginning the experiment to transition them to a photosensitive state. Six days of locomotor activity from these short days are shown in Figure 4.1. The birds experienced an increase in photoperiod to equinoctial conditions (LD 12:12) for eight days, before a further expansion to long days

(LD 18:6) for 79 days. The birds were then transcardially perfused with 4% paraformaldehyde and the left ovary weighed. One female's ovary was not collected due to an oversight.
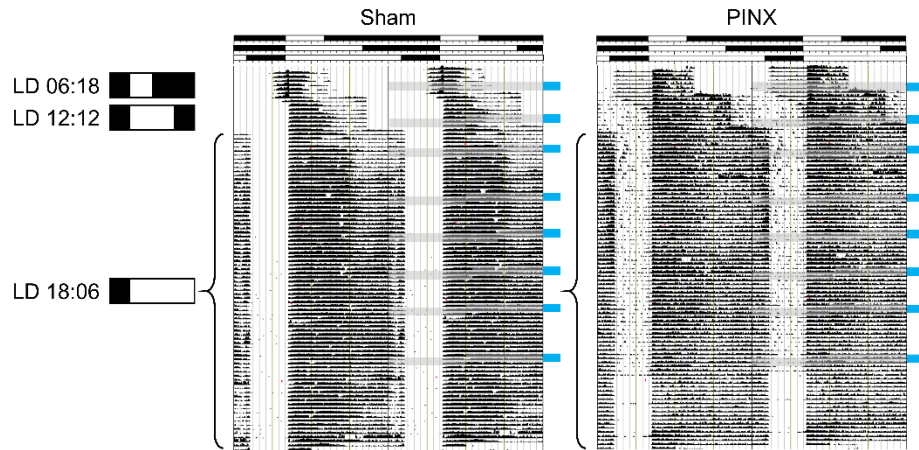
Figure 4.1 Double plotted actograms
*Double plotted actograms of a representative Sham (**A**) and PINX (**B**) female birds. Surgeries occurred 10 weeks before, activity records not shown for this portion of the experiment. Birds began in a 6 hours of light, 18 hours of darkness (6:18 LD) photoperiod: the transition to 12:12 is indicated by an arrow. The second arrow indicates the transition to 18:6. Blue shading on the right portion of the actograms indicates the vocal recordings sampled throughout the experiment.*

All animal care and procedures were approved by University of Kentucky's Institutional Animal Care and Use Committee and comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

4.2.2   Data collection and analysis

Locomotor activity was continuously recorded from each cage's IR detector with VitalView data acquisition system in 5-minute bins (STARR Life Sciences Corp., Oakmont, PA). At eight stages, 48 hours of continuous audio was recorded using Raven Pro 1.4 multi-channel recorder (Cornell Lab of Ornithology, Ithaca, NY) from 2 MOTU 8pre firewire audio interface recording units (MOTU, Inc., Cambridge, MA): end of short days, end of LD 12:12, and six recordings roughly every ten days throughout long days (7, 17, 28, 34, 42, and 56 days into LD 18:6).

Vocalizations were extracted using Raven Pro 1.4, generating timestamps for each vocalization, that could be totaled (Figure 4.2A) and plotted as mean by hour of each bird's daily activity (Figure 4.4A-D & I-L). The locomotor activity deflections coincident with the vocal recordings were also plotted as such (Figure 4.2B, Figure 4.4E-H & M-P).

For 5 of the 8 vocal recording sessions (recordings 06:18, 12:12, 18:06 02, 04, and 06), sonograms were produced in Raven Pro 1.4 software, visually and aurally inspected, and manually placed into categories based on criteria as in Wang et al. (2014) and Harpole et al. (2020)(Figure 4.3). Each bird's repertoire was examined for the first 100 morning vocalizations, when available, for the short days, the LD 12:12, and the second, fourth, and sixth recordings of long days. One Sham female did not reach 100 vocalizations for the first two and a half hours the morning of the recording on the short days (56 vocalizations), LD 12:12 (72), and second long days recording (64). A second Sham female only vocalized twice in the first two and a half hours of the short days recording.

We plotted the total repertoire produced at each sampled recording (Figure 4.3B). We also calculated the complexity of the expressed single syllable call repertoire (Harpole et al. 2020) such that a '0' would indicate all 'simple' calls associated with "quiet, chirping noises ("social song")" generally produced by house sparrows in flocks for contact, absent any sexual component. A '1' would indicate all 'complex' calls, described as being used by males to attract a mate and establish a territory (Figure 4.3C)(Anderson 2006b; Summers-Smith 2009). The complex call category includes the most common vocalization of the house sparrow, the 'chirrup.'

Daily parameters of phase angle and alpha were calculated from looking at raw data in spreadsheets (Figure 4.5). Onsets were determined as the time at which the percentile data exceeded 0.01 for each bird and each behavior, preceding an activity bout of three 5-minute samples also greater than 0.01. Offsets were determined as the last value following the primary activity bout where the percentile value was greater than 0.10, with no more of a gap in inactivity than three five-minute bouts. These criteria were informed and confirmed with graphs and actograms; the values were most often apparent. Phase-angle (ψ) between activity onset and lights on and between activity offset and

lights. Daily durations of locomotor activity and vocalization behavior (α) were determined from the length of time between onset and offset of each behavior.

4.2.3 Analytical statistics

Statistical analyses were calculated in Graph Pad Prism 6 (GraphPad Software, San Diego, CA), two-way repeated measures ANOVA with both Tukey's multiple comparison tests for pairwise comparisons across time (week-to-week, lowercase letters on Figures 4.2, 4.3, and Figure 4.5), and Sidak's multiple comparison test to compare the two experimental groups (asterisks on Figure 4.3B, Supplemental Figure 4.4). To analyze the effect of surgery on ovary size at sacrifice we used an ordinary unpaired two-tailed t-test.

Cohen's d values were calculated manually in Microsoft Excel from the difference in means between two experimental groups or timepoints of interest divided by the same two groups' pooled standard deviation. Results are presented as mean ± standard error.
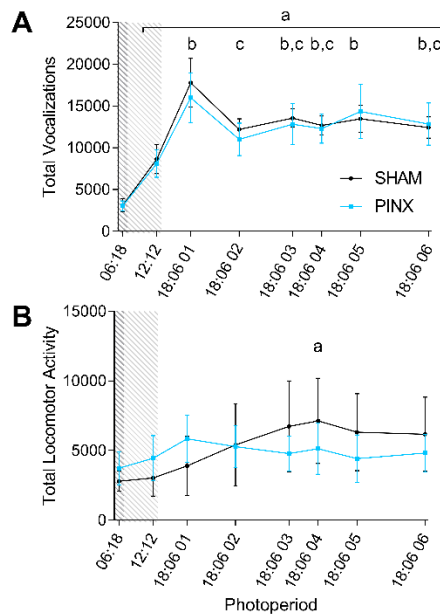
Figure 4.2 Activity totals
*Total counts of vocal (**A**) or locomotor (**B**) activity across the 48-hour recordings. A lowercase letter is a significant effect of time (p<0.05) at the corresponding time as found by repeated measures two-way ANOVA and Tukey's post-hoc test, from the time point denoted by the letter: a= different from 06:18 timepoint; b= different from 12:12 timepoint; c= different from 18:06 01.*

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4.3 Results

4.3.1 Vocalization totals

The birds vocalized more as they transitioned from short to long days (Figure 4.2A)(Two-way repeated measures ANOVA: Interaction $F_{7, 70} = 0.279$, $P = 0.96$; Time $F_{7, 70} = 26.2$, $P < 0.0001$, Treatment $F_{1, 10} = 0.0322$, $P = 0.861$). In short days, the birds vocalized 3083 ± 25 times. As the birds experienced increases in photoperiod, they vocalized more than the initial short day sample (Tukey's multiple comparison test, all P-values ≥ 0.0004, Cohen's d's ranging from 14.5 to 50.7). Seven days after transitioning to long days (LD 18:6) produced a relative peak of $16,889 \pm 897$ vocalizations.

In contrast, the amount of locomotor activity varied only slightly week-to-week, as sampled (Figure 4.2B)(Two-way repeated measures ANOVA: Interaction $F_{7, 56} = 1.675$, $P = 0.13$; Time $F_{7, 56} = 2.492$, $P = 0.0266$, Treatment $F_{1, 8} = 0.01969$, $P = 0.8919$). There was but one pairwise difference, the birds produced more locomotor activity at the fourth long day recording than they had in short days. (Tukey's multiple comparison test, $P = 0.0433$, Cohen's d = 2.6).
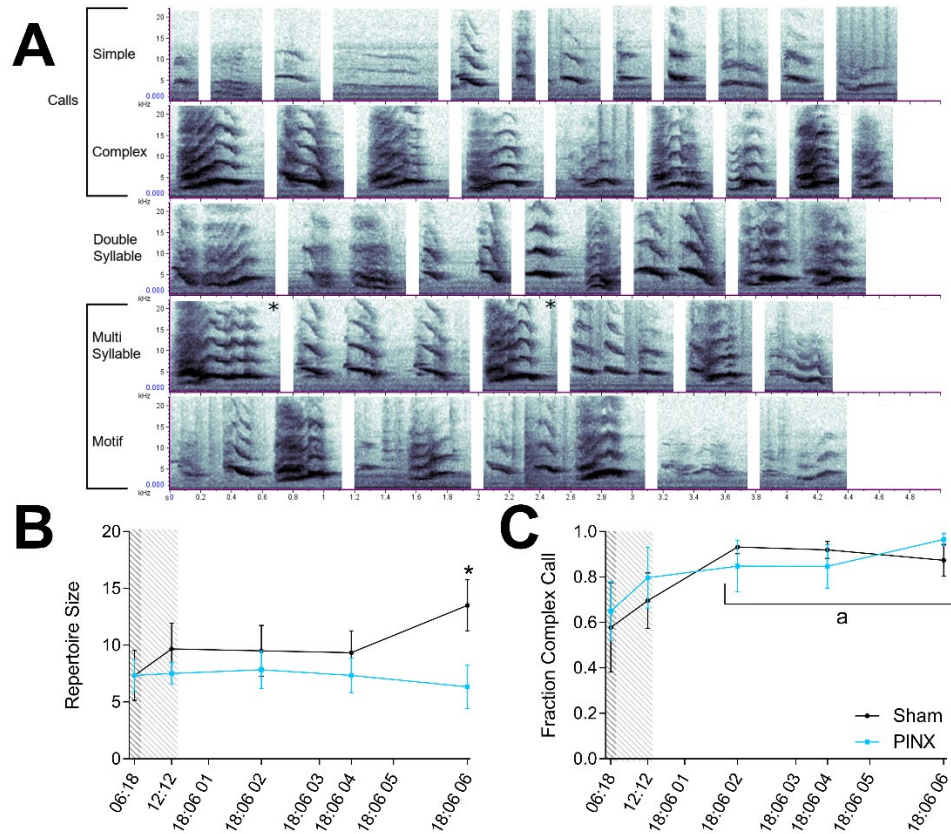
Figure 4.3 Repertoire

   *Representative female house sparrow sonograms of calls, which were split into the categories of 'simple' and 'complex,' double-syllable vocalizations (DS), and multiple-syllable vocalizations, which also include motifs (**A**). Axes are approximate as this composite figure was sourced from multiple birds and instances of our sonogram software. Asterisks (*) denote a type of call that is female-unique, so far as we have not seen it in males in our lab in similar experimental conditions.*

   *The repertoire produced by each bird at each quantified recording, averaged by surgical group (**B**). An asterisks (*) is a significant effect of surgery at the corresponding time as found by repeated measures two-way ANOVA and Sidak's multiple comparisons post-hoc test: *=p<0.05.*

   *Of the vocalizations classified as calls, the complexity at each time point, wherein a value of 1.0 would indicate all calls sampled are classified as the 'complex' type and 0.0 would indicate all calls sampled are of the 'simple' category (**C**). A lowercase letter is a significant effect of time (p<0.05) at the corresponding time as found by repeated measures two-way ANOVA and Sidak's post-hoc test, from the time point denoted by the letter: a= different from 06:18 timepoint.*

4.3.2 Repertoire

The first 100 vocalizations of the dawn chorus from five of the eight recordings were visually and aurally inspected. Sham birds had a sum repertoire of 23 ± 10 vocalization types and PINX birds 16 ± 5, although an unpaired t-test revealed these Sham repertoires were not significantly higher ($F_{5, 5} = 3.459$, $P = 0.1994$).

The female house sparrows produced a diverse and dynamic vocal repertoire (Figure 4.3A). Wang and colleagues (2014) described three categories of vocalizations for male house sparrows based on the number of components: single syllable calls, double syllable vocalizations, multi-syllable vocalizations and motifs. All three categories were represented by females as well, albeit skewed towards shorter, single syllable vocalizations.

Female house sparrows produced a vocal subtype that was not expressed by individually housed males in similar experimental conditions: a modulation of a subtype we identify as the "chirrup" (see the haystack shaped sonogram vocalizations, e.g. Figure 4.3A, Complex Calls 1st, 3rd, 7th, 8th vocalizations are consensus examples, however a number of these complex calls could be considered chirrups, Supplemental Video 4.1) with a number of additional peaks (see Figure 4.3A, Multi-syllable 1st and 3rd vocalizations, denoted by an asterisk *).

A vocal subtype known as the "cheep" is considered by some a song of the house sparrow. The female house sparrows in our study also did not produce this subtype, although it has been observed rarely in the wild (Will 1973; Nivison 1978). This is produced by males in similar experimental conditions. The females also did not produce any vocalizations of the "chatter/rattle" subtype of multi-syllable vocalization associated with threat and alarm.

4.3.2.1 Photoperiodic repertoire dynamics

A weak effect between PINX and Sham birds was revealed by our measures in the weekly expression of repertoire (Figure 4.3B)(Two-way repeated measures ANOVA: Interaction $F_{4, 40} = 2.145$, $P = 0.092$; Time $F_{4, 40} = 1.007$, $P = 0.4151$, Treatment $F_{1, 10} =$

1.533, $P = 0.2439$). Fifty-six days into long days at the sixth recording, Sham animals produced on average a repertoire of 13.50 ± 2.26 vocalization types, significantly more ($P = 0.0479$, Cohen's d = 1.39) than the 6.33 ± 1.91 types produced by PINX birds.

4.3.2.2 Call complexity

As birds experienced the change from short days to long days, they produced single-syllable calls at a higher complexity (Figure 4.3C, Supplemental Video 4.2). In the short days, the birds produced $61.4 \pm 3.6\%$ complex calls. In long days (LD 18:6), the complexity increased (long day recording 02 & 04, P-values 0.0295 & 0.0354, Cohen's d = 5.02 & 5.25), ending at $91.9 \pm 4.6\%$ ($P = 0.0127$, Cohen's d = 5.24).

4.3.3 Daily distribution

Birds lacking a pineal gland distributed their behavior differently from Sham operated birds at a couple of time points, always in the first 6 hours of light (Figure 4.4). In short days, the PINX birds produced their peak vocalization an hour later than sham animals ($P = 0.0097$) and had a reduced peak in 12:12 (two P-values ≤ 0.03). In long days, the vocalization distribution patterns converged between experimental groups. At the third and fourth recording during long days, the PINX birds produced locomotor activity with a morning peak that was absent in the more diffusely distributed behavior of the Sham birds (three P-values ranging from 0.0017-0.0125).

(next page) Daily distributions of vocalization and locomotor activity under different photoperiodic conditions. Each graph represents 48 hours of continuous recording, averaged together by hour, in surgical groups, from the labelled timepoint. Both activities are presented as percentile of each individual bird and day's daily total, e.g. a value of 0.1 would indicate 10% of that bird's daily activity occurred during the corresponding hour. The top graph of each pair is vocalization (A-D, I-L), the bottom is locomotor activity (E-H, M-P). An asterisks is a significant effect of surgery at the corresponding time as found by repeated measures two-way ANOVA and Sidak's multiple comparison post-hoc test: *=p<0.05; ** = p<0.01 .
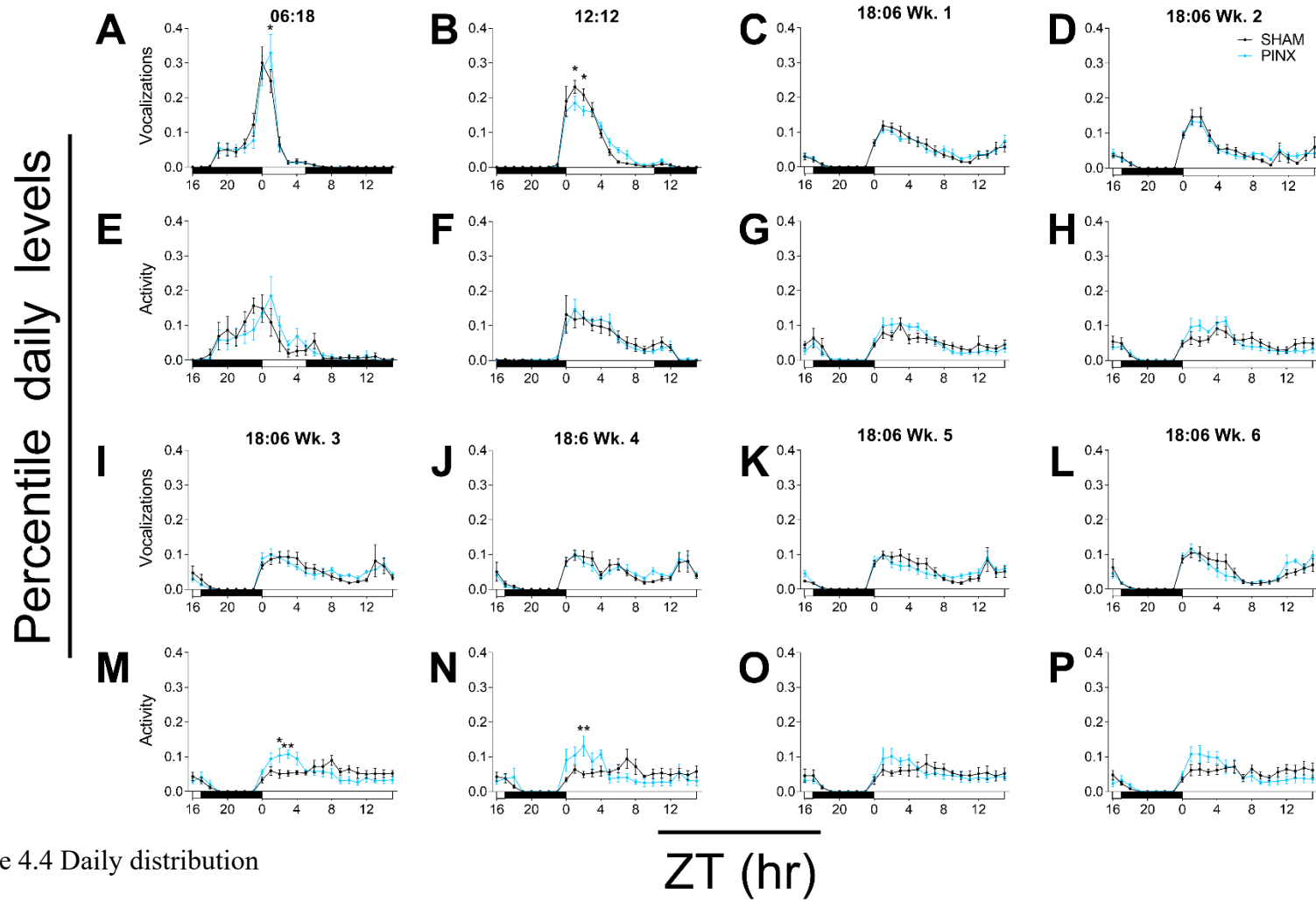
Figure 4.4 Daily distribution

4.3.4 Diurnal parameters

In the short photoperiods, the birds began their vocalization -4.94 ± 0.10 hr and locomotor activity -4.03 ± 0.33 hr before lights on (Figure 4.5A & D). As the experienced photoperiod increased, both sets of activity onsets became more closely associated with the timing of lights on, significantly different from the early values occurring in short days (P-values <0.0001). The vocalization phase angles in long days were also closer to 0 than the values of -0.167 ± 0.04 hr in LD 12:12 (P-values <0.0001), but not for locomotor activity.

Behavioral offsets were well associated with lights off in all photoperiods, all relationships close to zero with no effect of time or surgery (Figure 4.52B & E)(Vocalization: two-way repeated measures ANOVA: Interaction $F_{7, 70} = 1.319$, $P = 0.2543$; Time $F_{7, 70} = 1.456$; $P = 0.1975$, Treatment $F_{1, 10} = 0.1961$, $P = 0.6673$ | Activity: two-way repeated measures ANOVA: Interaction $F_{7, 56} = 0.897$, $P = 0.520$; Time $F_{7, 56} = 0.4548$, $P = 0.8627$; Treatment $F_{1, 8} = 1.269$, $P = 0.2925$).

Related to the early activity onsets, in short photoperiods the duration (α) of vocalization behavior was 11.74 ± 0.015 hr and locomotor behavior 11.26 ± 0.75 hr, longer than the 6 hours of daylight they were experiencing (Figure 4.5C & F). The long day alphas for both behaviors were significantly longer than both the short days and LD 12:12 (all P-values<0.0001).

4.3.5 Ovaries

At the end of the experiment, the animals were sacrificed and the left ovary was collected. The ovaries all appeared small except for one, in the PINX group, which was mature. Sham birds' ovaries weighed 0.128 ± 0.048 g while PINX birds weighed 0.160 ± 0.091 g. A student's t-test revealed no significant difference between the weights of the ovaries ($F_{5, 4} = 3.550$, $P = 0.4922$).
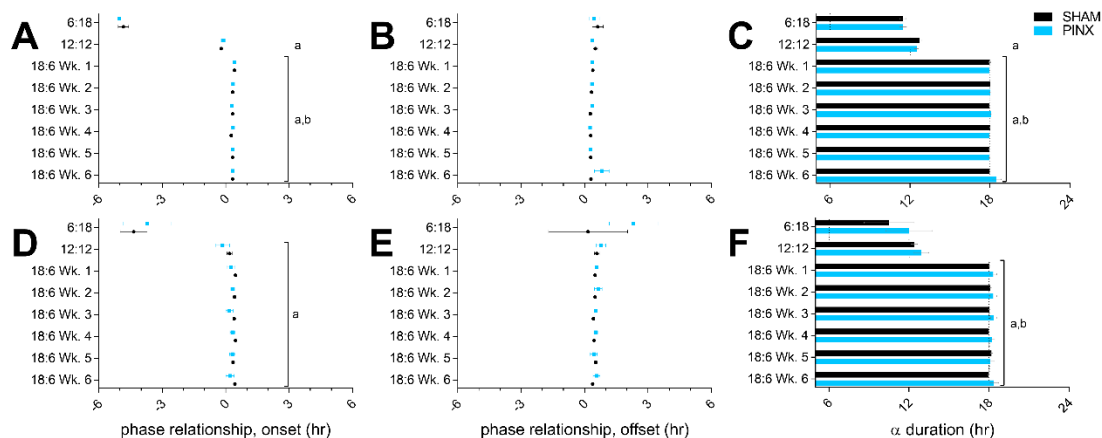
Figure 4.5 Diurnal parameters

*Vocalization and locomotor activity diurnal entrainment measures. The left column is vocalization (**A**, top) and locomotor activity (**D**, bottom) phase relationships to the onset of light, respectively. The middle column is the vocalization (**B**, top) and locomotor activity (**E**, bottom) phase relationships to the offset of light. The right column is vocalization (**C**, top) and locomotor activity (**F**, bottom) lengths of daily activity or alpha. A lowercase 'a' indicates that value is significantly different from the 06:18 time point's value; a 'b' indicates that value is significantly different from the 12:12 time point's value, all with p<0.0001 from a repeated measures two-way ANOVA with a Tukey's multiple comparison post-hoc test.*

4.4   Discussion

Female house sparrows express a diverse repertoire, intersecting much of what has been heard in male house sparrows in similar lab conditions. As the photoperiod lengthens from short days to long days, the birds' vocal behavior changes state: from vocalizing less in short days, with simple calls, to more frequent vocalizations and an increase in call complexity in long days. In contrast to what has been shown in male house sparrows, removing the pineal gland has little effect on the timing of these dynamics.

The photoperiodic dynamic in vocalization that we observed was on the order of what was heard from male house sparrows. Wang and colleagues (2014) measured a seasonal increase in vocalizations from ~7,777 ± 1,039 vocalizations in short days to ~18,879 ± 1,933 vocalizations in long days. In the current study, female birds began by producing 3,083 ± 25 vocalizations in short days and to peaking with 16,889 ± 897 vocalizations upon transition to long day conditions. Wang and colleagues also found that

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pinealectomized male house sparrows had an earlier but less sustained increase in vocal state (2014). Female birds showed no difference in this aspect, and only a few scant differences in surgical groups at all (Figure 4.3B, Figure 4.5). A previous study reported that pinealectomy in house sparrows produced a change in phase relationship in regards to locomotor activity, causing a bird to begin its activity bout earlier in anticipation of the lights-on cue than before its surgery (Gaston 1971). In contrast to this study, we saw no effect of pinealectomy on phase relationship in either behavior we measured (Figure 4.5).

Demonstrations of photoperiodic dynamics in female songbirds within a single season are rare in captivity (Pavlova et al. 2007). Seasonal dynamics have been measured more frequently in several species in the wild, in the sizes of the song control nuclei (Kirn et al. 1989; Deviche and Gulledge 2000; Jawor and MacDougall-Shackleton 2008) and the rate of singing (e.g. Brunton et al. 2016, Magoolagan et al. 2019). The female seasonal dynamic we show here in isolated birds may be low compared with what happens in the field, as female birds are thought to prefer several factors beyond photoperiod for seasonal induction (Ball and Ketterson 2008).

Similarly, the female house sparrows' repertoire was more diverse than what is appreciated but differed from males in important ways. Female house sparrows did not produce any vocalizations of the "cheep" subtype, considered to be a song of male house sparrows (Nivison 1978). Male house sparrows also produce "musical song" comprised of several vocal subtypes and repeated in the same pattern. The female birds in this study also produced some vocalizations with this syntactical complexity, frequently with two notes, and occasionally three. Catchpole and Slater (2008) also recognize that there is overlap with a simple song and a complex call. We saw a change in the types of calls produced from those associated with social flocking to calls involved with mate attraction (simple to complex calls), the latter of which could be classified as non-musical song. Thus, house sparrows could be added to the growing list of songbirds with females that sing.

Males have been recorded producing the vocalization type classified by such names as "churr." "chattering," or "rattling." This vocalization is generally associated with a threat or alarm call (Summers-Smith 2009). This vocal subtype has been observed

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in the wild by both males and females (Nivison 1978; Anderson 2006b) but was absent in this study.

The presence of a pineal gland had no effect on the reproductive state, as the excised ovaries of the birds were all generally small except for one that appeared fully developed. The reason males have a larger photoperiodic response could be due to sexual dimorphisms in the brain. Females possess smaller song control nuclei than do males (Bottjer et al. 1989). Female house sparrows do not feature melatonin receptor binding in the diminished HVC, Area X or RA; only displaying some binding in the MAN (Whitfield-Rucker and Cassone 1996), thus making it worthy of consideration in mediating the small effect of pinealectomy we observed in female repertoire size. Lesions of the MAN in male zebra finches has no effect on adult males with already crystallized song, based on visual and aural inspection of sonograms (Bottjer et al. 1984). However, the LMAN is activated with early immediate gene expression upon singing in both juveniles and adult male zebra finches (Jarvis and Nottebohm 1997) and exhibits electrophysiological activity while adult male zebra finches were singing (Hessler and Doupe 1999). Further, in some other species of passerine birds, there are no sex differences in size between the male and female LMAN: e.g. in cowbirds, *Molothrus ater* (Hamilton et al. 1997), and Bengalese finch, *Lonchura striata* (Tobari et al. 2005). These similarities shown in females suggests a role for the LMAN in song perception and sexual selection of vocalizations by the female, as there was a positive correlation between LMAN size and ability to discriminate effective male songs in cowbirds (Hamilton et al. 1997). The small effect of pinealectomy on female repertoire size could be mediated in the LMAN, or some unidentified part of the brain. Additionally, the question remains whether a long duration of melatonin could prevent the observed expansion in call complexity in female house sparrows, as was demonstrated in males (Harpole et al. 2020).

There is a growing effort in avian birdsong and vocalization research, as well as all model organism research, to include females (Langmore 1998; Riebel 2003; Odom et al. 2014; Riebel et al. 2019). This includes the new database to record and gather recordings of female birds singing in their natural habitat (Odom et al. 2017).There is

considerably more strain on females than on males in vertebrate reproduction. In many avian species, including house sparrows, males help in incubation and feeding of the young (Anderson 2006c). Even so, the resources needed to produce the egg and to lay it, both of which are costly in resources and time to recovery, make the energetic requirements for females greater than males (Ball and Ketterson 2008). Thus, should a mistake in timing occur, females would suffer the consequences more dramatically than would a male. The data presented here add to a growing literature that passerine avian female vocalizations are more sophisticated than initially thought (Riebel 2003; Odom et al. 2014; Riebel et al. 2019), these processes in half of a species' population should not be ignored. Even so, there are several songbird species that exhibit song behavior in both males and females. For example, male and female northern cardinals, *Cardinalis cardinalis*, duet during the breeding season. In this species, song control nuclei in both sexes are larger during spring than during winter, even though male song control nuclei are larger than those in the female birds (Jawor and MacDougall-Shackleton 2008). This dimorphism of male's larger song control nuclei is consistent across nearly all passerine birds, including house sparrows (Whitfield-Rucker and Cassone 1996). Female song is more common in tropical species, where competition among intraspecifics is high (Ball 2016). As in the cardinal, in duetting rufous and white wrens, *Thryothorus rufabus*, bay wrens, *T. nigricapillus* (Brenowitz and Arnold 1986), and African bush shrikes, *Laniarius fundebris* (Gahr et al. 1998), males continue to express larger HVC and RA than do females, even though the song repertoires among the sexes in these species are similar.

Female birds are thought to require additional environmental cues for seasonal induction, such as temperature, changes in diet, and availability of nesting material (Ball and Ketterson 2008). Thus, single-year captive demonstrations of female vocal development are even more rare than their male counterparts mentioned above (Pavlova et al. 2007). This study shows that for female house sparrows, experiencing an increase in photoperiod is sufficient to bring about a change in vocal state.

4.5   Acknowledgements

Additional files:

Supplemental Video 4.1: Demonstration of female-unique call types.
Supplemental Video 4.2: Demonstration of the call complexity switch.

CHAPTER 5.   CONTEXT AND NOVELTY INCREASE STRENGTH OF AUDITORY CUES AS A CIRCADIAN ZEITGEBER IN SONGBIRDS

5.1   Introduction

The canonical "cue giver" or *zeitgeber* to a free-living vertebrate's circadian system is the light cycle imposed by the rising and setting of the sun. However, there are likely evolutionary advantages to be able to anticipate rhythmic phenomena beyond light. As such, several modes of non-photic entrainment have been discovered, including temperature cycles (Pittendrigh 1960), food availability (Richter 1922; Stephan et al. 1979), drug administration (Honma et al. 1987), exercise (Edgar and Dement 1991), humidity (Pittendrigh 1950), air pressure (Hayden and Lindberg 1969), turbulence (Naylor 1996), and electromagnetic fields (Dowse and Palmer 1969); there are several possible types of sensory input to transmit ambient temporal cues to the central clock apparatuses. Several of these zeitgebers could be grouped together by causing a change in arousal of the organism, which interacts with its central oscillators (Johnson et al. 2003).

Social cues can also act as zeitgebers for animals that live in communities, potentially for the purpose of synchronizing clocks across the society (Davidson and Menaker 2003; Mistlberger and Skene 2004; Favreau et al. 2009; Castillo-Ruiz et al. 2012). Social cues are likely more important to animals that live in constant dark and temperature conditions. For example, a beaver (*Castor canadensis*) family that lived in their lodge in near constant darkness and temperature, synchronized their collective activity with a period of 27 hours (Bovet and Oertli 1974). Leaf-nosed bats (*Hipposideros speoris*) that were experimentally trapped in a light-tight cave exhibited anticipatory behavior to sunset, likely from social cues from the turbulence of wings in flight or ultrasonic vocalizations from its free-living conspecifics (Marimuthu et al. 1978; Marimuthu et al. 1981).

In other social organisms that detect and live in light-dark cycles, olfactory signals can synchronize rhythms. Fruit fly individuals (*Drosophila melanogaster*) have more robust rhythms when housed with conspecifics than they do separately, and arrhythmic mutant or phase shifted "visitors" can disturb this synchrony, which is probably reliant on olfactory cues (Levine et al. 2002). Teresa Lee and colleagues

characterized the influence of what turned out to be olfactory social cues on the diurnal South American rodent, the degu (*Octodon degus*): circadian re-entrainment is accelerated when phase advanced females (or castrated male degus) are housed with an entrained female donor (Goel and Lee 1995a; Goel and Lee 1995b; Goel and Lee 1997; Governale and Lee 2001; Jechura et al. 2003). There is some evidence that rats' (*Rattus norvegicus*) entrainment to a light pulse is enhanced when the olfactory signal of cedar oil is present, which opens the possibility that a social-specific olfactory cue could function in a similar manner (Amir et al. 1999). However, in sum social entrainment does not seem to be an important zeitgeber to rodents (Mistlberger and Skene 2004).

Aural cues can entrain social rhythms as well. Male common marmosets (*Callithrix jacchus*) free-running in constant light responded to the sound of conspecifics housed in the same room by expressing pseudo-splitting of their rhythms or relative coordination to phase mismatched free-running or light-dark entrained individuals (Erkert et al. 1986). The strength of this zeitgeber of acoustic contact can be enhanced when aural cues from an individual the subject is socially familiar to is presented, a littermate or pair bonded mate (Erkert and Schardt 1991).

There are four peer-reviewed studies which have previously shown that songbirds can entrain to rhythmic presentation of audio cues (Menaker and Eskin 1966; Gwinner 1967; Lohmann and Enright 1967; Reebs 1989). In sum these papers suggest that almost any acoustic cue can act as a weak zeitgeber to the locomotor activity clock. It is difficult to parse from the performed experiments if the social context or novelty of the sounds are involved in this phenomenon, or if the acoustic cues simply cause a broad arousal to a free-running bird. These studies have not been revisited in a couple of decades, so much that all audio cues were presented from analogue tape.

In the current experiment, we utilize the model organism the zebra finch (*Taeniopygia guttata*) and its very clearly delineated song from call to determine if social context affects the strength of these aural zeitgebers and affecting the magnitude of the arousal. We investigate which components of the presented audio affects the clock, such as the length of the presentation, novelty, and social context.

5.2 Methods

5.2.1 Animal care and ethics

Zebra finches (n= 85: 47 male, 38 female) were taken from the colony maintained at the University of Kentucky Ecological Research and Education Center (EREC) in Lexington, KY. Birds (12 per session: 6 male, 6 female) were individually housed in cages (30.5 × 41 × 42 cm) in their own cabinet (55.9 × 47.6 × 47.6 cm) with perches, at a constant ambient temperature of 22.2 ± 1 °C. Birds were provided with *ad libitum* food (2:1 white millet seed to chicken starter crumble) and water. LED arrays in each cabinet provided timed white light (40 µW/cm^2), and constant green-tinted night light (>1×10^{-4} µW/cm^2). Constant background white noise (average power = 21 dB, frequency range from 0 to 10 KHz, measured within the cabinets) played from speakers outside the cabinets to increase acoustic isolation between birds. Locomotor activity was continuously recorded with an IR detector in each cage with VitalView data acquisition system in 5-minute bins (STARR Life Sciences Corp., Oakmont, PA).

All animal care and procedures were approved by University of Kentucky's Institutional Animal Care and Use Committee and comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

5.2.2 Playback and experimental timeline

Each cabinet was fitted with a Tymphany 2" Full Range Neodymium Driver speaker (San Rafael, CA). These 12 speakers were connected to a Dayton Audio MA1240 Multi-zone 12 Channel Amplifier (Springboro, OH), and the respective audio sources connected through its line-in jack. Playback volumes were standardized by measuring the average power of sounds in sonograms recorded to memory in Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY).

(A)

(B) Single song

Reversed song

Pseudorandom tones

Live colony monitoring   15-25 zebra finches
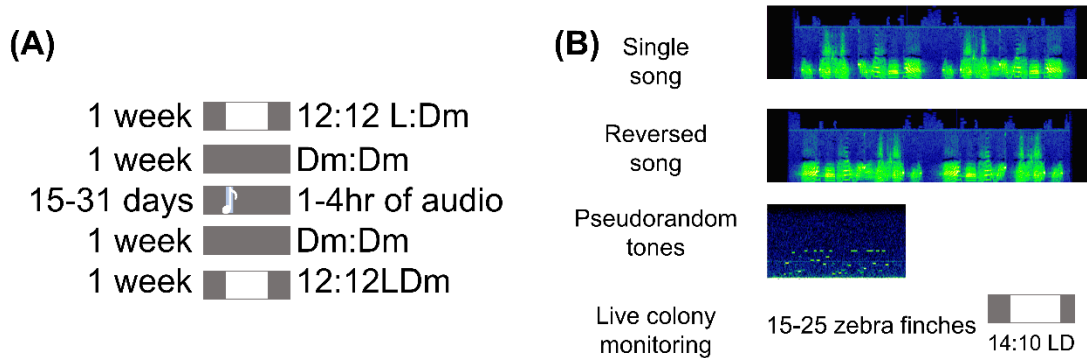
Figure 5.1 Experimental timeline

Experimental timeline for each trial (A), and representative sonograms of the various audio sources (B).

Each set of experiments was limited to 12 birds maximum due to the number of channels available from the playback amplifier. They followed the following format (Figure 5.1): birds were acclimated to their cabinet and entrained to a 12 hr light : 12 hr dark (12:12 LD) light cycle for at least 7 days. The birds were then released into constant dim light (Dm:Dm) for at least 7 days. Dim light is required for zebra finches as they most often do not survive constant dark conditions, in our experience. Following this, audio of the specific cue was played through the speakers in the cabinets every day at the time of previous lights on for the length of several weeks (15-31 days), for the indicated duration (1-4 hrs). After this period, audio cues were ceased and the birds were again allowed to free run in Dm:Dm, completing the trial.

We presented several types of audio cues across the various trials (Figure 5.1). The "single song" cue was a single wildtype zebra finch song "Wildtype_example1.mp3" which we found online(Buchen 2009)), from the colony of Fehér and colleagues (Fehér et al. 2009), chosen so that it would be novel to our colony. This single song was spaced at a natural cadence from a randomly selected zebra finch we recorded at 16 songs per minute, repeated for the specified duration, at 63 dB. This was presented to birds for a one hour duration (n= 9, 5 males, 4 females), two hour-duration (n= 11, 6 males, 5 females), or four hour duration (n= 17, 9 males, 8 females).

The second audio cue was the same song from the "single song," only reversed, to remove any appropriate syntactical information, (n= 11 , 6 males, 5 females).

The third audio cue was random tones roughly along the same frequencies (500-9,000 Hz) and duration of a song (~2 s) of the single syllable zebra finch song from the Korg DS-10 Plus synthesizer (Xseed Games, Torrance, CA) loaded into a Nintendo DS Lite video game system (Nintendo of America, Redmond, WA), and played through the speakers at 63 dB. The rationale behind this cue was that it would contain many of the same auditory properties of zebra finch song but lack many of the organisms' signatures. As this cue was randomly generated for each instance, it was also more novel than the single song or reverse song. The sample size was n= 9, 5 males, 5 females.

The fourth cue was live monitoring from our indoor zebra finch breeding colony. A Røde NT3 microphone was placed in the room, connected to a MOTU 8pre Firewire audio interface (Cambridge, MA) to power the microphone, and the audio-out line connected to the amplifier, playing through the speakers with the loudest vocalizations captured by the microphone at 70 dB. The zebra finch colony housed 15-25 zebra finches of various ages and both sexes, from nestling to 4 years old, which were housed in a photoperiod of 14:10 LD. The sample size was n= 28 , 16 males, 12 females.

5.2.3   Data analysis

Actograms were generated using ClockLab Analysis 5 software (Actimetrics, Willmette, IL) (Figure 5.2). To calculate the effect on the clock by this weak zeitgeber, actograms were double-plotted, and the activity onsets compared by eye with a one-day window. These were qualified using three degrees of certainty: no entrainment, perturbed but not necessarily entrained, and entrained (Figure 5.3). For this paper we only considered the highest certainty as a "day entrained," which was plotted per the total days the cue was presented.
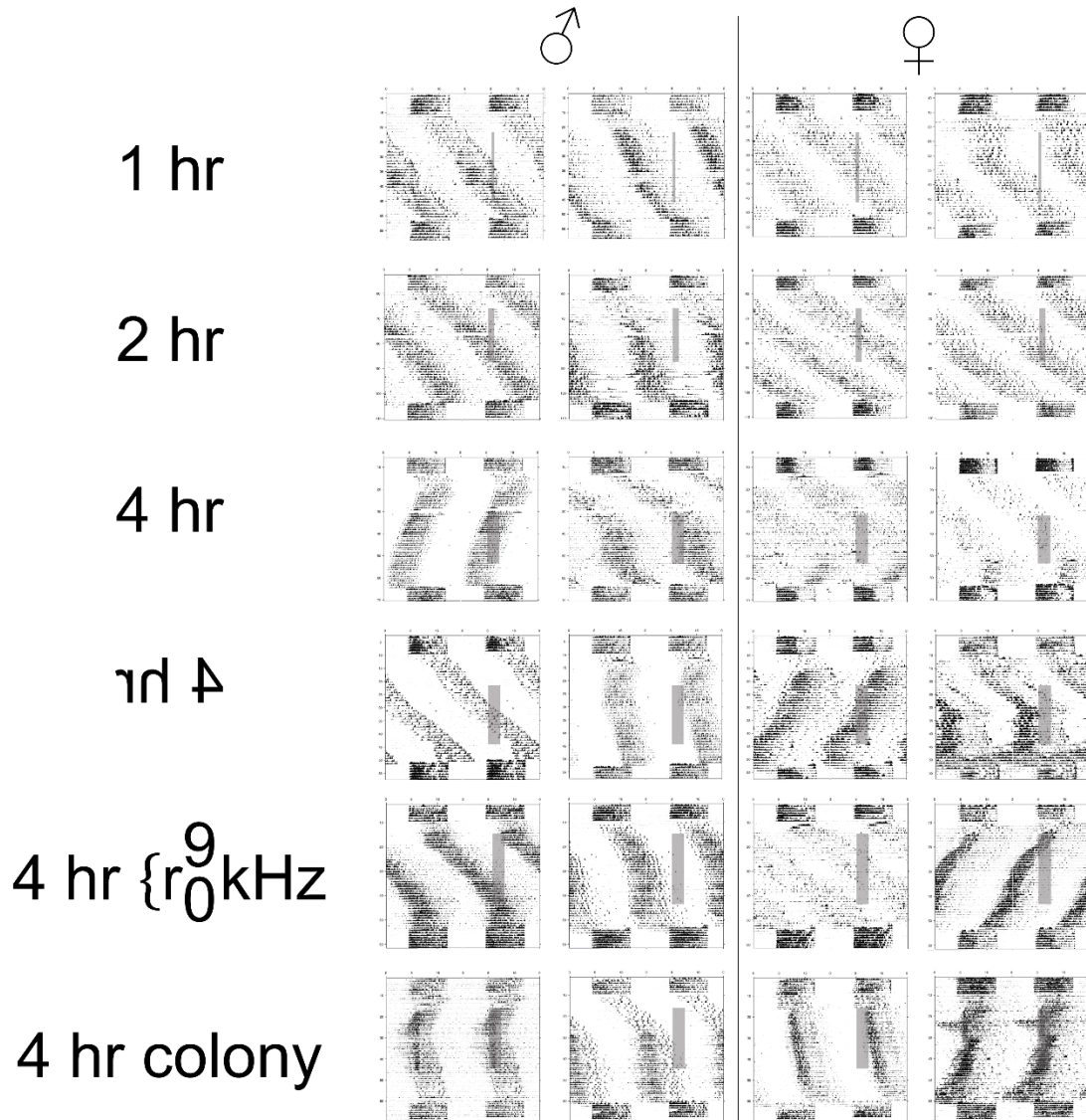
Figure 5.2 Representative actograms

Representative actograms from the various trials: a single zebra finch song (1 hr, 2 hr, 4 hr), that same song reversed (4 hr reversed), pseudorandom tones (4 hr with a frequency of 0.5 to 9kHz), and 4 hr of live colony monitoring from zebra finches entrained to a light-dark cycle (4 hr colony).

5.2.4 Statistical analysis

Ordinary one-way ANOVA of the percentage of days entrained when the audio cues were present was performed with GraphPad Prism 8 (San Diego, CA). Cohen's d values were calculated manually in Microsoft Excel from the difference in means between two experimental groups of interest divided by the same two groups' pooled standard deviation.

Results are presented as mean ± standard error.

5.3 Results

When a phase shift caused by the zeitgeber is not large enough to fully entrain the period imposed by the weak zeitgeber, "relative coordination" occurs (Pittendrigh and Daan 1976a; Pittendrigh and Daan 1976b; Pittendrigh 1981; Johnson et al. 2003). This manifested itself as some of the birds only showed an effect when their free-running period's activity onset approached coincidence with the day's rhythmic presentation of audio: they would entrain for several days before again expressing their endogenous free-running period.

Other birds expressed relative coordination in a manner consistent with "unstable phase entrainment" (Figure 5.3), wherein examining the data with a day-by-day window, the activity onsets would coincide, but the phase would 'jump' around when looking at a larger window of several days. This scalloped and/or hopping pattern is likely the result of the phase shifting effects of the audio cues onto the pacemaker, and the pacemaker repetitively failing to stably entrain.
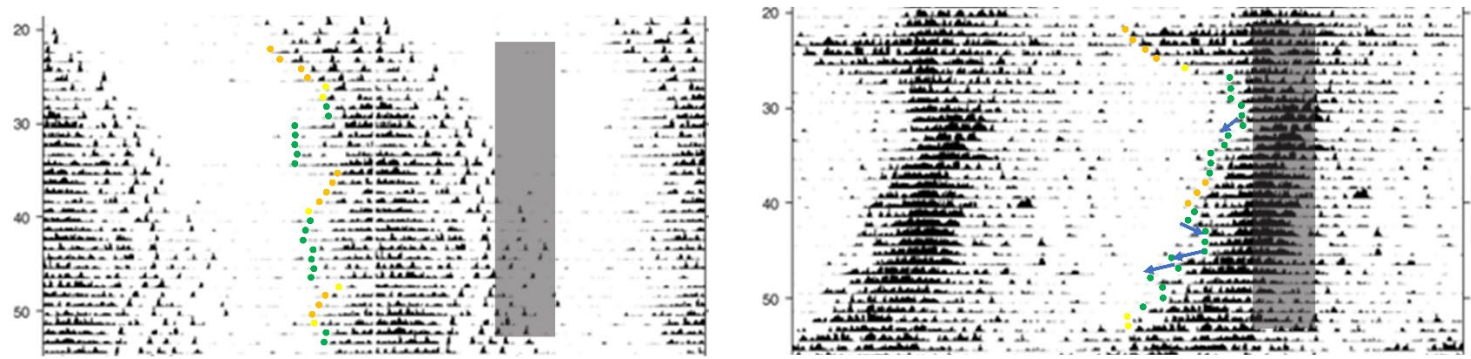
Figure 5.3 "unstable phase entrainment examples

(previous page) Examples of "unstable phase entrainment," the coloured circles indicating the certainty from orange (no entrainment), yellow (perturbation but not considered entrainment), and green (entrained). Blue arrows on the left actogram portion emphasize moments when the bird remained entrained, but the phase changed.

5.3.1 Days entrained

The birds receiving a rhythmic presentation of four hours of live monitoring from an LD entrained colony was significantly higher than several other types of audio, with $50.31 \pm 4.12\%$ days entrained (Figure 5.4). Birds receiving this rhythmic presentation entrained more than those that received two ($27.08 \pm 4.71\%$ days entrained) and four hours ($22.12 \pm 4.74\%$ days entrained) of single song playback (One-way ANOVA $F_{(5, 79)}$, p= 0.0308, Cohen's d= 1.14, and p= 0.0005, Cohen's d= 1.34, respectively). The live colony monitoring cue also significantly entrained the clock more than the cue of four hours of random tones ($25.97 \pm 6.66\%$ days entrained, One-way ANOVA $F_{(5, 79)}$ p= 0.0387, Cohen's d= 1.14).
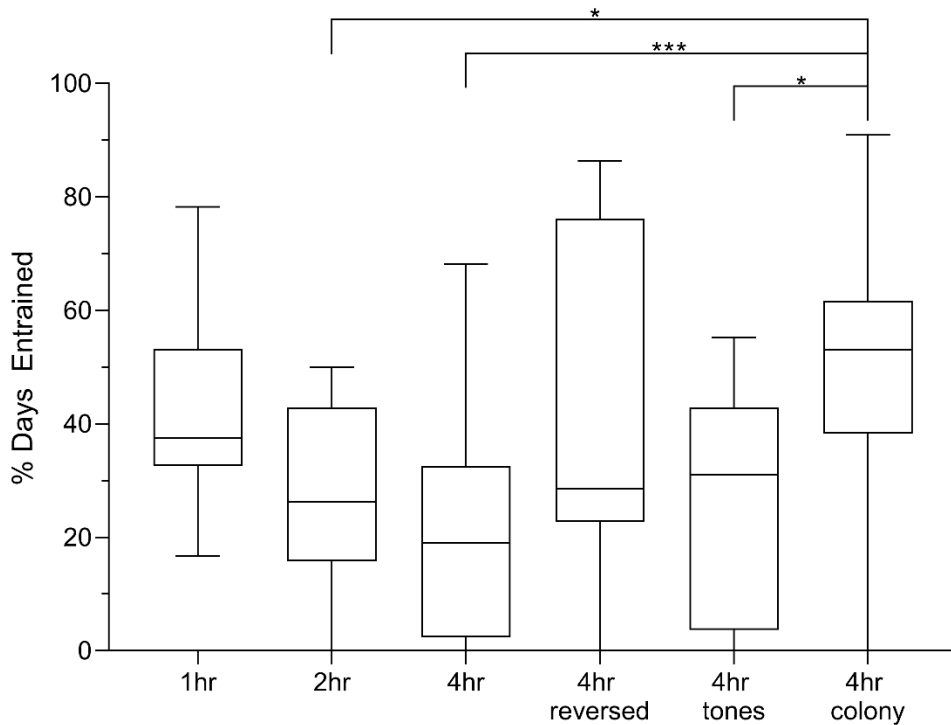
Figure 5.4 Percent days entrained

The percentage of days entrained across the various trials: a single zebra finch song (1 hr, 2 hr, 4 hr), that same song reversed (4 hr reversed), pseudorandom tones (4 hr tones), and 4 hr of live colony monitoring from zebra finches entrained to a light-dark cycle (4 hr colony). Boxplots were drawn using the Tukey method, such that the line represents the median, the box the inter-quartile range, and the whiskers the range, with outliers not visually represented if exceeding 1.5 times the inter-quartile range. * indicates a one-way ANOVA p-value of 0.0387 or less, and *** a p-value of 0.0005.

5.4   Discussion

We found that numerous modes of zebra finch-associated audio act as a weak zeitgeber to the clock underlying locomotor activity rhythms. Novel, contextually appropriate conspecific vocalizations affect the clock more than random tones or a repeated single song, as the strongest zeitgeber we presented was live monitoring from a colony of zebra finches entrained to a light-dark cycle. This suggests that the transduction of this weak zeitgeber to the central clock may be integrated through pathways underlying the perception of social cues.

This study joins four previous peer-reviewed studies on acoustic circadian entrainment in songbirds. In 1966, Menaker and Eskin showed half of their five each male and female house sparrows entrained or were moderately affected by rhythmic presentation of a taped 4.5 hour recording of a conspecifics colony's dawn and dusk choruses (Menaker and Eskin 1966). Gwinner showed three female siskins (*Spinus spinus*) and a male serin (*Serinus serinus*) entraining their locomotor activity to a 12:12 song:silence cycle in otherwise constant environmental conditions, and re-entraining, over the course of several days, to the stimulus after its presentation was advanced 6 hrs (Gwinner 1967).

Instead of conspecific vocalization recordings, Lohmann and Enright investigated mechanical buzzers or white noise generated by an oscilloscope as potential zeitgebers. In the first experiment, 4 greenfinches (*Chloris chloris*) and 1 chaffinch (*Fringilla coelebs*) were presented 12:12 loud mechanical noise:silence cycles in constant light conditions; this auditory stimulus showed varying degrees of acting as a weak zeitgeber, with the birds ranging from strong entrainment to relative coordination, to very little effect in the chaffinch. Their second experiment was transitioning from constant light and silence to more than a week of continuous mechanical noise, then cycling again; the birds (three greenfinches and two chaffinches) had a longer free-running period in the noise sections than constant light (Lohmann and Enright 1967).

This series of reports was finally followed up in 1989 when Reebs tested conspecific playback on house sparrows in constant dark conditions, using rhythmic presentations of 2 hr of conspecific dawn chorus vocalizations and a control of raking his thumbnail across the cage. The four male birds entrained to both stimuli, and the 3 females showed mostly relative coordination to the cues. Reebs's second experiment constructed a phase response curve to the thumbnail cage disturbances, revealing a sensitivity to the late subjective night phase. Reebs concluded that conspecific vocalizations and human cage disturbance were of the same mechanism: one of general arousal from sleep, without the necessity of social context (Reebs 1989).

An interesting aspect of our findings is that the 4 hr of colony playback was not statistically different from 1 hr of a single, repeated zebra finch song. Thus, the zebra

finches might habituate to the presented audio. Habituation to a single song playback has been well studied in songbird models, wherein repeated playback of the same instance of conspecific song has a reduction in response on subsequent repeats (Dong and Clayton 2009). This can occur using behavioral responses. In wild, free-living white-crowned sparrows (*Zonotrichia leucophrys*), playback of a novel song caused a behavioral disturbance in male birds, the response to which reduced on subsequent presentations (Verner and Milligan 1971). The response to the various vocalization subtypes of white-crowned sparrows was then parsed by Petrinovich and Patterson, showing that some vocalizations were more likely to deteriorate the conspecific's response, including male song (Patterson and Petrinovich 1979; Petrinovich and Patterson 1979; Petrinovich and Patterson 1980; Petrinovich and Patterson 1981; Petrinovich and Patterson 1982; Dong and Clayton 2009).

Habituation can also be measured looking at immediate early gene expression in the auditory forebrain of songbirds, parts of the brain that are associated with song learning in juveniles and song production and perception in adults (Mello 2004). The caudomedial neostriatum (NCM) is the portion of the auditory forebrain that is best characterized in this regard, which is located near Field L, the primary auditory area in the avian telencephalon (Mello and Clayton 1994). Habituation to the familiarity of a song can accrue with several sessions of 1 hr playback over the course of a single day, or 1 hr playbacks every day for a week (Mello 2004). The latter is relevant to these results, as it may belie the reduced response to 2hr and 4hr presentations of the same song. The zebra finch behavioral response to song playback correlates well with what is known in immediate early gene expression in the NCM; upon hearing a novel song, the bird will sit quietly and listen, only occasionally moving their head, for on average around 20 minutes. Upon a subsequent presentation the next day, the birds latency to return to normal behavior shortens significantly, to seven minutes (Stripling et al. 2003).

Additionally, the single song played in reverse was a somewhat surprisingly strong zeitgeber. In the electrophysiology playback literature, reversed playback is used as a negative control (Doupe et al. 2004) and was expected to be as such in this as well.

This finding confirms the finding from previous studies that social context or appropriateness is not necessarily required for entrainment to aural cues.

As this research was performed on zebra finches, which favor nonphotic cues such as rainfall in their seasonal induction of the breeding condition (Zann et al. 1995), one might expect their perception of social cues to be relatively consistent across their life. It is possible that a photoperiodic breeder is more sensitive to these rhythmic presentations of audio cues in either a photosensitive or photostimulated state. Petronovich and Patterson found that life history stages strongly affected the behavioral responses to song playback in white-crowned sparrows, such that females that were brooding eggs were less responsive (Patterson and Petrinovich 1979; Petrinovich and Patterson 1979). As this phenomenon may include mechanisms underlying social perception and song distinction, including the NCM, this output could function as a measure of social receptivity. Further, it has been shown that supplemental colony sounds have caused female zebra finches to lay eggs earlier, more synchronously with other females, and with larger clutch sizes (Waas et al. 2005), so audio cues may interact with the hypothalamic-pituitary-gonadal axis, in addition to the clock.

5.5   Acknowledgements

CHAPTER 6.   CONCLUSION

6.1   Summary of presented research

The study of seasonal and circadian rhythms is historically linked. Colin Pittendrigh, one of the three architects of the modern field of circadian rhythms, with Jurgen Aschoff and Franz Halberg, was first inspired to pursue the subject by a lecture on a migratory bird's sun compass. In this lecture, Kramer discussed how the sun compass of a European starling, *Sturnus vulgaris,* would shift approximately 15° per hour over the course of the day when kept indoors, showing evidence for an internal clock that could compensate for the movement of the sun across the sky (Kramer 1952; Pittendrigh 1993). It is fascinating that knowledge of a bird's ability to navigate via the sun's diurnal movements across the sky preceded what is now known about circadian rhythms, as introduced in Chapter 1. Now, we know that the avian circadian clock indeed underlies this phenomenon, as birds housed in constant dim light shift their flight orientation gradually across the subjective days, correlated with their free-running period of locomotor behavior (Hoffmann 1960; Cassone and Westneat 2012). Beyond the historic intellectual link, the two are linked mechanically. Photoperiodic induction in birds is controlled through time measurement via the circadian clock. This is accomplished, at least in part, through the external coincidence model in which birds have a critical time-of-day during which if light occurs, as an example 11.5 hours after dawn, it will trigger their photostimulation. In the preceding chapters, I and my co-authors detailed experiments investigating the role of pineal melatonin in circadian or seasonal rhythms, directly or as an investigation of the central control of these biological rhythms.

The primary pacemaker in oscine passerine birds is considered the pineal gland, and its main secreted hormone melatonin thought to be the driver of this function. The question is outstanding if pineal melatonin performs this task via synchronizing the molecular clock genes in the peripheral organs. To test this, we compared the abundance of clock genes and a marker for metabolism at different times of day, at increasing days of exposure to constant dark conditions in house sparrows, *Passer domesticus* (Chapter 2). The results revealed that the removal of the pineal gland has a profound effect on the

locomotor behavior rhythm, but a minimal effect on the cycling of rhythms of molecular clock genes in the peripheral organs.

Pineal melatonin is also implicated in the photoperiodic control of vocalization in birds, although, unlike mammals, it does not affect the primary sexual characteristics, the gonads. As long durations of melatonin, simulating the long winter nights, prevent the photoperiodic expansion of the song control system of male birds, we tested whether this intervention would alter their vocalization behavior. We pinealectomized 24 wild-caught, photosensitive, male house sparrows and presented them with a short or long duration of melatonin, or a vehicle control, for ~7 weeks (Chapter 3). Birds that received the long duration of melatonin remained in a winter-like vocal state; these birds developed neither much of a dawn chorus, nor a dusk chorus, and did not produce many single-syllable vocalizations associated with territory defense and mate attraction. These results were independent of any effect on the gonads.

With the increasing evidence that the pineal gland modulates seasonal dynamics in vocalization in male birds, we measured any effect on females. Wild caught, photosensitive female house sparrows experienced an increase in photoperiod; this correlated with an increase in vocalization rate and a switch from simple to complex calls. The vocalizations were quite similar to those produced by males in similar laboratory conditions. There was very little effect of pinealectomy on these outputs (Chapter 4). These data add to the literature that temperate passerine female vocalization behavior is more complex and dynamic than previously appreciated.

Vocalization behavior is not only an output of the biological clocks, but an input as well. Audio of vocalization behavior can act as a weak zeitgeber to the circadian clock, as passerine birds free-running in constant dark conditions can entrain to these cues when rhythmically presented. We followed up on this somewhat obscure field of literature by playing a variety of audio cues across several parameters, demonstrating that social context and novelty improve the strength of this zeitgeber (Chapter 5).

Part of the Aves class's appeal to humans is its species diversity. There are ~10,000 extant species of bird, and ~6,500 of these are passerine birds (Dale et al. 2015; Gill et al. 2020). This proves both a fascination and a frustration in avian research. How

easily can the concepts underlying the data in this dissertation generalize to the Aves class, passerine order, or between families within the passerine order? One can integrate these ideas across taxonomic divisions more easily when considering these variables as ratios that have been distributed throughout evolutionary time. The contribution of the retinae to circadian organization has been distributed differently multiple times: pinealectomy abolishes the circadian rhythm of locomotor activity in Passeriformes but not Galliformes; yet Galliformes become arrhythmic with both enucleation and pinealectomy (Underwood and Siopes 1984), as the retinae produce melatonin that accounts for a percentage of the serum concentrations (Underwood et al. 1990). Further, in the suboscine passerine European starling, pinealectomy alone again does not suffice to abolish the circadian rhythm in locomotor activity (Gwinner 1978). The seasonal induction of the Galliforme Japanese quail, *Coturnix japonica*, has been described in detail by Takashi Yoshimura, yet the details of this process may not occur in Passeriformes, as the rhythms in Dio2 and Dio3 appear not to be mimicked in European starling (Bentley et al. 2013). Zebra finches, *Taeniopygia guttata*, are considered opportunistic breeders, as they live in tropical areas. This was often considered in *opposition* to the temperate birds who are considered photoperiodic; however this designation is now being appreciated as a different ratio of contributing factors to seasonal induction; wherein opportunistic breeders have a different ratio of how non-photic cues such as rainfall or food or nest material availability are required to change their reproductive state (Perfito et al. 2008; Perfito et al. 2015). This could even vary across the sexes of birds within the same species, where it is thought that females require more environmental or social cues to initiate their more energetically costly reproductive state (Ball and Ketterson 2008). Since avian adult neurogenesis was discovered in the canary, *Serinus canaria*, an open-ended learner that undergoes a sensorimotor learning stage each year and can add syllables to their repertoire beyond adolescence, this adding of syllables was a hypothesis for the purpose underlying the seasonal expansion of the song control system (SCS) size (Nottebohm et al. 1986). However, the SCS grows in close-ended learners and birds that are thought not to learn their vocalizations at all (Tramontin and Brenowitz 1999); although it is possible that these species do learn subtle components of their vocalizations that are as of yet unnoticed, as in house sparrows

potentially learning to add extra harmonic frequencies onto their vocalizations from a tutor (Nivison 1978). The seasonal and diurnal dynamics for birds' vocalization are beginning to be appreciated in birds thought to have rudimentary vocalizations, including birds like the house sparrow, who some would say do not even produce a "song" by a strict definition. Similarly, the songs and vocalizations of female birds are also becoming better appreciated.

This chapter will discuss how the research presented within this dissertation may integrate with what is known about other model passerine birds and reveal some directions that future research could take to further explore these phenomena.

6.2   Circadian and diurnal patterns in vocalization

There are many ways in which the molecular circadian clock does not behave in a manner consistent with the properties of circadian clocks as determined in the mid-twentieth century at the organismal level (Johnson et al. 2003). The molecular clock certainly is endogenously generated, free-runs with a period of approximately 24 hours, and persists in constant conditions (Partch et al. 2014). The molecular clock can respond to environmental cues such as light: most models associate light's induction of the Period genes as the external interface with the environment (Jolley et al. 2014), although this may vary from organ to organ (Pett et al. 2018). Period 2 is a candidate molecule as an interface to environmental non-photic cues, as it contains, in addition to the normal clock gene promoter regions, a cAMP response element, a glucocorticoid response element, and a ligand-bound glucocorticoid receptor (Travnickova-Bendova et al. 2002; Segall et al. 2006). The periodicity of circadian clocks must also run be somewhat resistant to changes in ambient temperature; there are some experimental data on temperature compensation of the molecular clocks of *Drosophila* and the *Arabidopsis* clock (Gould et al. 2006; Kidd et al. 2015), and it has been explored through modeling, however this criterion for circadian rhythms remains quite mysterious. Further, the ability for the molecular clock to perform some elements of entrainment, such as transients and/or after-effects when transitioning from various light regimes, and the ability for organisms to entrain to skeleton photoperiods, are unproven (Johnson et al. 2003). One should consider

the limitations of what we currently know about the molecular clock when considering circadian phenomena at the organismal level.

6.2.1   Diurnal and life history rhythms in vocalizations of the zebra finch

The results reported in Chapters 3 and 4 of this dissertation may be generally applicable to oscine passerine birds at large, including the zebra finch. With the canary, the zebra finch is the most studied model organism for vocalization research, but it is considered an opportunistic rather than photoperiodic breeder. Zebra finches, and many other tropical birds, remain in a physiological halfway "readiness to breed" state that can be further stimulated with an appropriate non-photic cue, such as rainfall or food abundance (Zann et al. 1995). This definition is incomplete, as zebra finches are still sensitive to increases in photoperiod (Perfito et al. 2007; Perfito et al. 2008; Perfito 2010). It remains to be investigated if water availability or an increase in photoperiod has more of an effect on the reproductive system of zebra finches. Regardless, the term "opportunistic breeder" should more accurately be considered a photoperiodic breeder where non-photic cues play a larger role than the stimulating ratio in temperate zone male birds. This component of zebra finches makes them appealing for laboratory research however, since their more constant in-between state allows them to breed readily in captivity and express more consistent behavior than a seasonal breeder with more distinct breeding and non-breeding states.

Zebra finches are also often considered having "crystallized" song—male juvenile birds learn their song during a critical period between 25 and 120 days post hatch (dph) (Immelmann 1969; Zann 1996). The first subphase of this period is 'sensory learning,' wherein the juvenile hears a conspecific mature tutor produce their song that forms the template it will then imitate in the second subphase, the 'sensorimotor learning' phase, at ~30 dph. Herein, the juveniles will produce a type of highly variable babble, termed subsong, that will become more recognizable in the next phase as 'plastic song,' before ultimately 'crystallizing' during the period of 90-120 dph. Crystallized zebra finch song generally consists of multiple short introductory syllables, followed by two to seven motifs in a row, each consisting of a stereotyped sequence of syllables that is unique to the individual male. The fact that there is a period of learning followed by adulthood

wherein no further syllables are added makes zebra finches an appealing model for investigating song learning but not necessarily adult intraindividual variation. The single sensorimotor learning stage during adolescence classifies zebra finches as a closed-ended learner; house sparrows are thought to be closed-ended learners as well. There are songbirds, such as the canary, that undergo a sensorimotor learning stage each year, adding syllables before crystallizing again, as juveniles and through adulthood. Birds such as these are considered open-ended learners or age-unlimited vocal learners (Brenowitz and Beecher 2005). However, as with how Chapters 3 and 4 found dynamic complexity in presumed-uninteresting adult house sparrow vocalizations, careful quantifications of zebra finch vocalizations reveal both diurnal and life-history related rhythms in vocalizations of these closed-ending learners.

6.2.1.1 Juvenile zebra finches

Juvenile birds express a diurnal rhythm of vocal parameters. Johnson and colleagues found that as juvenile birds in the critical period for learning express variable diurnal distributions of when during-the-day they vocalized (Johnson et al. 2002); when birds were producing subsong and plastic song (36–70 dph), they did so with distinct morning and evening peaks. However, when singing during song crystallization and adult song (70–365 dph), they did so with a morning-concentrated diurnal peak characteristic of adult zebra finches. This pattern of varying diurnal distributions of vocalization persists if the juvenile is deafened or socially isolated without a tutor (Ohgushi et al. 2015). Further, learning zebra finches' performances vary over the course of the day in the early plastic song phase. These juvenile zebra finches would express a morning session of less-structured song, as measured by Weiner entropy variance and variance of other features that capture the richness of acoustic structure within a syllable. This morning session would improve after bouts of practice by late morning, stay at about that level for the rest of the light phase until deteriorating again overnight (Derégnaucourt et al. 2005). These night-morning diurnal deteriorations would fade as the juveniles approached crystallization. The song structure of learning juvenile zebra finches as measured by "Kullback–Leibler (K-L) distance" deteriorates and recovers several times throughout the day, the highest amplitude oscillation occurring in the morning, similar to

the evening-morning deterioration-recovery measured by Derégnaucourt and colleagues' entropy variance measure (Ohgushi et al. 2015). These advanced analyses of vocalization often use more general terms such as "song structure" in their discussions, which track poorly between papers from different investigators and measures; even a seemingly simple result such as "song length" can vary from investigator to investigator depending on how the laboratories decide to handle the vocal extraction. A recent treatise on diurnal dynamics of bird vocalizations implores the usage of nearest neighbor statistics in order to quantify these differences, as they can be subtle, change along more than a single parameter, and the various parameters are likely to be species-specific (Kollmorgen et al. 2020).

6.2.1.2 Adult zebra finches

There are also diurnal rhythms to adult zebra finch vocalization production. Adult zebra finch behaviors, including vocalizing and perch-hopping, are expressed mostly in the morning in a strong diurnal pattern; acoustic activity generally peaking at ZT=5 and declining until lights off (Ollason and Slater 1973; Rashotte et al. 2001; Johnson et al. 2002). This diurnal pattern of production persists in sham-operated control birds in constant dim light conditions (Wang et al. 2012). The acoustic parameters 'fundamental frequency' and 'amplitude' follow a diurnal pattern in adult zebra finches' harmonic syllables, these values rising in the morning, peaking at mid-day, and then falling until lights-off (Wood et al. 2013). The length of an individual's song can also vary across the day, with the shortest song at ZT=4, and increasing until ZT=11; these length dynamics are subtle to human observers, at only ~6 milliseconds / 1.5% of the total motif duration (Glaze and Troyer 2006). Song tempo increases such as this occur along this diurnal pattern, as well as in the presence of a female, wherein a male bird produces a "directed" song that is faster than its "undirected" counterpart produced in isolation or in the presence of only other males; both causes of increase in tempo correlate well to increasing brain temperature in the HVC (Aronov and Fee 2012). In constant conditions, the circadian rhythms of adult zebra finch song, call, and locomotor activity become arrhythmic in pinealectomized birds at different rates, suggesting that these three behaviors are under control of separable circadian oscillators (Wang et al. 2012). It is

unclear if these diurnal rhythms are consistent with the dawn choruses seen by other passerines in the wild. In Chapter 5, the colony audio presentation consisted of only the morning vocalizations of the light-dark entrained zebra finches. Presenting audio from entrained birds at different times-of-day to free-running birds in constant dim light could quantify if the birds perceive a difference in these vocal properties.

6.2.1.3 Life history rhythms

Both male and female zebra finches exhibit a call-type switch during life history stage transitions in response to social cues while forming pairs. Individual zebra finches fitted with telemeter audio recorders as "backpacks" were switched from same-sex to both-sex group housing. As the newly introduced birds formed pairs when nest material was introduced, both males and females produced more calls of the 'cackle' subtype, from the onset of reproductive activities to the early nest stage. The 'whine' subtype peaked from the early to late nest stages (Gill et al. 2015). Canaries and the tui, *Prosthemadera novaeseelandiae*, fortify their repertoires when photostimulated to favor mate-desired syllables (Voigt et al. 2001; Hill et al. 2015). Chapters 3 and 4 also reveal a call-type switch in house sparrows. In zebra finches this behavior may require social cues or the addition of nest material rather than solely an increase in photoperiod. This could be tested by comparing the repertoires of individually-housed male zebra finches in short and long day conditions and checking for "cackles."

6.2.1.4 Melatonin sensitivity

Zebra finch vocalizations have been demonstrated so far to be sensitive to melatonin on two timescales –upon chronic administration and in circadian rhythms. Injection of the melatonin receptor 1B antagonist S20928 at lights-off shortens the next day's song and motif length, as well as a change to song syllable length that varied across individuals; this iteration of analysis did not measure the diel tempo variation seen later by Glaze and Troyer in untreated birds (Jansen et al. 2005; Glaze and Troyer 2006). Melatonin administered to zebra finch brain slices decreased the firing-rate of neurons in the RA (Jansen et al. 2005). Injection of 3 µg of melatonin into juvenile zebra finches producing song at ZT=4 reportedly caused the birds to take a daytime nap and a

deterioration of their morning practice improvement similar to what is seen from night-morning intervals (Derégnaucourt et al. 2005). Adult zebra finches exposed to two weeks of constant light or pinealectomized vocalize shorter song motifs (Derégnaucourt et al. 2012). Melatonin administration via a dermal cream lengthened the song in intact, sham-operated, or pinealectomized zebra finches (Derégnaucourt et al. 2012). It is my impression that when melatonin was present in the drinking water for the experiment in Chapter 3 in house sparrows, melatonin also lengthened the duration of the calls produced. In a teleost fish, the plainfin midshipman, *Porichthys notatus*, constant light conditions shortened the duration of its hum vocalization, that was rescued by administration of a melatonin analog (Feng and Bass 2016). Constant light or pinealectomy also adversely affected learning in juvenile zebra finches, as the produced vocalizations attempting to imitate the tutor were too short (Derégnaucourt et al. 2012). Pinealectomized adult zebra finches that are arrhythmic in the behaviors of calls, song, and locomotor behavior from pinealectomy and several days spent in constant dim light conditions have the three behaviors re-entrain to rhythmic presentation of melatonin at different rates; with locomotor activity first, singing second, and call third (Wang et al. 2012). These three behaviors in birds in LL or pinealectomized birds introduced to constant dim light conditions also become arrhythmic at different rates (Wang et al. 2012).

The literature of diurnal variation and melatonin sensitivity tend to focus on non-circadian explanations for these phenomena, and rarely measure their outputs in constant conditions to see if they free-run. The discussed literature investigating the evening morning-deterioration in juvenile learning birds, and others (Shank and Margoliash 2009), make compelling arguments that melatonin is affecting vocalization in a sleep-dependent manner. Melatonin is known as a sleep-promoting molecule in diurnal animals, and intravenous infusions of melatonin do so to pigeons (Mintz et al. 1998). A zebra finch's RA will fire during sleep in patterns that closely matches those that occur during daytime singing (Dave and Margoliash 2000). Sleep is also involved with the consolidation of memory (Maquet 2001). However, sleep is controlled by both a homeostatic hourglass as well as the circadian clock (Borbély 1982). An experiment with sleep deprivation could potentially divorce the two and investigate the clock's

contribution to the evening-morning deterioration in juvenile zebra finches. Further, using a daytime intraperitoneal injection of melatonin to induce sleep may be effective, but may affect the clock or metabolism of some tissues as well. The daily distribution shifts seen throughout vocal learning stages (Johnson et al. 2002) and during the day (Ohgushi et al. 2015) are attributed to energetic hourglass phenomena; where the production of subsong or plastic song are so costly to the individual they take a break during the day and overnight, or that the syllable K-L distance deteriorates over short vocal breaks and recovers upon practice several times during the day. This is likely true, but the circadian clock could gate these hourglasses and contribute to the emphasis on the morning. In adult birds, the diurnal change in tempo may be caused by increases in brain temperature in the HVC (Aronov and Fee 2012); it is unclear whether this brain temperature rhythm is circadian and if it would free-run in constant conditions. Melatonin is known to decrease core body temperature in humans (Cagnacci et al. 1992; Kräuchi et al. 1997; Kräuchi et al. 2006), and pinealectomy abolishes the nocturnal hypothermia experienced in intact house sparrows (Binkley et al. 1971). It is possible that the lengthening effect of chronic melatonin administration on zebra finch syllables could be caused in part due to temperature decreases.

6.3   Seasonal and photoperiodic control of vocalization

The best studied species regarding seasonal development of vocalization are the canary, the species wherein avian adult neurogenesis was discovered, and New World/American sparrows such as the song sparrow, *Melospiza melodia*, and white-crowned sparrow, *Zonotrichia leucophrys*. Canaries are in the taxonomic family Fringillidae and the American sparrows Passerellidae; however, the vocal properties of these species share some important similarities, to the extent that American sparrows were once classified as finches. These species vocalizations all contain a trill element, or a syllable that is repeated in a series rapidly. Further, these species produce more "stereotyped" vocalizations during long day breeding conditions than in short days. Stereotypy is synonymous with 'low variability,' and can refer to temporal parameters such as duration of a vocal element like a syllable or motif, acoustic parameters such as minimum frequency, and/or syntax, or the order of which vocal elements are produced.

The ability to produce a high-quality, consistent vocal performance is desirable to mates (Botero et al. 2009; Byers et al. 2010; Sakata and Vehrencamp 2012). An important distinction is that canaries are open-ended learners, adding syllables to their repertoire, while song and white-crowned sparrows are closed-ended and the syllables they produce crystallize while they are juveniles. These species are well studied regarding when during the season these vocal dynamics occur, but to my knowledge very little is known about if there are diurnal dynamics of these parameters.

6.3.1 Timeline of photoperiodic induction

6.3.1.1 Timeline of events in New world sparrows and house sparrows

As shown in white-crowned sparrows, there are four major elements to photoperiodic induction of seasonal vocal behavior: increase in vocalization rate, maturation of the gonads, increase in size of the SCS, and peak changes in acoustic parameters of vocalization (Figure 6.1). The timing of these events throughout simulated seasons suggests much about the regulation of these behaviors and physiologies. Laboratory studies on this species are generally conducted with both an increase in photoperiod to photosensitive birds, as well as the addition of exogenous testosterone in a silastic capsule, as increases of photoperiod alone in captivity generally results in testosterone levels (peaking ~1.5ng/mL) that are lower than those measured in the wild (peaking at ~4ng/mL)(Lam and Farner 1976; Wingfield and Farner 1978; Smith et al. 1995). In these studies, the events occur in the following order: upon photostimulation, first the vocalization rate increases at about three days, and peaks at around day six. However, at day six when the rate has reached its maximum, song stereotypy is still low, not peaking until day 10 (Meitzen et al. 2009). The HVC was large, at 94% of its full breeding-season size, by day 7 in long days; the RA and Area X grew more slowly and were not large until the next sacrifice at day 20 (Tramontin et al. 2000); these nuclei were the same size they were at day 44 of a similar study (G. Troy Smith et al. 1997).

The experimental design of the long day treatments combined with exogenous testosterone obscures how the timing of the recrudescence of the gonads factors into this

timeline of event. This combined treatment keeps the testes in a regressed state, as the gonadotropins are inhibited through negative feedback. The timing of their recrudescence must come from other captive studies where photoperiod alone was manipulated; FSH and LH increase after one day of exposure to 20:4 LD (Wingfield et al. 1996), resulting in a ~0.089 mg/day growth rate, such that testes will have increased from 4-5mg in short days to 164mg on day 18 and 370mg by day 30 of long day conditions (Yokoyama et al. 1978). Plasma testosterone levels begin to rise after 15 days in long day conditions and peak at around day 28 (Lam and Farner 1976). The question is still outstanding of how these two sequences of events align. In the wild, there is evidence in the song sparrow that the increase in the SCS size precedes the recrudescence of the gonads and may coincide with or occur before the increase in plasma testosterone (Tramontin et al. 2001).

It is interesting that vocalization rate occurs first, well before the gonads are fully mature. This is theorized to occur based on signaling in the medial preoptic nucleus (POM)(Ball and Balthazart 2010). In female canaries, the POM volume begins to increase within one day of photostimulation and testosterone capsule implantation, while it took 21 days for the HVC, RA, and Area X to significantly increase (Shevchouk et al. 2017). Photostimulated, castrated male canaries with testosterone cannulated to the POM increased their singing rate, but did not produce the stereotypy increases that birds receiving exogenous, peripheral testosterone, or intact birds from other studies (Alward et al. 2013; Alward et al. 2016). Interestingly, the testosterone infusion into the POM also increased the size of the HVC and RA (Alward et al. 2013; Alward et al. 2016), potentially through activity-dependent processes wherein singing behavior helps to grow these nuclei (Sartor et al. 2005). Singing activity has been shown to increase brain-derived neurotrophic factor in the HVC, in addition to testosterone (Li et al. 2000; Alvarez-Borda and Nottebohm 2002). Canaries with testosterone infused at only the POM did not develop the increase in stereotypy, suggesting that the POM belies the motivation to sing but not the increase in acoustic parameters seen in intact birds (Alward et al. 2013; Alward et al. 2016). This adds to the data suggesting that the increase in the SCS results in the increase in stereotypy but not vocalization rate.
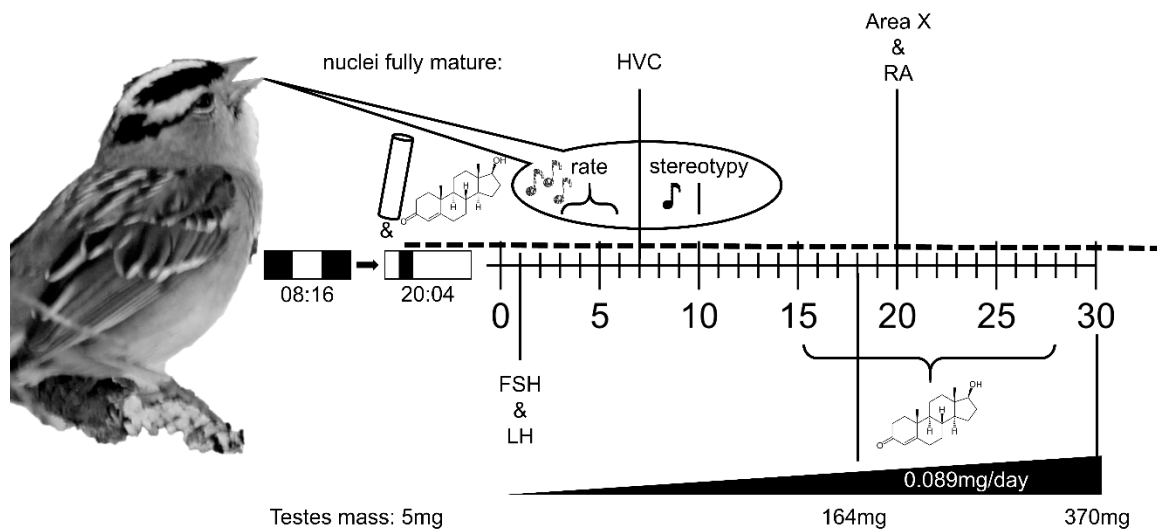
Figure 6.1 Timeline of seasonal events in white-crowned sparrow

The horizontal, dashed line indicates a change in experimental protocol (above = with exogenous testosterone and photostimulation, below = photostimulation alone). The alignment of these two timelines may not be exactly as pictured in free-living birds. Photograph credit: Kenneth Cole Schneider

The timeline for these events in the house sparrow, the primary model for this dissertation, fits a similar template. The timeline is less resolved than that known in white-crowned sparrows, but one can suppose that the general order of events is conserved. The photoperiodic increase in vocalization rate precedes changes in complexity. To my knowledge, it is unclear whether stereotypy is a dynamic parameter in house sparrow vocalizations, but other measures of complexity develop after an increase in vocalization rate. Vocalization rate increases after one week in 12:12 after being in a photosensitive state and 08:16 LD in Wang and colleagues' 2014 study, as well as Chapter 3 and 4 of this dissertation. The call-type switch and development of the dusk chorus was first measured in pinealectomized birds 4 weeks into 12:12 conditions (Chapter 3), and two weeks into long day (18:06) conditions in intact and pinealectomized female house sparrows (Chapter 4), all after the increase in vocalization rate. The complexity here was measured at a two-week resolution, so these events could have happened earlier. The SCS is large after 8 weeks in long day conditions (Whitfield-Rucker and Cassone 2000) and after two weeks in 12:12 and four weeks in LL (Cassone

et al. 2008). The SCS was large very rapidly in white-crowned sparrows with long days and exogenous testosterone, and the various nuclei recrudescing at different rates,: the HVC at day 7 and RA and Area X by day 21 (Tramontin et al. 2000); so it is possible that this occurs more rapidly than measured in house sparrows. Testes size is increased after one week in birds transitioned from short days of 08:16 to 16:08 LD, from around 4-5 mg to over 10mg, and were over 100 mg by day 21 (Donham et al. 1982). With 8 weeks in long days, testes can reach up to 500mg (Whitfield-Rucker and Cassone 2000). LH peaked at day 7, and reduced at day 21, but still higher than control birds, before falling to levels similar to those of birds remaining in short days (Donham et al. 1982). Plasma testosterone levels were high by day 21 (Donham et al. 1982). Beak darkness, a correlate of plasma testosterone in male house sparrows (Laucht et al. 2010), increased after one week in 12:12 and one week in 18:06 (Wang et al. 2014), or three weeks in 12:12 (Chapter 3).

6.3.1.2 Possible mechanisms of the timeline of seasonal induction

Steroid hormones, testosterone most among them, are the primary signal in photoinduction of the breeding condition. This has been reviewed extensively (Ball and Balthazart 2010; Alward et al. 2018). Testosterone cycles seasonally and is higher at times when birds are breeding (Nottebohm 1981; G Troy Smith et al. 1997). Androgen receptors are expressed through the SCS, except for in Area X (Arnold et al. 1976; Balthazart et al. 1992; Smith et al. 1996; Bernard et al. 1999; Soma, Sullivan, et al. 1999), as well as estrogen receptors (Gahr et al. 1993; Bernard et al. 1999). AR and ER in the HVC are required for the increase in song stereotypy (Meitzen, Moore, et al. 2007). The seasonal increase in serum testosterone contributes to the structural and electrophysiology of the SCS (Nottebohm 1981; Meitzen, Perkel, et al. 2007). Castration severely impairs the ability for the recrudescing SCS to reach full size (Bernard et al. 1997; G. Troy Smith et al. 1997; Bentley et al. 1999; Whitfield-Rucker and Cassone 2000). Exogenous testosterone applied to castrated males or males in short-day conditions induce SCS growth (Johnson and Bottjer 1993; Rasika et al. 1994; Bernard and Ball 1997; G. Troy Smith et al. 1997). Replacement of testosterone of birds that were

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both castrated and housed in short days conditions led to the increase in vocalization rate and the size of the HVC (Sartor et al. 2005). It is also important to note that plasma testosterone levels may not always correlate with sex steroid levels in the brain. The brain can synthesize its own steroids, creating high local levels that would not correlate with levels in the plasma (Vanson et al. 1996; Schmidt et al. 2008; Pradhan et al. 2010). Further, an increase in sensitivity to steroids could also play a role: there is evidence that the expression of ARs can be increased in the breeding season of spring (Gahr and Metzdorf 1997; Soma, Hartman, et al. 1999; Fraley et al. 2010).

However, there are numerous suggestions of one or more non-steroidal causes of induction in these seasonal events. The SCS still grows in castrated birds upon photostimulation: in white-crowned sparrow (G. Troy Smith et al. 1997), tree sparrow (*Spizella arborea*)(Bernard et al. 1997), house sparrow (Whitfield-Rucker and Cassone 2000), and European starling (Bentley et al. 1999). There are two populations of song sparrows which live at the same latitude but different altitudes, one living along the coast and one in the mountains. The coastal birds had their gonads recrudesce 2 weeks earlier than the population in the mountains, however their SCS were large in both groups at the early timepoint (Tramontin et al. 2001). The SCS grows in 2-3 month old, photorefractory dark-eyed juncos exposed to long days, despite low levels serum testosterone (Gulledge and Deviche 1998). These studies, plus the timeline displayed in Figure 6.1 and discussed previously show a partial gonadal testosterone independence of the growth of the SCS. Further, white-crowned sparrows housed with females in long days had larger SCS nuclei than those housed individually, however this increase was not a result of plasma testosterone (Tramontin et al. 1999). The singing-dependent SCS size increases could have contributed here, as the birds housed with a female did sing at a higher rate (Tramontin et al. 1999; Li et al. 2000; Alvarez-Borda and Nottebohm 2002).

This dissertation proposes that this non-steroidal stimulation comes from disinhibition of the suppressive long durations of melatonin seen in the winter. There is melatonin binding throughout the SCS in house sparrows (Whitfield-Rucker and Cassone 1996), zebra finches (Gahr and Kosar 1996), and European starlings (Bentley and Ball 2000; Bentley 2003). Melatonin receptor mRNA is also present in the SCS of the zebra

finch and blackcap, *Sylvia atricapilla* (Fusani and Gahr 2015). SCS melatonin receptor mRNA expression is higher in long days in European starling (Bentley et al. 2013). Photostimulated and castrated European starlings implanted with silastic melatonin capsules did not grow their SCS, unlike photostimulated birds with blank capsules (Bentley et al. 1999). Administration of long durations of melatonin prevent the photoperiodic expansion of the SCS in house sparrow (Cassone et al. 2008), and the Indian weaver bird, *Ploceus philippinus* (Surbhi et al. 2015). Chapter 3 also adds to this evidence, in that long durations of melatonin prevent the change in vocal state seen in short duration or control birds, of the development of a dawn and dusk chorus, as well as a call-type switch to calls associated with mate attraction and territory defense. Pinealectomized, photosensitive house sparrows that are exposed to long day photoperiods produce an earlier yet unsustained increase in the vocalization rate and complexity than sham birds, that also responds more slowly to a decrease in photoperiod (Wang et al. 2014). The more sustained development of vocalization rate and complexity expressed by sham operated birds suggests a role for melatonin in the maintenance of these behaviors, in addition to the induction of them. In zebra finch and the seasonally breeding blackcap, there is melatonin binding and mRNA for receptors Mel1a and Mel1b in the POM (Fusani and Gahr 2015). Cytochrome $P450_{7\alpha}$ (CYP7B), the enzyme that converts pregnanalone to 7α-hydroxypregnanlone, a neural steroid proposed to mediate the effect of melatonin on avian behavior, is also expressed in the POM (Tsutsui et al. 2008). However, in Chapter 3, there was no effect of a long duration of melatonin on the vocalization rate in house sparrows. None of these studies saw an effect of melatonin on serum testosterone levels or the size of the gonads.

This can lead to the following scenario of photoperiodic induction. Upon photostimulation, after one day, FSH and LH increases. This could be due to a mechanism similar to that seen in Japanese quail, wherein clock genes keep time in the medial basal hypothalamus, and light reaching the deep-brain photoreceptors within this tissue coinciding with the inducible phase stimulate the release of these hormones from the pituitary. These hypothalamic-pituitary hormones then reach the gonads, beginning their recrudescence. Three-to-five days after photostimulation, the increase in vocalization rate occurs, likely mediated through the POM. After the increase in

vocalization rate, changes occur in the complexity of the vocalizations. This is likely mediated through the potentially rapid recrudescence in the HVC, that is synergistically enhanced by a combination of testosterone, the act of singing, and disinhibition of long durations of melatonin. The recrudescence of Area X and the RA occurs after that of the HVC, by day 21. The peak of serum testosterone levels occurs between day 15 and 28, potentially through some threshold of gonadal size and maturity. The growth of the testes and the serum testosterone levels are likely unaffected by melatonin. However, the timing and maintenance of when during the simulated season the increase in vocalization rate is sensitive to melatonin (Wang et al. 2014), which suggests an interaction with the POM. Further, the timing and maintenance of when during the year vocal complexity occurs is also sensitive to melatonin (Wang et al. 2014), and long durations of melatonin prevent the recrudescence of the SCS (Cassone et al. 2008) and the development of a vocal state consistent with the breeding season (Chapter 3). This implies that the disinhibition of long durations of melatonin on the SCS and vocal complexity plays a role in these sequences of secondary sexual characteristics.

6.4   Seasonal control of diurnal events: the dawn chorus

6.4.1   The dawn chorus

A recurring theme in the experiments presented here was a notion of the "dawn chorus." This features prominently in Chapter 3, wherein a long duration of melatonin prevented a photoperiodic increase in dawn chorus behavior, and in Chapter 5 when presentation of a live dawn chorus from zebra finches increased the strength of the zeitgeber of rhythmic audio presentations. In the wild, this behavior is defined by an intense bout of energetic singing, lasting ~20 to 60 minutes, before sunrise (Ulltang 2018). Dawn and dusk choruses are considered systematically different in passerine bird behavior from vocalizations occurring during the bulk of the day (Staicer et al. 1996; Lein 2007). Concentrating behavior to these times of day is not limited to the avian taxonomic class, either, for example as demonstrated in cicadas (Young 1981) and anole lizards (Ord 2008). Further, dawn chorus can differs from daytime singing not only in rate, but complexity: as examples, in field sparrows *Spizella pusilla* (Nelson and Croner

1991), chipping sparrows *Spizella passerine* (Liu and Kroodsma 2007), and the tui (Hill et al. 2018), the complexity of vocalizations differs during the dawn chorus from other, daytime vocalizations. The onset of the dawn chorus affects fitness, and may correlate with the age of the bird; free-living, young blue tits, *Cyanistes caeruleus*, sang later than older male birds, and this correlated to the earlier singing, older birds having more sexual partners and a better chance at extrapair paternity (Poesel et al. 2006). The dawn chorus is much more researched, but the dusk chorus is presumed to be of similar regulation.

There has been evidence in field studies that the dawn chorus is under seasonal control. For many of the taxonomic order, the dawn chorus is often absent at the beginning of a breeding season and then develops as the season progresses (Staicer et al. 1996). Seasonal variation in dawn complexity has been demonstrated less, as studies tend to focus on one or other. In blue grosbeaks, *Guiraca caerulea*, male birds increase their song complexity in correlation with their mate's fertility, which is relieved upon decrease of fertility (Ballentine et al. 2003). A switch between calls to vocalizations involved with mate attraction in the dawn chorus, as the breeding season advances has been demonstrated in wild Dupont's Larks, *Chersophilus duponti* (Pérez-Granados et al. 2018). Dawn choruses are also produced in the non-passeriforme, the common and lesser cuckoo (*Cuculus canorus* and *C. poliocephalus*), but their nocturnal vocalizations were only present in the early breeding season (Yoo et al. 2020). On occasion, the dawn chorus is studied incidentally, as sampling the first vocalizations of the day is convenient; it is advisable to realize that the rate and complexity of vocalization is dynamic throughout the day, the season, and the combination of the two.

Staicer and colleagues outlined twelve non-exclusive hypotheses explaining the dawn chorus in their book chapter, ranging from intrinsic, environmental, to social mechanisms (Staicer et al. 1996). Among the social hypotheses is that the dawn chorus is more for territory establishment, while the bulk of daytime singing is involved with mate attraction (Liu 2004); the dawn chorus fulfils a need to reestablish one's territory after a bout of sleeping and an increase in territorial invasions at dawn (Staicer et al. 1996; Hill 2014). This ethology could have been present in the photoperiodism study in Chapter 3—birds receiving a short duration of melatonin or control had a change in behavioral state

that allowed them to consider their individual cage as a territory worthy of defense, while long duration birds experienced an intrinsic willingness to form a flock. While the sum of many these factors likely contributed to the evolution of this phenomenon, our results provide evidence that many of them are not required for a seasonal development of a dawn and dusk chorus. Our experimental design did not allow for variable foraging conditions, territorial invasions, or variable light intensities; dawn's worse conditions for foraging have been hypothesized as a function of the dawn chorus (Hutchinson 2002; Ulltang 2018), but that factor has been removed in Chapter 3's experiment's ad libitum food availability. This suggests, at least in part, an intrinsic origin of dawn chorus behavior. However, testosterone may not be involved. The production of the dawn chorus does not require rhythmic expression of gonadal testosterone in Japanese quail, as the diel rhythm in calling persists upon castration and replacement with constitutive testosterone via silastic capsule (Wada 1983). The results of Chapter 3 are consistent with the hypothesis that in house sparrows, the reducing duration of melatonin could induce the development of the breeding season's dawn and dusk chorus.

6.4.2 Possible mechanisms

A mechanism that could belie this effect of long melatonin duration is the molecule's ability to decrease retinal sensitivity. The timing at which a particular species joins their voice to the local chorus in the dim light of dawn is characteristic (Wright 1913; Allard 1930; Berg et al. 2006). The inter-species timing has been correlated to eye size (Thomas et al. 2002) and more advanced measures of retinal sensitivity (McNeil et al. 2005). Retinal sensitivity in birds is modulated by melatonin. Melatonin binds in the visual system in several avian orders and species, including house sparrow (Cassone and Brooks 1991; Cassone et al. 1995). The visual system is also one of the largest sites of melatonin receptor expression in the central nervous system (Rivkees et al. 1989; Reppert et al. 1995). Melatonin injection during the day suppresses 2-DG uptake to levels consistent with nighttime in the visual system (Cassone and Brooks 1991) and daily administration of melatonin in the drinking water restores rhythms of 2-DG uptake in the visual system of pinealectomized house sparrows (Lu and Cassone 1993a). In chickens and pigeons, administration of exogenous melatonin during the day reduces the amplitude

of the electroretinogram b-wave (Lu et al. 1995), and constant administration of melatonin abolishes rhythmicity of a-wave and b-wave implicit times and b-wave amplitude (McGoogan and Cassone 1999; Wu et al. 2000). Changes in vision modulating the seasonal control of sexual behavior was recently described in medaka fish, *Oryzias latipes*. Medaka males seasonally develop a brighter orange-red coloration for nuptial displays by an increase in melanophores and xanthophores. In parallel, seasonally appropriate increases in water temperature stimulate medaka females to upregulate their eyes' long-wave sensitive opsin genes, increasing their preferences for this coloration in potential mates (Shimmura et al. 2017).

This evidence could lead to the non-exclusive hypothesis that a reduction in the length of nights relieves the inhibition of the retinal sensitivity caused by long durations of melatonin. This supposition is simplest to explain a change in vocal timing in natural sunrises; birds "notice" the dim early dawn light more as their retinae get more sensitive in springtime. This could also have been present in Chapter 3's experimental design, despite have a square wave of light intensity. In the experimental design, the long duration birds had melatonin in the drinking water removed right before lights. The elimination half-life for melatonin injections is around 72 minutes in the serum of chickens, and significantly shorter in the brain (Cassone and Menaker 1984). The absorption and elimination of melatonin administered orally via the drinking water may differ. In the ERG studies done in our lab, effect of injected melatonin occurred 30 minutes after injection, so we know that the inhibitory effects of melatonin on the visual system can last at least that long (Lu et al. 1995).

A second non-exclusive hypothesis could be that there is a functioning circadian clock in the SCS that enables circadian patterns of behavior, and a seasonal or photoperiodic dynamic thereof. As mentioned before, the rhythms of song, call, and locomotor behavior are under the control of separable circadian oscillators that are differentially sensitive to melatonin in zebra finch (Wang et al. 2012). Clock genes are also expressed throughout the brain, including the SCS. Despite its assumed importance to the avian circadian organization, pineal melatonin itself has little effect on clock gene expression in the tissues measured (Karaganis et al. 2009, Chapter 2). It is also becoming

clear that the pineal's contribution to the neuroendocrine loop model for the avian circadian clock is not mediated through clock genes in the pineal gland itself; in cultured chick pinealocytes, the production and secretion of melatonin damps after 3-10 days in DD (Zatz and Mullen 1988; Li and Cassone 2015). *In vivo* and *in vitro*, sympathetic input to the pineal gland inhibits the production of melatonin and synchronizes its diurnal pattern (Cassone and Menaker 1983; Zatz and Mullen 1988; Li and Cassone 2015). However, administration of norepinephrine to cultured pinealocytes does not alter the clock gene expression (Li and Cassone 2015). Melatonin's inhibition of the breeding season's vocal state may not be mediated through a direct impact of clock gene expression but may be through altering the clock's contribution to its outputs. Since the dawn and dusk choruses did not free-run in constant dark conditions in Chapter 3, they may be the interaction of a clock and the cues of lights-on and lights-off.

Support for the circadian system and melatonin underlying the timing of the morning vocalization bout can come from evidence in the Galliform order. The circadian clock underlies the timing of rooster crowing, as the behavior free-runs in constant dim light conditions (Shimmura and Yoshimura 2013). There are social influences to the timing of the rooster crow, as the birds subordinate to the highest ranking rooster in the pecking order wait for him to announce the dawn first (Shimmura et al. 2015). The production of these calls is produced in response to the neurotransmitter cholecystokinin in the intercollicularis (ICo) of the midbrain (Shimmura et al. 2019). Galliformes lack the Passeriform SCS, but the midbrain's ICo is considered the head of the vocal control system, as it projects to the nuclei in the hindbrain that control the vocal organ and respiratory systems (Shaw 2000). There are melatonin receptors in at least the ICo of Japanese quail (Aste et al. 2001). Japanese quail calls produced at night are longer than those during the day (Derégnaucourt et al. 2009). Constant light conditions caused the call of the Japanese quail to become shorter, as it did with zebra finch songs, and this modulation was relieved by a return to a light-dark cycle (Derégnaucourt et al. 2012).

A third hypothesis is that long durations of melatonin inhibit behavioral outputs. The inverse of this hypothesis is how melatonin operates in the nocturnal vocalizations of the plainfin midshipman fish; these fish vocalize at night and this vocalization free-runs

in constant dark conditions (Feng and Bass 2016). In constant light conditions, the vocalization is abolished, but its production returns upon constant administration of 2-iodomelatonin (Feng and Bass 2016). This influence of melatonin is likely mediated through the receptor Mel1b expression throughout the brain, including the parts of the brain associated with vocal control (Feng et al. 2019). In diurnal birds, chronic melatonin administration has been reported to prevent birds from vocalizing (Derégnaucourt et al. 2005). This difference, and other diversities in melatonin's effects, could be mediated through differential effects between melatonin receptor subtypes. In rat SCN, Mel1a receptors increase the $GABA_A$ receptor-mediated influx of ions, but in rat hippocampus, Mel1b subtypes inhibit the current (Wan et al. 1999). Further, melatonin may have a general inhibitory effect on overall metabolism and body temperature. In the nocturnal rat, melatonin increases the ATP production of mitochondria (Martin et al. 2000; Martín et al. 2002). One could speculate that melatonin would have an opposite, inhibitory effect in a diurnal animal. In house sparrows, pinealectomy removes the nocturnal hypothermia component of the circadian rhythm in body temperature (Binkley et al. 1971). Nocturnal hypothermia in diurnal animals is considered a means of an organism saving energy (Saarela and Reiter 1994). Long durations of melatonin administered to pinealectomized house sparrows did not affect body weights when compared to birds receiving a short duration or control treatments, so any energetic inhibition did not necessarily extend to body composition as we measured it (Chapter 3).

6.5   Prospects for future research

The position of the pineal gland in the hierarchy of the avian circadian system needs to be re-evaluated (Cassone, Paulose, Harpole, et al. 2017). Our lab and others consider the pineal gland the primary circadian pacemaker, as it is required for circadian rhythmicity of locomotor behavior (Gaston and Menaker 1968; Cassone and Menaker 1984), and this is presented in contrast to the central control of mammalian circadian rhythms (Bell-Pedersen et al. 2005). In mammals melatonin administration has little effect on clock genes in the SCN (Poirel et al. 2003), but does inhibit metabolism and electrical activity of the SCN (Cassone et al. 1988; Shibata et al. 1989). This is the case in birds as well, as pinealectomy in sparrows abolishes rhythms of metabolism in the SCN

(Lu and Cassone 1993a). Administering melatonin inhibits SCN metabolic activity in house sparrows and chickens (Lu and Cassone 1993b; Cantwell and Cassone 2002), and daily administration can re-entrain formerly arrhythmic house sparrows and zebra finches (Lu and Cassone 1993a; Gwinner et al. 1997; Wang et al. 2012). However, melatonin has little effect on clock gene expression in the Japanese quail mSCN (Yasuo et al. 2002); neither continuous administration of melatonin nor acute injection affected the circadian patterns of *per2*, *per3*, or *clock* in the mSCN. Further, the chicken pineal gland or retinae have a limited effect on the clock genes in peripheral rhythms, decreasing the amplitude of rhythms in most tissues, and altering the phase of *bmal1*, *per3*, and *cry1* (Karaganis et al. 2009). The data in Chapter 2 are consistent with this in house sparrows. Sympathetic input disrupts patterns of melatonin production and release from the pineal gland (Cassone and Menaker 1983), and administration of norepinephrine to arrhythmic pinealocytes *in vivo* re-entrains their rhythms of melatonin release (Zatz and Mullen 1988; Li and Cassone 2015). However, this effect of norepinephrine is brought about without altering the clock genes of the pinealocytes (Li and Cassone 2015). This evidence suggests that the avian pineal gland may act as a general inhibitor of behavior and metabolism, and may function as managing the outputs of a circadian clock that is more like those of mammals than previously thought.

A difficulty with studying dynamics in avian vocalizations is that humans simply are not adept at noticing differences in these presentations. Much of the research in this dissertation was enabled by the technology of the sonagram, wherein one can visualize changes in frequency and intensity of sounds, slowing them down for human visual inspection; the differences noticed are perceptible to human ears, but are much more striking when presented with a visualization. There is evidence that birds are indeed capable of distinguishing small changes in temporal structure and frequency (Dooling and Prior 2017). The repertoire analyses in Chapter 3 and 4 were sampled every two weeks, rather than the one-week resolution for the occurrence analyses, and for one to three times of day, and were performed via visual and aural inspection. If one wanted to further investigate the mechanism underlying this call type switch, development of a more objective analysis would be valuable. An automatic classification of the house sparrow call types would enable a higher resolution of analysis and may reveal dynamics

in "stereotypy" like those seen in canaries or New world sparrows, which were not measured in this research,

In parallel with the seasonal expansion of vocal states, birds also experience a dynamic in the physical movements of "dancing" associated with courtship displays (Halfwerk et al. 2019). Male zebra finches approach their potential mate with a series of hops, forming a zig-zag shape via a series of 180° turns, and performing a series of 'beak wipes.' The moves of 'hop,' 'beak wipe,' and 'turn-around,' were arranged in a non-random fashion, strongly associated with the production of song (Ullrich et al. 2016). Similarly, java sparrows, *Lonchura oryzivora*, add in non-vocal bill clicks to the beginning of their songs and at the start of certain song notes, and in this species the timing of this resembles that of the birds song tutor, suggesting these elements might be learned (Soma and Mori 2015). Male superb lyre-birds, *Menura novaehollandiae*, have four characteristic dance patterns that pair exclusively to their four song types, but can produce the song without the accompanying dance (Dalziell et al. 2013). As with much of vocalization research, more complexity in these displays exists beyond unaided human perception. The black manakin (*Xenopipo atronitens*) bird's hopping behavior was revealed to be a backflip when filmed and slowed using high-speed cameras (Lindsay et al. 2015). The blue-capped cordon-bleu finch (*Uraeginthus cyanocephalus*) was also revealed to perform a rhythmic "tap dancing" with its feet during courtship displays (Ota et al. 2015). House sparrows produce solicitation displays as well, consisting of hops, a stretched posture of its neck and head, raising and fanning of the tail, lowered, shivering wings, and the puffing of breast feathers (Anderson 2006b), although to my knowledge it has not been studied in the detail as the aforementioned studies on passerine dance displays. The neural mechanisms underlying these behaviors, and the potential for them to vary over the day or the seasons remains to be discovered.

The SCS is involved with the production of song, but it is also involved with song perception. Seasonal changes in perception have been documented in female teleost fish: medaka females enrich their retinae with LWS opsins, presumably to be able to select for males with orange-red colorations (Shimmura et al. 2017). Also, female midshipman fish will only exhibit phonotaxis to male vocalizations when they are carrying unlaid eggs

(Brantley and Bass 2010). This is also presumed to occur in female passerine birds (Maney and Pinaud 2011; Ball et al. 2020). Bilateral lesion of the HVC in female canaries led to a loss of ability for a female to distinguish heterospecific to conspecific vocalizations; females with exogenous estradiol capsules and lesioned HVC would perform copulation solicitation displays (CSD) indiscriminately to both white-crowned sparrow and canary song (Brenowitz 1991). These CSD displays are the preferred noninvasive output for the measure of female perception of song, and. The SCS's RA projects to the nXIIts, which innervates the syringeal muscles, as well as to respiratory premotor neurons in the nucleus retroambigualis (RAm), which in turn innervate the expiratory muscles that allow the syrinx to produce sound (Sturdy et al. 2003). The RAm was discovered to also innervate the motoneurons underlying cloacal positioning in both males and females, which is likely connecting to CSD in females (Wild and Botelho 2015).

Thus, the disinhibition of long durations of melatonin may alter the perception of birds. Chapter 4 of this dissertation explored the idea female house sparrow vocalizations were more like those of male's that previously appreciated. It is possible that long durations of melatonin presented to these birds as in the males in Chapter 3 may gate the photoperiodic expansion of these vocalizations. Estradiol-treated female house sparrows will also perform CSD in response to male vocalization playback (M//oller 1988); it is possible that long durations of melatonin may alter this behavior as well. The results of Chapter 5, wherein rhythmic presentations of vocalization signals were able to entrain the circadian clock of zebra finches may be another option as a measure of vocal perception. It would be interesting to see if house sparrows in a similar experimental protocol are more sensitive to the audio presentations in a photosensitive or photostimulated state. Reebs used birds caught at different times of year in his sound entrainment study on house sparrows, but it is unclear if the photosensitivity of the birds played a role in the sensitivity of their clocks to these cues (Reebs 1989). Using sound entrainment as a measure gives one insight into the clock and the sensitivity to non-photic cues; one could imagine that the sounds of a colony may be a more important cue to birds in a winter-like state, and an individual's song may be more important in a simulated breeding state.

6.5.1 Songbirds as a model for autism

As birds experience puberty each year, transitioning from sexual immaturity, maturity, before regressing again, one could argue that they also transition from socially inept to adept and regress again. It is possible that this socially inept state in the non-breeding season could share characteristics with classical autism in humans. Autism disorder is a condition wherein humans have difficulty with reciprocal social interactions, perform repetitive behavior, and have narrow interests. Autism diagnosed is diagnosed 1 in 166 children (Fombonne 2009). There is evidence of sleep and circadian dysfunctions in those with this condition (Amos 2013; Logan and McClung 2019); parents of children with autism note that they have difficulty processing hunger and sleep in short bouts (Malow 2004; Hu et al. 2009; Glickman 2010). Sleep disturbances are frequently reported in children with autism, including long sleep latency and frequent night-time and early morning awakenings (Hoshino et al. 1984; Richdale and Prior 1995; Johnson 1996; Patzold et al. 1998; Richdale 1999; Schreck and Mulick 2000; Honomichl et al. 2002). In one study, fifty-two percent of children with autism experienced increased night time awakenings, compared with seven percent of their non-neurodivergent siblings (Horvath and Perman 2002). Clock gene expression may also be abnormal in autistic people, as patients with autism in a genome-wide association screen had polymorphisms in their *per1* and *Npas2* genes (Nicholas et al. 2007). Some researchers have suggested that there may be a seasonal component to the occurrence of problem behaviors associated with his condition (Hayashi 2001; Boso et al. 2010). Further, melatonin specifically may underlie some of these sleep and circadian dysfunctions. Patients with classical autism have reduced melatonin amplitude at night (Nir et al. 1995; Kulman et al. 2000; Tordjman et al. 2005; Tordjman et al. 2015), some cases of which may be result of polymorphisms in the promoter region for the gene encoding acetylserotonin methyltransferase / hydroxyindole O-methyltransferase, one of the rate-limiting enzymes in the production of melatonin from tryptophan (Melke et al. 2008). Administering exogenous melatonin to humans with autism may have therapeutic benefits (Tordjman et al. 2015), including improvement of sleep quality (e.g. Wirojanan et al. 2009; Wright et al. 2011; Malow et

al. 2012), and in some, a correlated improvement of daytime behavior (Paavonen et al. 2003; Giannotti et al. 2006; De Leersnyder et al. 2011; Malow et al. 2012).

Autism is a variable, complex, and very human disorder; as such, current animal models tend to be piecewise, modelling only part of the disease's characteristics. To model "reciprocal social interactions" in transgenic mice, researchers quantify juvenile behavior, e.g. nose-to-nose sniffing, following, pushing, or sexual behavior such as approach or vocalizations (Silverman et al. 2010; Banerjee et al. 2014). Mouse with the gene SHANK3 knocked-out have reduced social interactions of this manner (Peça et al. 2011). Repetitive behavior, and "narrow interests" / "insistence on sameness" in mice can be quantified from circling, jumping, and self-grooming behavior (Silverman et al. 2010). For example, the inbred mouse strain BTBR T+tf/J partake in longer self-grooming sessions that C57BL/6 mice (McFarlane et al. 2008). The wealth of genetic tools and specific strains available in mice make them quite powerful, and these models are certainly valid. However, songbirds could also function as powerful models for some conditions of autism (Panaitof 2012). Songbird vocalizations are learned, a relative rarity among animals, in contrast with the vocalizations of laboratory mouse and rats. Further, songbirds are one of the few orders of non-primates which have 'mirror neurons,' which activate when mimicking a conspecific (Mooney 2014). Outputs such as CSD and entrainment to rhythmic presentations to audio cues as in Chapter 5 involve arguably higher processing of social cues than those measured in rodent models.

Associating components of these avian behaviors with genes associated with autism would be the key to developing this argument. Autism is highly heritable but is genetically heterogeneous. There are genes that are associated with the development of language, which have shown to be impaired in some cases of autism, and are present in songbirds (Panaitof 2012). FoxP2 is a famously language-associated gene, shared with humans, birds, and midshipman fish (Scharff and White 2004; Pengra et al. 2018), and a mutation in this gene can cause language impairment (Lai et al. 2001). However, FoxP2 is not considered primary candidate as an autism susceptibility gene (Newbury et al. 2002); its close homolog FoxP1, however, is (An and Claudianos 2016). Also, downstream of FoxP2, CNTNAP2 is an autism susceptibility gene (An and Claudianos

2016) that is expressed in birds, in the LMAN and RA (Carmen Panaitof et al. 2010). Comparing the list of genes linked in cases caused with autism (An and Claudianos 2016) and genes that share a similar expression pattern in the brains of humans and birds (Pfenning et al. 2014) reveals several candidates: FOXP1 and CNTNAP2 are present, but there are also 11 other genes that are not as well-discussed in the literature, including MEF2C (expressed in RA), PAFAH1B1 (expressed in the pallium), and SATB2 (expressed in LMAN). ADNP is another autism associated gene that is enriched in male birds vs female birds (Kleiman et al. 2015). It remains to be seen if some of these candidate genes are expressed in a photoperiodic dynamic or are sensitive to melatonin or correlate to performance in a behavioral output such as song entrainment of CSD in response to vocalization playback.

Melatonin is an interesting hormone, as its receptors are widespread, and it does not always participate in 'traditional' inhibitory/excitatory feedback loops at distant sites as with other hormones. This dissertation helps to describe some of these functions. Chapter 2 adds to a growing literature suggesting that the central organization of the circadian clock in birds may not be as different from mammals as once thought; that a central hypothalamic pacemaker may orchestrate the molecular clock throughout the body, while pineal melatonin can affect outputs of this clock such as metabolism and behavior. Among these behaviors sensitive to melatonin are the vocalization behavior in male and female house sparrows, which were explored in Chapters 3 and 4. Whatever this central clock may be, it is also sensitive to the input of social behavior, as investigated in Chapter 5. Review of the literature suggests that many of these principles might extend to Passeriformes at large, and humans may share some of these mechanisms.

APPENDICES

APPENDIX 1. PIEZOELECTRIC SLEEP DETECTION IN A SONGBIRD

1.1   Introduction

Administration of melatonin to a diurnal vertebrate generally results in the promotion of sleep, including birds. Intravenous infusions of melatonin to pigeons (*Columba livia*) promote sleep (Phillips and Berger 1992), and melatonin injected intramuscularly to zebra finches (*Taeniopygia guttata*) caused them to fall asleep via visual inspection within 15 minutes (Hahnloser et al. 2002; Derégnaucourt et al. 2005).

The pineal gland is considered a critical component of the central control of circadian rhythms in oscine passerine birds (Cassone, Paulose, Harpole, et al. 2017). In this taxonomic order, the primary source of systemic melatonin comes from the pineal gland (Janik et al. 1992). In Galliform and Columbiform birds, the retinae also contribute melatonin to the systemic circulation (Underwood et al. 1990), and thus the pineal and eyes are necessary for overt rhythmicity in locomotor activity rhythms (Nyce and Binkley 1977; Underwood and Siopes 1984; Oshima et al. 1989). Surgical removal of the pineal gland causes loss of overt rhythmicity in oscine passerine birds in constant environmental conditions over the course of several days (Gaston and Menaker 1968). Transplantation of the pineal from an entrained bird to the eye cup of a pinealectomized, behaviorally arrhythmic individual restores rhythms with the phase of the donor (Zimmerman and Menaker 1979). Melatonin production and secretion is performed in the nighttime, as light inhibits these processes (Cassone 1990; Klein et al. 1997). Thus, constant light conditions (LL) can also cause behavioral arrhythmicity, presumably due to constant inhibition of melatonin production in the pineal (McMillan 1972; Binkley 1976). Constant light suppresses sleep in pigeons (Berger and Phillips 1989), and daily rhythmic, 12 hour infusions of melatonin restores the rhythms in sleep (Phillips and Berger 1992). Curiously, the sleep deprivation caused by LL in pigeons did not result in a subsequent sleep rebound when transitioning to constant dark (DD) conditions (Berger and Phillips 1994). Conversely, daytime infusions of melatonin caused inappropriate phase sleep bouts, with no effect on nighttime electroencephalogram (EEG) parameters (Mintz et al. 1998).

There are occasions in certain bird's life histories wherein sleep and the circadian clock are suppressed, and effects of this sleep loss are relatively minimal when compared with what occurs in sleep deprived mammals. It is possible that melatonin belies these phenomena. As was the case with pigeons (Berger and Phillips 1989), experimental suppression of avian of behavioral rhythms with constant light or surgical interventions causes constant behavior such that long bouts of sleep would be impossible, most notably in behavior records using perch hopping as an output (e.g. Gaston and Menaker 1968). Normally diurnal white-crowned sparrows (*Zonotrichia leucophrys*) transition and become nocturnal migrators twice per year, and during such a time their sleep is reduced by over 60% when compared to their resident states; this sleep deprivation is also less detrimental to their performance in a cognitive test when in the migratory state than not (Rattenborg et al. 2004; Jones et al. 2010). In other seasonal, nocturnal migrants, Swainson's thrushes (*Catharus ustulatus*) make up for their sleep debt by taking micro-naps during the daytime (Fuchs et al. 2009). At their Artic breeding grounds, male pectoral sandpipers (*Calidris melanotos*) severely suppress their sleep during an intense period of competition for fertile female birds, and this sleep deprivation seems not to affect their performance; in fact those which sleep the least have the highest reproductive success (Lesku et al. 2012). The great frigatebird (*Fregata minor*) is a marine feeder that cannot float or swim, thus they remain in flight for weeks at a time; telemetric EEG reveals that their sleep debt is relieved via unihemispheric sleep, often while circling in a rising air current (Rattenborg et al. 2016).

The gold standard for sleep detection in mammals and birds is EEG and electromyography (EMG). These have been performed on birds since the 1960s (Klein et al. 1964), and birds are the only taxa of animals besides mammals to exhibit both a REM and NREM state of sleep (Allada and Siegel 2008). However, the method is not preferable for all experimental designs and poses distinct challenges in studies on birds. These procedures are highly invasive, analysis-intensive, and due to the tethering of leads, restrictive and dangerous to the flight and natural behaviors of the animal. Further, surgeries such as removing the pineal gland of birds lowers the integrity of the bird's skull, wherein the EEG leads need to be drilled. Similar drawbacks and a desire for efficient mass detection of sleep phenotype in the mouse model led Donohue, O'Hara,

and colleagues to develop a non-harmful sleep/wake classification system using polyvinylidine difluoride (PVDF) piezoelectric sensors (Flores et al. 2007; Donohue et al. 2008). The piezoelectric sensors produce voltage deflection relative to the pressure applied to them, as such, the sleep detection algorithm capitalizes on how sleep produces a more regular respiratory pattern, which can be detected from the mouse asleep and breathing on the floor of its cage.

Perching is the characteristic behavior of birds in the taxonomic order "Passeriformes." In a cage lacking a nest, these birds will typically only sleep while grasping a perch. Applying the advantage of this trait, the present work describes the creation of a piezoelectric sensor-lined perch and the ongoing procedure to establish, train and validate a similar automatic system for real-time and harmless detection of sleep in birds. These experiments compare the deflections from piezoelectric material when the bird was considered asleep via (1) visual inspection via infrared goggles and (2) EEG patterns.

1.2   Methods

1.2.1   Development of a sleep detection algorithm using visual observation

Four zebra finches (2 male, 2 female) were individually isolated to a cage containing two piezo perches for separate trials, in a 12 hours of light, 12 hours of darkness cycle (12:12 LD).

Piezo perches were constructed: silver-coated polyvinylidine difluoride (PVDF) sheets (gift of Bruce F. O'Hara) were cut into ½-inch wide strips with a razor blade and epoxied to wooden dowels. The strips were linked in series using copper tape and affixed with a terminal on one end of the perch. The terminal was created using two conductive metal ring terminals, each on a respective side of the PVDF sheet, and held into plate using a non-conductive vinyl nut and screw. These two leads were soldered onto a 3.5 mm male stereo jack for placement into a custom low voltage amplifier (Signal Solutions, LLC, Lexington, KY), where the signals would be integrated to a PC via a National

Instruments NI-USB 6221 DAQ (Austin, TX), before being displayed and recorded as a voltage trace using MouseRec Software (Signal Solutions, Lexington, KY).

The individual zebra finches were each observed for 2-4 hours equally in both light and dark periods. The "night" observations were made using infra-red goggles. A laptop in a cabinet with its screen dimmed and polarized with a privacy screen was running custom labelling software (BirdMonitor, Signal Solutions LLC, Lexington, KY), such that the visual observer could correlate the signal's produced by the animal's movements to one of four labels: ("Active", "Sleep", "Quiet Rest", "No Observation"). This laptop had an output via a National Instruments generator that would input to another channel on the NIDAQ and produce a characteristic, consistent voltage assigned to the selected label.

The "Active" state was defined as lateral movement across the perch, or any gross body movement while stationary exceeding shifting of the head. "Quiet Rest" was marked when the bird sat in place, only moving the head with the eyes open. The state of "Sleep" was recognized when the bird sat essentially motionless with a characteristic tucking of the body close to the perch and head pointed directly forward or tucked against the body. The eyes would open and close transiently, typically staying closed for no longer than 30 seconds. Slight bobbing of the entire body and transient motions include quick flutters of the wings and a rapid opening and closing of the beak lasting about a second, though these actions were rare. Finally, "No Observation" was used for when the bird was not on a perch, though practically this state is similar to Active as the bird would never rest or sleep off of the perch and vibrations from the cage floor would also be picked up by the perches.

Raw piezo data manually marked with a state was extracted for the development of an automated system for interpreting voltage traces. The system is composed of two main parts: the features and the classifier. Features are physical properties of the signal and resulting waveforms, which can be used interchangeably but eventually honed to an optimal array for efficiency at differentiating states. They are implemented by the classifier program to score raw voltage trace data.

A portion of the human-marked data was used to train the algorithm, which is then used against the remaining data to quantify the effectiveness of the system.

Among the features considered for the present evaluation are the following waveform properties; these do not represent a comprehensive list: Envelope, specifically the number of peaks and dynamic range of the envelope; power spectral density function – specifically, the 14-18 Hz region of a normalized spectrum which can be associated with transients occurring during the active state; and the autocorrelation function – period and specifically, max peak in the 0.16 – 1.6 s range ($\approx$6.25 – 0.625 Hz) indicative of a regular breathing pattern.

Due to corruption of data from the initial block of birds, results displayed are based on an *n* of 1.

1.2.2   Development of a sleep detection algorithm using EEG

We also performed an association of piezoelectric signals from birds while they were tethered to a six lead EEG headmount. EEG surgeries were adapted from the Pinnacle Technology (Lawrence, KS) suggested 2 EEG, 1 EMG surgery for mouse: the bird is anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine. The bird's feathers are clipped from atop the skull. The head is secured in a stereotaxic instrument and placed on a heated platform. A rostral-caudal incision is made with a scalpel to expose the skull. Five pilot holes are tapped with a fresh 23-gauge needle, and 70% ethanol-sterilized stainless-steel screws with leads are implanted into the skull to the level of the dura. The sixth lead pressed against the outside of the skull to capture the gross motor movements of the head of the bird. The screws were secured to the skull using Integrity Dental Cement (Dentsply Sirona, Mississauga, ON, Canada), and cured via UV light, protecting the exposed parts of the bird from the damaging light with several layers of sterile gauze. These leads were then soldered to an Omnetics A8393-001 connector (Omnetics, Minneapolis, MN), and this secured with a second round of Integrity cement and cured again with UV light. Any excess incision can be closed with a suture if needed. Antibiotic ointment is applied to the surgical site, and the bird allowed to recover on a heating pad until responsive. Acetaminophen is administered in the drinking water as an analgesic for two days following the surgery.

For EEG measurements, the birds' attached headmount was plugged into a Neuro8BP100 headstage (Triangle Biosystems, Durham, NC), leading to a low-torque, 10-channel commutator (Dragonfly Industries, Ridgeley, WV). The analog signal will then be converted to digital and further amplified by an ADInstruments PowerLab 16/30 (Colorado Springs, CO), interfacing with LabChart software. EEG recordings will occur in a custom built cylindrical, grounded, stainless steel cage with the piezoelectric perch obstructed beneath so the bird cannot tangle itself and the tethered cable. The visual observer will confirm that the eyes of the bird are closed and that the automatic EEG scoring confirms the bird is asleep before labelling the piezo data. A visual observer can also observe the states of the bird as in the above section.

This would require an additional state that the piezoelectric system may need to have documented in order to differentiate this state that has not been reported in terrestrial animals (Rattenborg et al. 2000; Bobbo et al. 2006). Based on preliminary data, perceived sleep can be achieved with both eyes closed in an apparent non-vigilant state with an observer very near the cage.

1.3   Results

1.3.1   Visual observation

The gross voltage patterns from the birds living in its cage with two piezo perches correlates well with what is expected in diurnal patterns of locomotion. Figure A1.1 represents a largely unmarked trace, recorded for roughly 4 days. Daytime active periods are evident by the presence of higher voltage transients while nighttime rest and sleep is relatively unperturbed, exhibiting low voltage signals.

Preliminary training of an algorithm also looks promising. Figure A1.2A is a representative 8 second window of the voltage trace seen when the bird's activity is classified as active. Transient spikes are noticeably sharper, frequency and amplitude are highly variable, though the amplitude of this view is atypically diminished. Quiet Rest in Figure A1.2B features steadier amplitude and a visibly rhythmic stretch indicative of regular breathing and a lack of movement. The Sleep state shown in Figure A1.2C is of

even lower amplitude with a seemingly longer period, likely due to a slower respiration rate. Biphasic peaks, as present, have also been observed in mouse sleep piezo data.
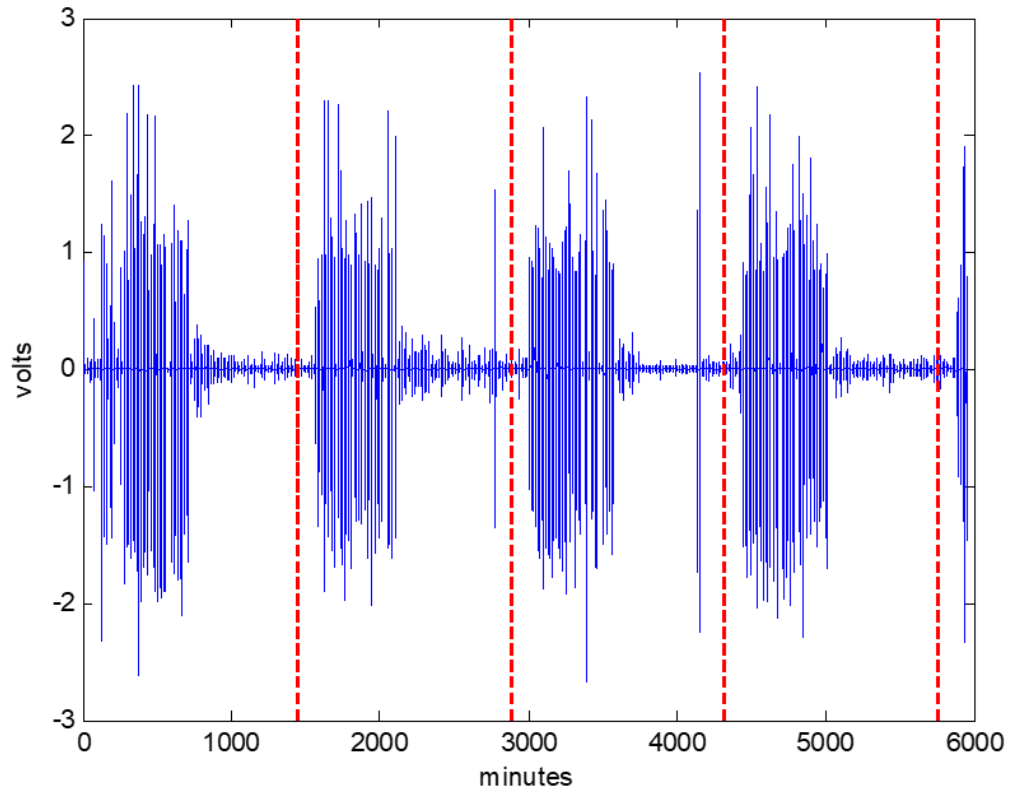
Figure A1.2 Gross piezoelectric deflections
Gross voltage deflections as a zebra finch remained in a cage with piezo-electric covered perches for approximately 4 days.

The 14-18 Hz region instrumental in identifying the active state is strongly present in the spectrogram of the Active state in Figure A1.2D. As for Quiet Rest in 2E, note a declining pattern of maximum peaks beginning around 3 Hz and falling below 2 Hz – a tempting interpretation being that of slowing respiration as sleep overtakes, the state of Quiet Rest essentially may never be observed other than adjacent to the Sleep state. The Sleep spectrogram (Figure A1.3F) suggests a heightening of activity below 2 Hz, though this is yet unclear due to noise.
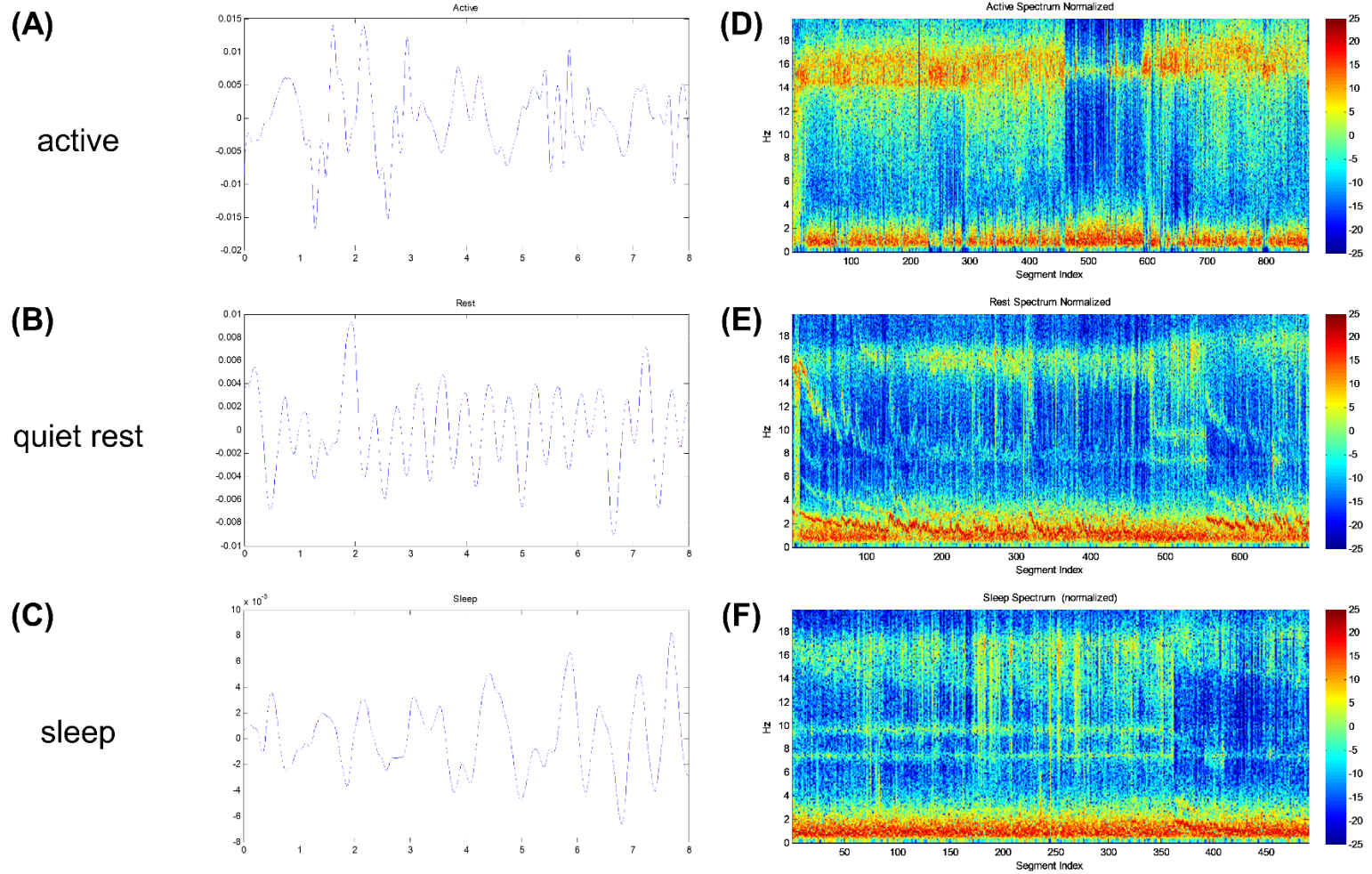
Figure A1.2 Sleep detection states and spectrograms

(previous page) Eight seconds of representative piezoelectric signal of the behavioral states (A) Active (B) Rest, and (C) Sleep, and their resulting normalized spectrograms (D-F).

Table A1.1 Percent error between various states

	% error
Active classified as Sleep or Rest	0.088 ± 0.005
Sleep or Rest classified as Active	0.09 ± 0.006
Sleep classified as Rest	0.23 ± 0.007
Rest classified as Sleep	0.29 ± 0.007

1.3.2   EEG anecdotes

The dental cement used for the surgery would fail after the bird would apply pressure through tugging against the cable when the headmount was plugged in. A more permanent cement should be used in the future. When testing this surgery, we initially used a dental cement that was thermogenic while curing and produced strong odorous fumes; either or a combination of these factors caused death.

Further, the weight of the cable when plugged in to the headmount caused the bird subject to adopt a crouched posture. Worse, the weight also discouraged the bird from sleeping on a perch. I attempted to counter this by adding piezo pads to the floor of the cage, but these issues prohibited progress on this project. If EEG on free-moving zebra finches is desired, perhaps telemetric technology will improve to create a lighter responder, or the weight of the headmount cable should be offset with a spring or similar apparatus.

1.4   Discussion

Training a sleep detection algorithm that is acceptably as accurate as visual inspection seems very possible. Training such an algorithm that is as accurate as EEG

would require a bit more engineering. A bird larger than a zebra finch may relieve some of the issues of our EEG apparatus, as well.

The biggest hurdle in refining the visual inspection-level algorithm is in distinguishing Quiet Rest and Sleep. The amplitude of the sleep signal is quite close to the noise floor of the system and if the trends are substantiated, sleep respiration rates may be in the range of 1 Hz or lower. Increasing the area of the piezo strip and tuning the sensitivity of the amplifier should increase the signal to noise ratio. The 8 Hz rhythm, faintly seen in Figure A1.2D and A1.2E, is most likely an artifact and could be removed through an increase in sampling rate as it may be due to aliasing. The birds also occasionally exhibited unihemispheric or "Vigilant Sleeping" during the observation, as evinced by having one eye open in a sleep posture (Rattenborg et al. 2000; Bobbo et al. 2006). An additional state should be added to the BirdRec program to account for this behavior.

The system might also be improved by adding sensor to the system to recognize when the bird is on a particular perch and extract only that data.

Noninvasive, higher-throughput sleep detection would be a very valuable addition to avian behavioral research. This would allow for some outstanding, basic questions to be answered, such as is the pineal gland required for sleep. This automated sleep detection algorithm could also run in real time, and trigger the movement of the perches only during sleep to minimize stress and cause sleep deprivation, to see if zebra finches are resistant to sleep deprivation, and if the pineal gland belies this phenomenon.

1.5 Acknowledgements

APPENDIX 2. THE EFFECT OF MELATONIN DURATIONS ON HOUSE
SPARROWS IN CONSTANT LIGHT CONDITIONS

2.1   Introduction

        Chapter 3 of this dissertation experimentally suppressed male house sparrows'
endogenous melatonin via removal of the pineal gland, allowing us to artificially
determine the length of melatonin presence via its availability in the drinking water.
Despite experiencing photostimulation, birds receiving a long duration of melatonin,
simulating winter conditions, did not develop an advanced vocal state as in birds
receiving a short duration of melatonin or control (Chapter 3). House sparrows
maintained in constant light which received a long duration of melatonin also did not
have as large of brain regions associated with song production as birds receiving a short
duration or control (Cassone et al. 2008). The current experiment endeavored to
experimentally suppress melatonin another way and follow a similar experimental
protocol to Chapter 3. Intact passerine birds kept in constant bright light (LL) conditions
have the rhythmicity of their circadian locomotor activity abolished, similar to
pinealectomized birds in constant darkness conditions, likely due to light's inhibitory
effect on melatonin synthesis and release removing the temporal dynamics of melatonin
(McMillan 1972; Binkley 1976). As a companion to the study from Cassone and
colleagues in 2008, and the behavioral outputs from Chapter 3: male house sparrows
were kept in constant light conditions, and melatonin presented to 1/3 of them for a short
daily duration, 1/3 a long daily duration, and 1/3 a vehicle control. Most birds failed to
entrain to these rhythmic presentations, in contrast to similar experimental designs.

2.2   Methods

        Adult male house sparrows (n=18) were captured via mist net in central Kentucky
and moved to an outdoor aviary, with food (2:1 white millet seed and chick starter) and
water *ad libitum* at the University of Kentucky Ecological Research and Education
Center field station. Captures occurred in the winter months up to two months preceding
the experiment. In the short day on February 10th (10.62 hrs of light, 13.38 hrs of
darkness and twilight at 38° N), birds were transferred indoors (constant 22.2°C) to

isolation cabinets, each with timer-controlled white LED lights (40 μW/cm^2, cycling for 12 hours of light; 12 hours of darkness, 12:12 LD), a microphone (either a Røde NT3 or an AKG Perception 170), and an infrared detector to measure locomotor activity. Beginning in this short winter photoperiod ensured that the birds commenced the study in a photosensitive state.

The birds experienced the following experimental procedure (Figure A2.1): After acclimating to their new cages for nine days, the light cycle was transitioned to constant light for 2 weeks. After this two-week period the short duration group (n=6) received 200 μg/mL melatonin in 0.5% ethanol daily for 6 hours. The long duration group (n=6) received 200 μg/mL melatonin daily for 14 hours. Fresh water was available ad libitum in times when treated water was not present. This melatonin concentration (200 μg/mL = 0.86mM) is sufficient to restore behavioral rhythmicity in melatonin: water cycles and result in an plasma melatonin amplitude similar to intact physiology (Heigl and Gwinner 1994). The birds received this concentration of melatonin for three weeks, however, the behavior was not sufficiently entraining to this dosage, so we increased it to 400 μg/mL for four additional weeks. The control group (n=6) had their drinking water replaced daily with 0.5% ethanol, throughout. Throughout these treatments, each dish was refreshed weekly and when necessary as consumed, and stock solutions replaced weekly.

Locomotor activity was continuously recorded from each cage's IR detector with VitalView data acquisition system in 5-minute bins (STARR Life Sciences Corp., Oakmont, PA). At nine stages, 48 hours of continuous audio was recorded using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY): the two weeks of LL before treatments started, the three weeks of LL with control and low dosage concentration of melatonin, and the four weeks of LL with control and high dosage concentration of melatonin. Vocalizations were extracted using Raven Pro 1.4, generating timestamps for each vocalization. The locomotor activity deflections coincident with the vocal recordings were plotted as mean by hour of each bird's daily activity, and the amplitude of these rhythms calculated using modified cosinor analysis Circwave Batch (Gronigen, Netherlands).

2.3   Results

2.3.1   Behavioral totals

The birds receiving a control treatment increased their vocalization baseline more than birds receiving long durations of melatonin during weeks 3-5 in constant light conditions, however overall time nor treatment contributed to the variance of changes in behavior (Figure A2.2, Two-way ANOVA with Tukey's multiple comparison test pairwise p-values p= 0.0044, 0.0328, and 0.0484, respectively for weeks 3, 4, and 5).

2.3.2   Daily distribution and entrainment

The melatonin treatments did a poor job of entraining the birds locomotor behavior, and only a marginal effect on vocalization behavioral rhythms. This is apparent in visual observation of the actograms of the birds (Figure A2.1), the relatively flat daily distributions of behavior (Figure A2.3), and the calculated amplitudes of these rhythms (Figure A2.4). The melatonin presentations entrained (amplitude values higher than other behaviors) the vocal behavior for the birds receiving a long duration of melatonin in the LL low dose weeks 1 and 2, before falling in week 3. The increase in dosage generally managed to entrain the vocalization behavior of short duration and long duration birds, but with little effect on their locomotor activity.

Short   Long   Ctrl

LL

low dose

high dose

Figure A2.1 Representative actograms
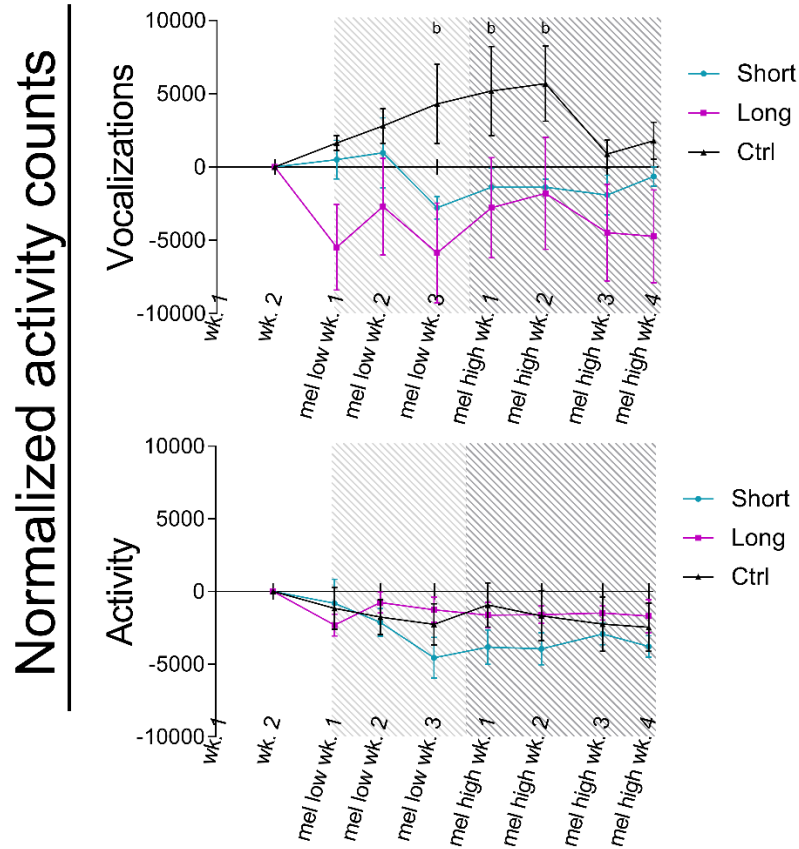
Figure A2.2 Change in behavioral totals

Change in behavioral activity totals for vocalization (top) and locomotor (bottom) behavior, using the first week in LL as a baseline. Lowercase letters indicate a difference between experimental groups (remaining panels, repeated measures Two-way ANOVA, Tukey's multiple comparison test, $p<0.05$): b = significant difference between Long and Control.

(following page) Daily distributions of vocalization and locomotor activity. Each graph represents 48 hours of continuous recording, as mean ± standard error, by hour, in treatment groups. Both activities are presented as percentile of each individual bird and day's daily total, e.g. a value of 0.1 would indicated 10% of that bird's daily activity occurred during the corresponding hour. The top graph of each pair is vocalization, the bottom is locomotor activity. Lowercase letters indicate a difference between experimental groups (repeated measures two-way ANOVA, Tukey's multiple comparison test, $p<0.05$): a = significant difference between Short and Control, b = significant difference between Long and Control, c = significant difference between Short and Long.
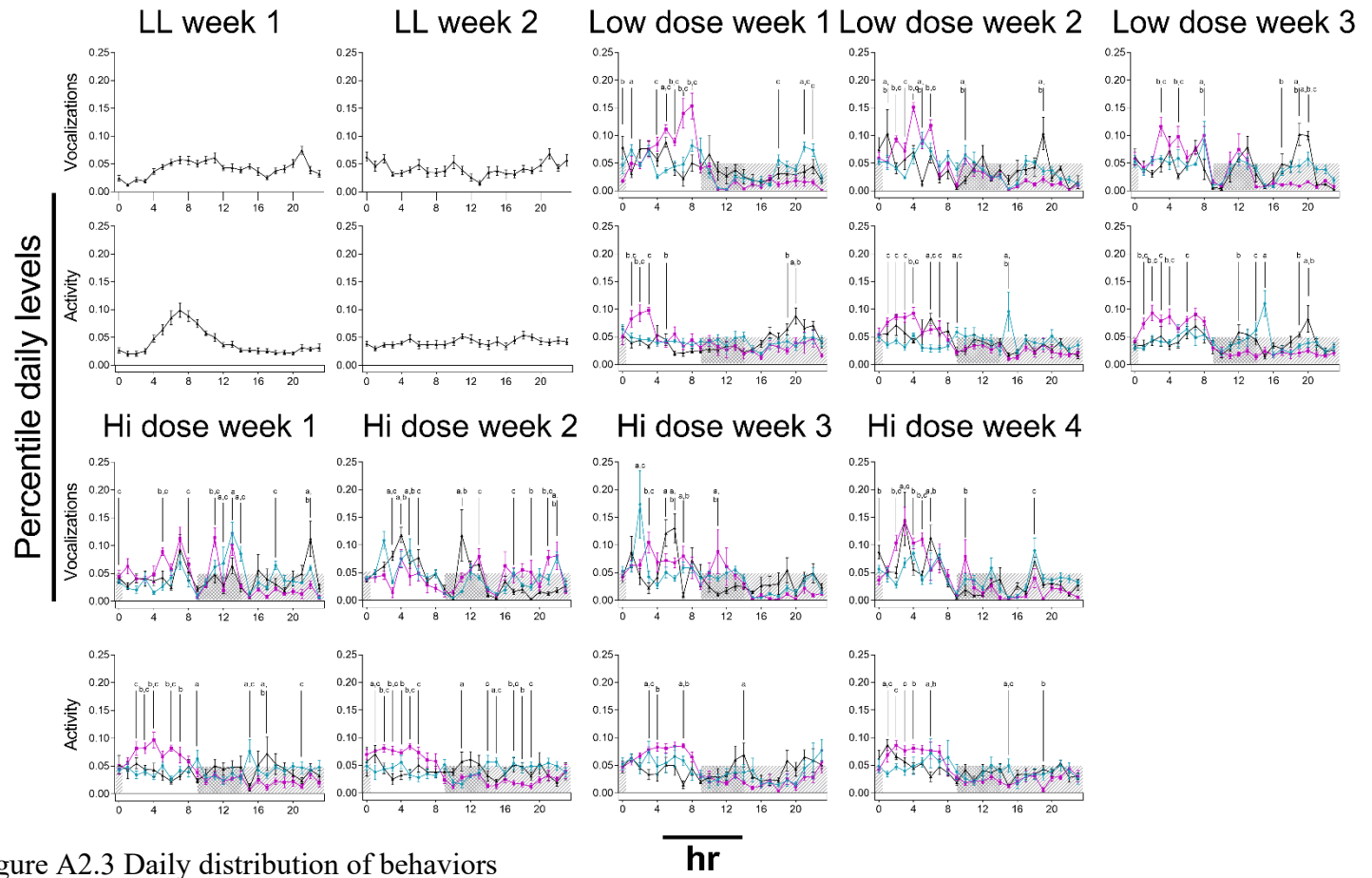
Figure A2.3 Daily distribution of behaviors
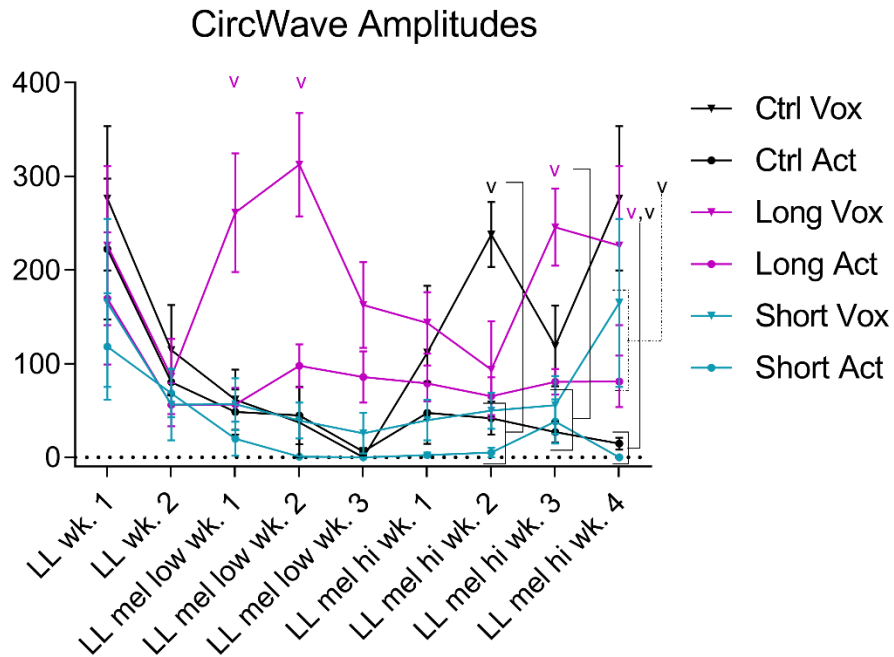
Figure A2.4 Amplitude
     Amplitude of the indicated behavior and experimental treatment, as measured from the cosinor function fit by Circwave software. Color-coded, lowercase 'v's indicate a significantly higher value than the bracketed behaviors when indicated, or all other groups when no bracket is present (repeated measures two-way ANOVA, Tukey's multiple comparison test, p<0.05).

2.4   Discussion

The rhythmic presentations of melatonin did a poor job of re-entraining birds which were rendered arrhythmic in their behavioral rhythms via constant light conditions. This is in contrast with experiments in zebra finch (Wang et al. 2012) and house sparrow (Cassone et al. 2008), despite using the same general protocol. Cassone and colleagues did use 8 hours of melatonin for their short duration group, while this experiment used 6 hours (2008). Our initial prediction was since male house sparrows receiving a long duration of melatonin in constant light conditions had smaller song control nuclei than others, that their vocal complexity would also be lower (Cassone et al. 2008). Based on this negative result indicating that melatonin was not properly affecting the birds as had been shown previously, no repertoire analyses were performed on these data.

2.5 Acknowledgements

APPENDIX 3. OUTDOOR STUDY OF A PHOTOPERIODIC CALL-TYPE SWITCH IN HOUSE SPARROWS, *PASSER DOMESTICUS*

3.1   Introduction

Chapter 3 and 4 of this dissertation described a "call type switch" in house sparrow, *Passer domesticus*, which correlates in captivity to an increase in photoperiod from a photosensitive state in both male and female birds. In male birds, long durations of melatonin prevent this call-type switch, suggesting that melatonin gates this photoperiodic development. In the wild, photosensitive house sparrows in the fall and early winter live in large flocks and favor "quiet, chirping noises ("social song")" thought to function for contact, without any social component. In the spring time, the males produce more "complex calls," including the "chirrup," thought to be used by males to attract a mate and establish a territory (Anderson 2006b; Summers-Smith 2009).

This call type switch is described in the *Handbook of the Birds of the World* encyclopedia (Summers-Smith 2009), but to our knowledge not quantified in a systematic manner. Chapters 3 and 4 of this dissertation describe this call type switch in individually housed birds kept indoors, so this current study endeavored to see if this call type switch occurs in more natural housing conditions: birds kept housed with both sexes in an outdoor aviary.

3.2   Methods

On two consecutive years, two sessions of 48 hrs of vocal recordings were taken from birds housed in a mixed sex 10'x12' aviary at the University of Kentucky Ecological Research and Education Center field station, with water and food (2:1 white millet seeds to chick starter) *ad libitum*.

In 2019, initial recording was on the short day of February 9th (10.56 hrs of light, 13.44 hrs of darkness and twilight at 38° N), and then 36 days later on the longer day of March 17th (12.0 hrs of light, 12.0 hrs of darkness and twilight); this consisted of 2 adult males and 2 adult females. In 2020, initial recording was on the short day of February 19th (10.92 hrs of light, 13.08 hrs of darkness and twilight), and then 34 days later on the

longer day of March 23rd (12.28 hrs of light, 11.72 hrs of darkness and twilight); this colony consisted of different birds than the 2019 colony, 2 males and 3 females. As birds were outdoors, they also received ambient non-photic cues, such as temperature and humidity levels associated with changing of seasons.

Vocalizations were extracted using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY), generating timestamps for each vocalization. These were plotted were plotted by mean by hour for the sum vocalizations produced by the colony. Other species of wild birds that were near the aviaries were errantly extracted in this process, as they were difficult to distinguish with the automation available in this software.

Repertoire was analyzed by the first 200 vocalizations produced by the colonies in the morning, using the call-type criteria as established in Chapters 3 and 4 of this dissertation.

We also attempted to have house sparrows in an aviary, housed individually. These data are not included due to loss of the birds in these conditions and for the surviving birds not vocalizing consistently in the short-day conditions.

3.3 Results

The daily distribution data in short days were similar between the years (Figure A3.1). However, in the longer days, the 2020 colony expressed a large dawn peak, and a secondary peak at dusk (Figure A3.1D), while the 2019 colony expressed general diurnal behavior (Figure A3.1B). This difference between years is likely due to nearby birds of different species that were falsely extracted during the analysis, as the aviaries were two different ones and were further (2019) and nearer (2020) to a tree-line, and the analysis method is effective at extracting biotic sounds from silence and abiotic sounds, but not at distinguishing between species.

The complexity of the calls increased as birds experienced the change in seasons (Figure A3.2). As this was a sample size of essentially 2 as individual birds were indistinguishable with the current setup, no statistical analyses were performed. Vocal

complexity began at 30% or less complex calls in the short days of February and increased to 60% or higher in the longer days of March.
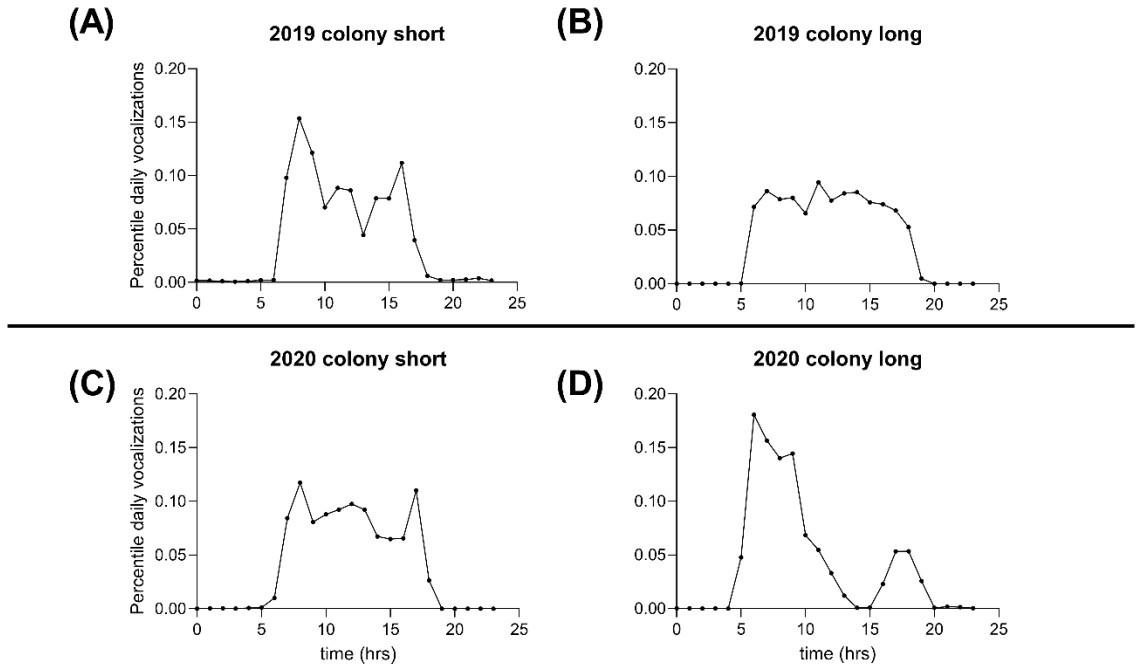
Figure A3.5 Daily distributions

Daily distributions of under different dates. Each graph represents 48 hours of continuous recording, presented as percentile of each individual bird and day's daily total, e.g. a value of 0.1 would indicated 10% of that bird's daily activity occurred during the corresponding hour.

colony repertoire

Fraction Complex Call vs Short/Long — Colony 19 (solid line), Colony 20 (dashed line)
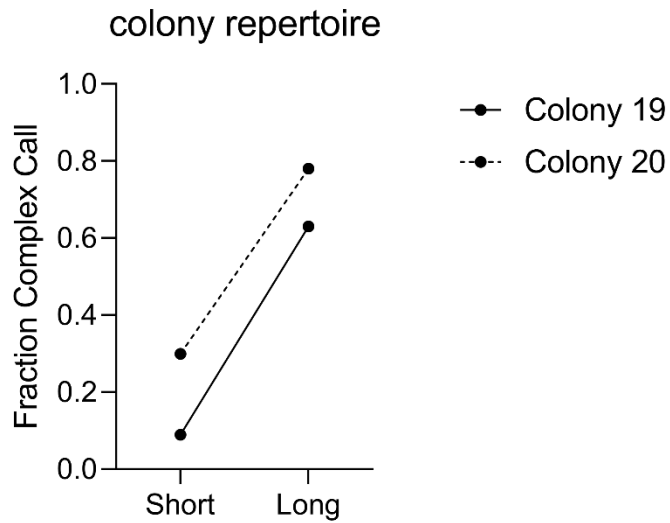
Figure A3.6 Repertoire

The complexity of calls within the 200 vocalizations of the morning; of the vocalizations classified as calls, the complexity at each recording, wherein a value of 1.0 indicates all calls sampled are classified as the 'complex' type and 0.0 indicates all calls sampled are of the 'simple' type.

3.4   Discussion

These results are encouraging that the call-type switch we noticed in Chapters 3 and 4 may be one that occurs in more natural settings. In Chapter 3, pinealectomized male birds receiving a short duration of melatonin or a control treatment developed a dawn and dusk chorus that was reduced or not present, respectively, when they were in a photosensitive state. This daily distribution result was not mirrored consistently here, but the vocal extraction certainly extracted neighboring wild birds of various species, lowering my confidence in this inconsistent result. The repertoire analysis of Figure A3.2 was performed manually, with aural and visual inspection of sonograms, thus there is confidence in this result that only house sparrow vocalizations were quantified. To further develop this idea, it would be best to develop a means of tracking individual birds that are living freely in their colony. This could be done with video or visual observation and leg bands, or perhaps using wild birds at nest boxes. A more technological solution could be the usage of telemetric microphone "backpacks" which have been used successfully in zebra finch colonies (Gill et al. 2015).

3.5   Acknowledgements

REFERENCES

Abraham U, Albrecht U, Brandstätter R. 2003. Hypothalamic circadian organization in birds. II. Clock gene expression. Chronobiol Int. 20(4):657–669. doi:10.1081/CBI-120022414.

Abraham U, Albrecht U, Gwinner E, Brandstatter R. 2002. Spatial and temporal variation of passer Per2 gene expression in two distinct cell groups of the suprachiasmatic hypothalamus in the house sparrow (Passer domesticus). Eur J Neurosci. 16(3):429–436. doi:10.1046/j.1460-9568.2002.02102.x.

Adachi A, Natesan AK, Whitfield-Rucker MG, Weigum SE, Cassone VM. 2002. Functional melatonin receptors and metabolic coupling in cultured chick astrocytes. Glia. 39(3):268–78. doi:10.1002/glia.10109.

Allada R, Siegel JM. 2008. Unearthing the phylogenetic roots of sleep. Curr Biol. 18(15):R670–R679. doi:10.1016/j.cub.2008.06.033.

Allard HA. 1930. The First Morning Song of Some Birds of Washington, D. C.; Its Relation to Light. Am Nat. 64(694):436–469. doi:10.1086/280329.

Altman J. 1962. Are New Neurons Formed in the Brains of Adult Mammals? Science (80-). 135(3509):1127–1128. doi:10.1126/science.135.3509.1127.

Altman J. 2011. The Discovery of Adult Mammalian Neurogenesis. In: Neurogenesis in the Adult Brain I. Vol. 17. Tokyo: Springer Japan. p. 3–46.

Alvarez-Borda B, Nottebohm F. 2002. Gonads and singing play separate, additive roles in new neuron recruitment in adult canary brain. J Neurosci. 22(19):8684–8690. doi:10.1523/jneurosci.22-19-08684.2002.

Alward BA, Balthazart J, Ball GF. 2013. Differential effects of global versus local testosterone on singing behavior and its underlying neural substrate. Proc Natl Acad Sci U S A. 110(48):19573–8. doi:10.1073/pnas.1311371110.

Alward BA, Cornil CA, Balthazart J, Ball GF. 2018. The regulation of birdsong by testosterone: Multiple time-scales and multiple sites of action. Horm Behav. 104(April):32–40. doi:10.1016/j.yhbeh.2018.04.010.

Alward BA, Madison FN, Parker SE, Balthazart J, Ball GF. 2016. Pleiotropic Control by Testosterone of a Learned Vocal Behavior and Its Underlying Neuroplasticity. eneuro. 3(1):ENEURO.0145-15.2016. doi:10.1523/ENEURO.0145-15.2016.

Amir S, Cain S, Sullivan J, Robinson B, Stewart J. 1999. Olfactory stimulation enhances light-induced phase shifts in free-running activity rhythms and Fos expression in the suprachiasmatic nucleus. Neuroscience. 92(4):1165–70. doi:10.1016/s0306-4522(99)00222-5.

Amos P. 2013. Rhythm and timing in autism: learning to dance. Front Integr Neurosci. 7(April):27. doi:10.3389/fnint.2013.00027.

An JY, Claudianos C. 2016. Genetic heterogeneity in autism: From single gene to a pathway perspective. Neurosci Biobehav Rev. 68(3):442–453. doi:10.1016/j.neubiorev.2016.06.013.

Anderson TR. 2006a. Annual Cycle. In: Biology of the Ubiquitous House Sparrow. Oxford University Press. p. 70–134.

Anderson TR. 2006b. Social Behavior and Vocalizations. In: Biology of the Ubiquitous House Sparrow. Oxford University Press. p. 283–295.

Anderson TR. 2006c. Breeding Biology and Reproductive Strategy. In: Biology of the Ubiquitous House Sparrow. Oxford University Press. p. 135–200.

Arendt J. 1998. Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev Reprod. 3(1):13–22. doi:10.1530/ror.0.0030013.

Arnold AP, Nottebohm F, Pfaff DW. 1976. Hormone concentrating cells in vocal control and other areas of the brain of the zebra finch (Poephila guttata). J Comp Neurol. 165(4):487–511. doi:10.1002/cne.901650406.

Aronov D, Fee MS. 2012. Natural Changes in Brain Temperature Underlie Variations in Song Tempo during a Mating Behavior. Coleman MJ, editor. PLoS One. 7(10):e47856. doi:10.1371/journal.pone.0047856.

Aschoff J. 1954. Zeitgeber der tierischen Tagesperiodik. Naturwissenschaften. 41(3):49–56. doi:10.1007/BF00634164.

Aschoff J. 1955. Tagesperiodik bei Mausestammen unter konstanten Umgebungsbedingungen. Pflugers Arch Gesamte Physiol Menschen Tiere. 262(1):51–59. doi:10.1007/BF00369733.

Aschoff J. 1960. Exogenous and Endogenous Components in Circadian Rhythms. Cold Spring Harb Symp Quant Biol. 25:11–28. doi:10.1101/SQB.1960.025.01.004.

Aschoff J. 1965. Circadian Rhythms in Man: A self-sustained oscillator with an inherent frequency underlies human 24-hour periodicity. Science (80-). 148(3676):1427–1432. doi:10.1126/science.148.3676.1427.

Aschoff J, Meyer-Lohmann J. 1954. Angeborene 24-Stunden-Periodik beim Kücken. Pflügers Arch - Eur J Physiol. 260(2):170–176. doi:10.1007/BF00363827.

Aste N, Cozzi B, Stankov B, Panzica G. 2001. Sexual differences and effect of photoperiod on melatonin receptor in avian brain. Microsc Res Tech. 55(1):37–47. doi:10.1002/jemt.1154.

Bailey MJ, Beremand PD, Hammer R, Bell-Pedersen D, Thomas TL, Cassone VM. 2003. Transcriptional profiling of the chick pineal gland, a photoreceptive circadian oscillator and pacemaker. Mol Endocrinol. 17(10):2084–95. doi:10.1210/me.2003-0121.

Bailey MJ, Beremand PD, Hammer R, Reidel E, Thomas TL, Cassone VM. 2004. Transcriptional profiling of circadian patterns of mRNA expression in the chick retina. J Biol Chem. 279(50):52247–54. doi:10.1074/jbc.M405679200.

Bailey MJ, Cassone VM. 2004. Opsin Photoisomerases in the Chick Retina and Pineal Gland: Characterization, Localization, and Circadian Regulation. Invest Ophthalmol Vis Sci. 45(3):769–75. doi:10.1167/iovs.03-1125.

Bailey MJ, Cassone VM. 2005. Melanopsin expression in the chick retina and pineal gland. Brain Res Mol Brain Res. 134(2):345–8. doi:10.1016/j.molbrainres.2004.11.003.

Bailey MJ, Chong NW, Xiong J, Cassone VM. 2002. Chickens' Cry2: molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. FEBS Lett. 513(2–3):169–174. doi:10.1016/S0014-5793(02)02276-7.

Ball GF. 2016. Species variation in the degree of sex differences in brain and behaviour related to birdsong: adaptations and constraints. Philos Trans R Soc Lond B Biol Sci. 371(1688):20150117-. doi:10.1098/rstb.2015.0117.

Ball GF, Balthazart J. 2010. Seasonal and hormonal modulation of neurotransmitter systems in the song control circuit. J Chem Neuroanat. 39(2):82–95. doi:10.1016/j.jchemneu.2009.08.005.

Ball GF, Ketterson ED. 2008. Sex differences in the response to environmental cues regulating seasonal reproduction in birds. Philos Trans R Soc B Biol Sci. 363(1490):231–246. doi:10.1098/rstb.2007.2137.

Ball GF, Madison FN, Balthazart J, Alward BA. 2020. How does testosterone act to regulate a multifaceted adaptive response? Lessons from studies of the avian song system. J Neuroendocrinol. 32(1):1–10. doi:10.1111/jne.12793.

Ballentine B, Badyaev A, Hill GE. 2003. Changes in song complexity correspond to periods of female fertility in blue grosbeaks (Guiraca caerulea). Ethology. 109(1):55–66. doi:10.1046/j.1439-0310.2003.00852.x.

Balthazart J, Foidart A, Wilson EM, Ball GF. 1992. Immunocytochemical localization of androgen receptors in the male songbird and quail brain. J Comp Neurol. 317(4):407–420. doi:10.1002/cne.903170407.

Banerjee S, Riordan M, Bhat MA. 2014. Genetic aspects of autism spectrum disorders: insights from animal models. Front Cell Neurosci. 8(February):58. doi:10.3389/fncel.2014.00058.

Bartell PA, Gwinner E. 2005. A separate circadian oscillator controls nocturnal migratory restlessness in the songbird Sylvia borin. J Biol Rhythms. 20(6):538–49. doi:10.1177/0748730405281826.

Beldhuis HJA, Dittami JP, Gwinner E. 1988. Melatonin and the circadian rhythms of feeding and perch-hopping in the European starling,Sturnus vulgaris. J Comp Physiol A. 164(1):7–14. doi:10.1007/BF00612712.

Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, Zoran MJ. 2005. Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet. 6(7):544–56. doi:10.1038/nrg1633.

Benoit J. 1935a. Stimulation par la lumière artificielle du développement testiculaire chez des canards aveugles par section du nerf optique. Comptes rendus l'Académie des Sci.(120):133–135.

Benoit J. 1935b. Stimulation par la lumière artificielle du développement testiculaire chez des canards aveugles par enucléation des globes oculaires. Comptes rendus l'Académie des Sci.(120):136–138.

Benoit J. 1964. THE STRUCTURAL COMPONENTS OF THE HYPOTHALAMO-HYPOPHYSEAL PATHWAY, WITH PARTICULAR REFERENCE TO PHOTOSTIMULATION OF THE GONADS IN BIRDS. Ann N Y Acad Sci. 117(1):23–32. doi:10.1111/j.1749-6632.1964.tb48157.x.

Bentley GE. 2001. Unraveling the enigma: the role of melatonin in seasonal processes in birds. Microsc Res Tech. 53(1):63–71. doi:10.1002/jemt.1069.

Bentley GE. 2003. Melatonin receptor density in Area X of European starlings is correlated with reproductive state and is unaffected by plasma melatonin concentration. Gen Comp Endocrinol. 134(2):187–192. doi:10.1016/S0016-6480(03)00261-2.

Bentley GE, Ball GF. 2000. Photoperiod-dependent and -independent regulation of melatonin receptors in the forebrain of songbirds. J Neuroendocrinol. 12(8):745–52.

Bentley GE, Perfito N, Calisi RM. 2013. Season- and context-dependent sex differences in melatonin receptor activity in a forebrain song control nucleus. Horm Behav. 63(5):829–835. doi:10.1016/j.yhbeh.2012.11.015.

Bentley GE, Van't Hof TJ, Ball GF. 1999. Seasonal neuroplasticity in the songbird telencephalon: a role for melatonin. Proc Natl Acad Sci U S A. 96(8):4674–9.

Berg KS, Brumfield RT, Apanius V. 2006. Phylogenetic and ecological determinants of the neotropical dawn chorus. Proc R Soc B Biol Sci. 273(1589):999–1005. doi:10.1098/rspb.2005.3410.

Berger RJ, Phillips NH. 1989. Continuous bright light suppresses circadian rhythms of body temperature and sleep in fed and fasted pigeons. Therm Physiol.:673–678.

Berger RJ, Phillips NH. 1994. Constant light suppresses sleep and circadian rhythms in pigeons without consequent sleep rebound in darkness. Am J Physiol. 267(4 Pt 2):R945-52.

Bernard DJ, Ball GF. 1997. Photoperiodic Condition Modulates the Effects of Testosterone on Song Control Nuclei Volumes in Male European Starlings. Gen Comp Endocrinol. 105(2):276–283. doi:10.1006/gcen.1996.6829.

Bernard DJ, Bentley GE, Balthazart J, Turek FW, Ball GF. 1999. Androgen Receptor, Estrogen Receptor α, and Estrogen Receptorβ Show Distinct Patterns of Expression in Forebrain Song Control Nuclei of European Starlings1. Endocrinology. 140(10):4633–4643. doi:10.1210/endo.140.10.7024.

Bernard DJ, Wilson FE, Ball GF. 1997. Testis-dependent and -independent effects

of photoperiod on volumes of song control nuclei in American tree sparrows (Spizella arborea). Brain Res. 760(1–2):163–169. doi:10.1016/S0006-8993(97)00277-1.

Binkley S. 1976. Comparative Biochemistry of the Pineal Glands of Birds and Mammals. Am Zool. 16(1):57–65. doi:10.1093/icb/16.1.57.

Binkley S, Kluth E, Menaker M. 1971. Pineal function in sparrows: circadian rhythms and body temperature. Science. 174(4006):311–314. doi:10.1126/science.174.4006.311.

Binkley S, Riebman J, Reilly K. 1978. The pineal gland: a biological clock in vitro. Science (80-). 202(4373):1198–1120. doi:10.1126/science.214852.

Blache D, Talbot RT, Blackberry MA, Williams KM, Martin GB, Sharp PJ. 2001. Photoperiodic control of the concentration of luteinizing hormone, prolactin and testosterone in the male emu (Dromaius novaehollandiae), a bird that breeds on short days. J Neuroendocrinol. 13(11):998–1006. doi:10.1046/j.1365-2826.2001.00722.x.

Bobbo D, Vallortigara G, Mascetti GG. 2006. Effects of social interaction on monocular/unihemispheric sleep in male and female domestic chicks. Biol Psychol. 73(3):213–9. doi:10.1016/j.biopsycho.2006.03.002.

Borbély AA. 1982. A two process model of sleep regulation. Hum Neurobiol. 1(3):195–204.

Boso M, Comelli M, Emanuele E, Podavini F, Marini M, Mancini L, Ucelli di Nemi S, Barale F, Politi P. 2010. Seasonal fluctuations in problem behaviors among young adults with autism and intellectual disability. Med Sci Monit. 16(5):CR213-6.

Botero CA, Rossman RJ, Caro LM, Stenzler LM, Lovette IJ, de Kort SR, Vehrencamp SL. 2009. Syllable type consistency is related to age, social status and reproductive success in the tropical mockingbird. Anim Behav. 77(3):701–706. doi:10.1016/j.anbehav.2008.11.020.

Bottjer S, Miesner E, Arnold A. 1984. Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science (80-). 224(4651):901–903. doi:10.1126/science.6719123.

Bottjer SW, Halsema KA, Brown SA, Miesner EA. 1989. Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. J Comp Neurol. 279(2):312–326. doi:10.1002/cne.902790211.

Bovet J, Oertli EF. 1974. Free-running circadian activity rhythms in free-living beaver (Castor canadensis). J Comp Physiol. 92(1):1–10. doi:10.1007/BF00696522.

Bradshaw WE, Holzapfel CM. 2007. Evolution of Animal Photoperiodism. Annu Rev Ecol Evol Syst. 38(1):1–25. doi:10.1146/annurev.ecolsys.37.091305.110115.

Bradshaw WE, Holzapfel CM. 2010. Light, Time, and the Physiology of Biotic Response to Rapid Climate Change in Animals. Annu Rev Physiol. 72(1):147–166. doi:10.1146/annurev-physiol-021909-135837.

Brandstätter R, Abraham U. 2003. Hypothalamic circadian organization in birds. I. Anatomy, functional morphology, and terminology of the suprachiasmatic region. Chronobiol Int. 20(4):637–55.

Brandstätter R, Abraham U, Albrecht U. 2001. Initial demonstration of rhythmic Per gene expression in the hypothalamus of a non-mammalian vertebrate, the house sparrow. Neuroreport. 12(6):1167–70. doi:10.1097/00001756-200105080-00023.

Brantley RK, Bass AH. 2010. Alternative Male Spawning Tactics and Acoustic Signals in the Plainfin Midshipman Fish Porichthys notatus Girard (Teleostei, Batrachoididae). Ethology. 96(3):213–232. doi:10.1111/j.1439-0310.1994.tb01011.x.

Brenowitz E. 1991. Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. Science (80-). 251(4991):303–305. doi:10.1126/science.1987645.

Brenowitz EA. 2004. Plasticity of the adult avian song control system. Ann N Y Acad Sci. 1016:560–85. doi:10.1196/annals.1298.006.

Brenowitz EA, Arnold AP. 1986. Interspecific comparisons of the size of neural song control regions and song complexity in duetting birds: evolutionary implications. J Neurosci. 6(10):2875–9. doi:https://doi.org/10.1523/JNEUROSCI.06-10-02875.1986.

Brenowitz EA, Beecher MD. 2005. Song learning in birds: diversity and plasticity, opportunities and challenges. Trends Neurosci. 28(3):127–32. doi:10.1016/j.tins.2005.01.004.

Bretzl H. 1903. Botanische Forschungen des Alexanderzuge. Leipzig, Germany: B.G. Teubner.

Brown AJ, Pendergast JS, Yamazaki S. 2019. Peripheral circadian oscillators. Yale J Biol Med. 92(2):327–335. doi:10.1016/b978-0-12-396971-2.00004-x.

Brunton DH, Roper MM, Harmer AMT. 2016. Female song rate and structure predict reproductive success in a socially monogamous bird. Front Ecol Evol. 4(MAR). doi:10.3389/fevo.2016.00013.

Brusco LI, García-Bonacho M, Esquifino AI, Cardinali DP. 1998. Diurnal rhythms in norepinephrine and acetylcholine synthesis of sympathetic ganglia, heart and adrenals of aging rats: Effect of melatonin. J Auton Nerv Syst. 74(1):49–61. doi:10.1016/S0165-1838(98)00134-9.

Buchen L. 2009. Culture May Be Encoded in DNA. Wired. https://www.wired.com/2009/05/songbirdculture/.

Bunning E. 1960. Opening Address: Biological Clocks. Cold Spring Harb Symp Quant Biol. 25(0):1–9. doi:10.1101/SQB.1960.025.01.003.

Byers J, Hebets E, Podos J. 2010. Female mate choice based upon male motor performance. Anim Behav. 79(4):771–778. doi:10.1016/j.anbehav.2010.01.009.

Cagnacci A, Elliott JA, Yen SS. 1992. Melatonin: a major regulator of the

circadian rhythm of core temperature in humans. J Clin Endocrinol Metab. 75(2):447–452. doi:10.1210/jcem.75.2.1639946.

Cailotto C, La Fleur SE, Van Heijningen C, Wortel J, Kalsbeek A, Feenstra M, Pévet P, Buijs RM. 2005. The suprachiasmatic nucleus controls the daily variation of plasma glucose via the autonomic output to the liver: are the clock genes involved? Eur J Neurosci. 22(10):2531–2540. doi:10.1111/j.1460-9568.2005.04439.x.

Cailotto C, van Heijningen C, van der Vliet J, van der Plasse G, Habold C, Kalsbeek A, Pévet P, Buijs RM. 2008. Daily Rhythms in Metabolic Liver Enzymes and Plasma Glucose Require a Balance in the Autonomic Output to the Liver. Endocrinology. 149(4):1914–1925. doi:10.1210/en.2007-0816.

Cantwell EL, Cassone VM. 2002. Daily and circadian fluctuation in 2-deoxy[(14)C]-glucose uptake in circadian and visual system structures of the chick brain: effects of exogenous melatonin. Brain Res Bull. 57(5):603–11.

Cantwell EL, Cassone VM. 2006a. Chicken suprachiasmatic nuclei: I. Efferent and afferent connections. J Comp Neurol. 496(1):97–120. doi:10.1002/cne.20935.

Cantwell EL, Cassone VM. 2006b. Chicken suprachiasmatic nuclei: II. Autoradiographic and immunohistochemical analysis. J Comp Neurol. 499(3):442–57. doi:10.1002/cne.21124.

Carmen Panaitof S, Abrahams BS, Dong H, Geschwind DH, White SA. 2010. Language-related cntnap2 gene is differentially expressed in sexually dimorphic song nuclei essential for vocal learning in songbirds. J Comp Neurol. 518(11):1995–2018. doi:10.1002/cne.22318.

Carouso-Peck S, Goldstein MH. 2019. Female Social Feedback Reveals Non-imitative Mechanisms of Vocal Learning in Zebra Finches. Curr Biol. 29(4):631-636.e3. doi:10.1016/j.cub.2018.12.026.

Cassone VM. 1988. Circadian variation of [14C]2-deoxyglucose uptake within the suprachiasmatic nucleus of the house sparrow, Passer domesticus. Brain Res. 459(1):178–82.

Cassone VM. 1990. Melatonin: time in a bottle. Oxf Rev Reprod Biol. 12:319–67.

Cassone VM. 1992. The pineal gland influences rat circadian activity rhythms in constant light. J Biol Rhythms. 7(1):27–40. doi:10.1177/074873049200700103.

Cassone VM. 2014. Avian circadian organization: A chorus of clocks. Front Neuroendocrinol. 35(1):76–88. doi:10.1016/j.yfrne.2013.10.002.

Cassone VM, Bartell PA, Earnest BJ, Kumar V. 2008. Duration of melatonin regulates seasonal changes in song control nuclei of the house sparrow, Passer domesticus: independence from gonads and circadian entrainment. J Biol Rhythms. 23(1):49–58. doi:10.1177/0748730407311110.

Cassone VM, Brooks DS. 1991. Sites of melatonin action in the brain of the house sparrow,Passer domesticus. J Exp Zool. 260(3):302–309.

doi:10.1002/jez.1402600305.

Cassone VM, Brooks DS, Kelm TA. 1995. Comparative distribution of 2[125I]iodomelatonin binding in the brains of diurnal birds: outgroup analysis with turtles. Brain Behav Evol. 45(5):241–256. doi:10.1159/000113553.

Cassone VM, Forsyth AM, Woodlee GL. 1990. Hypothalamic regulation of circadian noradrenergic input to the chick pineal gland. J Comp Physiol A. 167(2):187–92.

Cassone VM, Kumar V. 2015. Circadian Rhythms. In: Scanes CG, editor. Sturkie's Avian Physiology. Sixth. Elsevier. p. 811–827.

Cassone VM, Menaker M. 1983. Sympathetic regulation of chicken pineal rhythms. Brain Res. 272(2):311–7.

Cassone VM, Menaker M. 1984. Is the avian circadian system a neuroendocrine loop? J Exp Zool. 232(3):539–49. doi:10.1002/jez.1402320321.

Cassone VM, Moore RY. 1987. Retinohypothalamic projection and suprachiasmatic nucleus of the house sparrow, Passer domesticus. J Comp Neurol. 266(2):171–82. doi:10.1002/cne.902660204.

Cassone VM, Paulose JK, Harpole CE. 2017. Aging and the Circadian Control of the Gastrointestinal System: From the Brain to the Gut Microbiome (and Back). In: Jazwinski SM, Belancio VP, Hill SM, editors. Circadian Rhythms and Their Impact on Aging. Vol. 7. Cham: Springer International Publishing. (Healthy Ageing and Longevity). p. 83–101.

Cassone VM, Paulose JK, Harpole CE, Li Y, Whitfield-Rucker M. 2017. Avian Circadian Organization. In: Biological Timekeeping: Clocks, Rhythms and Behaviour. New Delhi: Springer India. p. 241–256.

Cassone VM, Paulose JK, Whitfield-Rucker MG, Peters JL. 2009. Time's arrow flies like a bird: two paradoxes for avian circadian biology. Gen Comp Endocrinol. 163(1–2):109–16. doi:10.1016/j.ygcen.2009.01.003.

Cassone VM, Speh JC, Card JP, Moore RY. 1988. Comparative Anatomy of the Mammalian Hypothalamic Suprachiasmatic Nucleus. J Biol Rhythms. 3(1):71–91. doi:10.1177/074873048800300106.

Cassone VM, Takahashi JS, Blaha CD, Lane RF, Menaker M. 1986. Dynamics of noradrenergic circadian input to the chicken pineal gland. Brain Res. 384(2):334–41.

Cassone VM, Westneat DF. 2012. The bird of time: cognition and the avian biological clock. Front Mol Neurosci. 5(March):32. doi:10.3389/fnmol.2012.00032.

Cassone VM, Yoshimura T. 2015. Circannual Cycles and Photoperiodism. In: Scanes CG, editor. Sturkie's Avian Physiology. Sixth. Elsevier. p. 829–845.

Castillo-Ruiz A, Paul MJ, Schwartz WJ. 2012. In search of a temporal niche: Social interactions. In: Progress in Brain Research. Vol. 199. 1st ed. Elsevier B.V. p.

267–280.

Catchpole C. K., Slater PJB. 2008a. Production and Perception. In: Bird Song: Biological Themes and Variations. 2nd ed. Cambridge: Cambridge University Press. p. 19–48.

Catchpole C. K., Slater PJB. 2008b. How Song Develops. In: Bird Song: Biological Themes and Variations. Cambridge: Cambridge University Press. p. 49–84.

Catchpole Clive K., Slater PJB. 2008. Bird Song. Second. Cambridge: Cambridge University Press.

Chabot CC, Menaker M. 1992. Circadian feeding and locomotor rhythms in pigeons and house sparrows. J Biol Rhythms. 7(4):287–99. doi:10.1177/074873049200700403.

Colwell CS. 2011. Linking neural activity and molecular oscillations in the SCN. Nat Rev Neurosci. 12(10):553–69. doi:10.1038/nrn3086.

Cox KH, Takahashi JS. 2019. Circadian clock genes and the transcriptional architecture of the clock mechanism. J Mol Endocrinol. 63(4):R93–R102. doi:10.1530/JME-19-0153.

Cumming BG, Wagner E. 1968. Rhythmic Processes in Plants. Annu Rev Plant Physiol. 19(1):381–416. doi:10.1146/annurev.pp.19.060168.002121.

Daan S. 2010. A History of Chronobiological Concepts. In: Albrecht U, editor. The Circadian Clock. New York, NY: Springer New York. p. 1–35.

Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M. 2015. The effects of life history and sexual selection on male and female plumage colouration. Nature. 527(7578):367–70. doi:10.1038/nature15509.

Dalziell AH, Peters RA, Cockburn A, Dorland AD, Maisey AC, Magrath RD. 2013. Dance choreography is coordinated with song repertoire in a complex avian display. Curr Biol. 23(12):1132–1135. doi:10.1016/j.cub.2013.05.018.

Dardente H, Hazlerigg DG, Ebling FJP. 2014. Thyroid hormone and seasonal rhythmicity. Front Endocrinol (Lausanne). 5(FEB):19. doi:10.3389/fendo.2014.00019.

Darwin C. 1881. The formation of vegetable mould through the action of worms: with observations on their habits. Appleton.

Dave AS, Margoliash D. 2000. Song replay during sleep and computational rules for sensorimotor vocal learning. Science. 290(5492):812–6. doi:10.1126/science.290.5492.812.

Davidson AJ, Menaker M. 2003. Birds of a feather clock together – sometimes: social synchronization of circadian rhythms. Curr Opin Neurobiol. 13(6):765–769. doi:10.1016/j.conb.2003.10.011.

Dawson A. 1991a. Photoperiodic control of testicular regression and moult in male House Sparrows Passer domesticus. Ibis (Lond 1859). 133(3):312–316.

doi:10.1111/j.1474-919X.1991.tb04575.x.

Dawson A. 1991b. Effect of daylength on the rate of recovery of photosensitivity in male starlings (Sturnus vulgaris). Reproduction. 93(2):521–524. doi:10.1530/jrf.0.0930521.

Dawson A. 1998. Photoperiodic control of the termination of breeding and the induction of moult in House Sparrows Passer domesticus. Ibis (Lond 1859). 140(1):35–40. doi:10.1111/j.1474-919X.1998.tb04538.x.

Dawson A. 2015. Avian Molting. In: Scanes CG, editor. Sturkie's Avian Physiology. Sixth. Elsevier. p. 907–917.

Dawson A, Follett BK, Goldsmith AR, Nicholls TJ. 1985. Hypothalamic gonadotrophin-releasing hormone and pituitary and plasma FSH and prolactin during photostimulation and photorefractoriness in intact and thyroidectomized starlings (Sturnus vulgaris). J Endocrinol. 105(1):71–7. doi:10.1677/joe.0.1050071.

Dawson A, King VM, Bentley GE, Ball GF. 2001. Photoperiodic Control of Seasonality in Birds. J Biol Rhythms. 16(4):365–380. doi:10.1177/074873001129002079.

Dawson A, Sharp PJ. 2007. Photorefractoriness in birds—photoperiodic and non-photoperiodic control. Gen Comp Endocrinol. 153(1–3):378–384. doi:10.1016/j.ygcen.2007.01.043.

Derégnaucourt S, Mitra PP, Fehér O, Pytte C, Tchernichovski O. 2005. How sleep affects the developmental learning of bird song. Nature. 433(7027):710–6. doi:10.1038/nature03275.

Derégnaucourt S, Saar S, Gahr M. 2009. Dynamics of crowing development in the domestic Japanese quail (Coturnix coturnix japonica). Proc R Soc B Biol Sci. 276(1665):2153–2162. doi:10.1098/rspb.2009.0016.

Derégnaucourt S, Saar S, Gahr M. 2012. Melatonin affects the temporal pattern of vocal signatures in birds. J Pineal Res. 53(3):1–14. doi:10.1111/j.1600-079X.2012.00993.x.

Deviche P, Gulledge CC. 2000. Vocal control region sizes of an adult female songbird change seasonally in the absence of detectable circulating testosterone concentrations. J Neurobiol. 42(2):202–211. doi:10.1002/(SICI)1097-4695(20000205)42:2<202::AID-NEU4>3.0.CO;2-G.

Devoogd TJ, Krebs JR, Healy SD, Purvis A. 1993. Relations between Song Repertoire Size and the Volume of Brain Nuclei Related to Song: Comparative Evolutionary Analyses amongst Oscine Birds. Proc R Soc B Biol Sci. 254(1340):75–82. doi:10.1098/rspb.1993.0129.

Dijk D-J, von Schantz M. 2005. Timing and Consolidation of Human Sleep, Wakefulness, and Performance by a Symphony of Oscillators. J Biol Rhythms. 20(4):279–290. doi:10.1177/0748730405278292.

Dong S, Clayton DF. 2009. Habituation in songbirds. Neurobiol Learn Mem. 92(2):183–8. doi:10.1016/j.nlm.2008.09.009.

Donham RS, Wingfield JC, Mattocks PW, Farner DS. 1982. Changes in testicular and plasma androgens with photoperiodically induced increase in plasma LH in the house sparrow. Gen Comp Endocrinol. 48(3):342–347. doi:10.1016/0016-6480(82)90146-0.

Donohue KD, Medonza DC, Crane ER, O'Hara BF. 2008. Assessment of a non-invasive high-throughput classifier for behaviours associated with sleep and wake in mice. Biomed Eng Online. 7:14. doi:10.1186/1475-925X-7-14.

Dooling RJ, Prior NH. 2017. Do we hear what birds hear in birdsong? Anim Behav. 124:283–289. doi:10.1016/j.anbehav.2016.10.012.

Doupe AJ, Solis MM, Kimpo R, Boettiger C a. 2004. Cellular, circuit, and synaptic mechanisms in song learning. Ann N Y Acad Sci. 1016:495–523. doi:10.1196/annals.1298.035.

Dowse HB, Palmer JD. 1969. Entrainment of circadian activity rhythms in mice by electrostatic fields. Nature. 222(5193):564–6. doi:10.1038/222564a0.

Driedzic WR, Crowe HL, Hicklin PW, Sephton DH. 1993. Adaptations in pectoralis muscle, heart mass, and energy metabolism during premigratory fattening in semipalmated sandpipers (Calidris pusilla). Can J Zool. 71(8):1602–1608. doi:10.1139/z93-226.

Ebihara S, Kawamura H. 1981. The role of the pineal organ and the suprachiasmatic nucleus in the control of circadian locomotor rhythms in the Java sparrow, Padda oryzivora. J Comp Physiol □ A. 141(2):207–214. doi:10.1007/BF01342667.

Edgar DM, Dement WC. 1991. Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. Am J Physiol. 261(4 Pt 2):R928-33. doi:10.1152/ajpregu.1991.261.4.R928.

Erkert HG, Nagel B, Stephani I. 1986. Light and social effects on the free-running circadian activity rhythm in common marmosets (Callithrix jacchus; Primates): social masking, pseudo-splitting, and relative coordination. Behav Ecol Sociobiol. 18(6):443–452. doi:10.1007/BF00300520.

Erkert HG, Schardt U. 1991. Social Entrainment of Circadian Activity Rhythms in Common Marmosets, Callithrix j. jacchus (Primates). Ethology. 87(3–4):189–202. doi:10.1111/j.1439-0310.1991.tb00246.x.

Favreau A, Richard-Yris MA, Bertin A, Houdelier C, Lumineau S. 2009. Social influences on circadian behavioural rhythms in vertebrates. Anim Behav. 77(5):983–989. doi:10.1016/j.anbehav.2009.01.004.

Fehér O, Wang H, Saar S, Mitra PP, Tchernichovski O. 2009. De novo establishment of wild-type song culture in the zebra finch. Nature. 459(7246):564–8. doi:10.1038/nature07994.

Feng NY, Bass AH. 2016. "Singing" Fish Rely on Circadian Rhythm and Melatonin for the Timing of Nocturnal Courtship Vocalization. Curr Biol. 26(19):2681–2689. doi:10.1016/j.cub.2016.07.079.

Feng NY, Marchaterre MA, Bass AH. 2019. Melatonin receptor expression in vocal, auditory and neuroendocrine centers of a highly vocal fish, the plainfin midshipman (*Porichthys notatus*). J Comp Neurol.(December 2018):1–16. doi:10.1002/cne.24629.

Flores AE, Flores JE, Deshpande H, Picazo JA, Xie XS, Franken P, Heller HC, Grahn DA, O'Hara BF. 2007. Pattern recognition of sleep in rodents using piezoelectric signals generated by gross body movements. IEEE Trans Biomed Eng. 54(2):225–33. doi:10.1109/TBME.2006.886938.

Follett BK, Mattocks PW, Farner DS. 1974. Circadian Function in the Photoperiodic Induction of Gonadotropin Secretion in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Proc Natl Acad Sci. 71(5):1666–1669. doi:10.1073/pnas.71.5.1666.

Fombonne E. 2009. Epidemiology of Pervasive Developmental Disorders. Pediatr Res. 65(6):591–598. doi:10.1203/PDR.0b013e31819e7203.

Fraley GS, Steiner RA, Lent KL, Brenowitz EA. 2010. Seasonal changes in androgen receptor mRNA in the brain of the white-crowned sparrow. Gen Comp Endocrinol. 166(1):66–71. doi:10.1016/j.ygcen.2009.08.001.

Franken P, Dijk D-J. 2009. Circadian clock genes and sleep homeostasis. Eur J Neurosci. 29(9):1820–9. doi:10.1111/j.1460-9568.2009.06723.x.

Fuchs JL. 1983. Effects of pinealectomy and subsequent melatonin implants on activity rhythms in the house finch (Carpodacus mexicanus). J Comp Physiol ??? A. 153(3):413–419. doi:10.1007/BF00612595.

Fuchs T, Maury D, Moore FR, Bingman VP. 2009. Daytime micro-naps in a nocturnal migrant: an EEG analysis. Biol Lett. 5(1):77–80. doi:10.1098/rsbl.2008.0405.

Fusani L, Gahr M. 2015. Differential expression of melatonin receptor subtypes MelIa, MelIb and MelIc in relation to melatonin binding in the male songbird brain. Brain Behav Evol. 85(1):4–14. doi:10.1159/000367984.

Gahr M, Güttinger H-R, Kroodsma DE. 1993. Estrogen receptors in the avian brain: Survey reveals general distribution and forebrain areas unique to songbirds. J Comp Neurol. 327(1):112–122. doi:10.1002/cne.903270109.

Gahr M, Kosar E. 1996. Identification, distribution, and developmental changes of a melatonin binding site in the song control system of the zebra finch. J Comp Neurol. 367(2):308–18. doi:10.1002/(SICI)1096-9861(19960401)367:2<308::AID-CNE11>3.0.CO;2-M.

Gahr M, Metzdorf R. 1997. Distribution and Dynamics in the Expression of Androgen and Estrogen Receptors in Vocal Control Systems of Songbirds. Brain Res Bull. 44(4):509–517. doi:10.1016/S0361-9230(97)00233-5.

Gahr M, Sonnenschein E, Wickler W. 1998. Sex differences in the size of the neural song control regions in a duetting species with similar songs in males and females. J Neurosci Methods. 18(3):1124–1131.

Garamszegi LZ, Eens M. 2004. Brain space for a learned task: Strong intraspecific evidence for neural correlates of singing behavior in songbirds. Brain Res Rev. 44(2–3):187–193. doi:10.1016/j.brainresrev.2003.12.001.

Garner WW, Allard HA. 1920. Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants. Mon Weather Rev. 48(7):415–415. doi:10.1175/1520-0493(1920)48<415b:EOTRLO>2.0.CO;2.

Gaston S. 1971. The influence of the pineal organ on the circadian activity rhythm in birds. In: Menaker M, editor. Biochronometry; Proceedings of a Symposium. Washington, D.C.: National Academies Press. p. 541–8.

Gaston S, Menaker M. 1968. Pineal Function: The Biological Clock in the Sparrow? Science (80-). 160(3832):1125–1127. doi:10.1126/science.160.3832.1125.

Giannotti F, Cortesi F, Cerquiglini A, Bernabei P. 2006. An Open-Label Study of Controlled-Release Melatonin in Treatment of Sleep Disorders in Children with Autism. J Autism Dev Disord. 36(6):741–752. doi:10.1007/s10803-006-0116-z.

Gill F, Donsker D, Rasmussen P. 2020. IOC World Bird List (v10.2). doi:10.14344/IOC.ML.10.2. https://www.worldbirdnames.org/.

Gill LF, Goymann W, Ter Maat A, Gahr M. 2015. Patterns of call communication between group-housed zebra finches change during the breeding cycle. Elife. 4(OCTOBER2015):1–23. doi:10.7554/eLife.07770.

Glaze CM, Troyer TW. 2006. Temporal structure in zebra finch song: implications for motor coding. J Neurosci. 26(3):991–1005. doi:10.1523/JNEUROSCI.3387-05.2006.

Glickman G. 2010. Circadian rhythms and sleep in children with autism. Neurosci Biobehav Rev. 34(5):755–68. doi:10.1016/j.neubiorev.2009.11.017.

Goel N, Lee TM. 1995a. Sex differences and effects of social cues on daily rhythms following phase advances in Octodon degus. Physiol Behav. 58(2):205–13. doi:10.1016/0031-9384(95)00051-j.

Goel N, Lee TM. 1995b. Social Cues Accelerate Reentrainment of Circadian Rhythms in Diurnal Female Octodon Degus (Rodentia-Octodontidae). Chronobiol Int. 12(5):311–323. doi:10.3109/07420529509057280.

Goel N, Lee TM. 1997. Olfactory Bulbectomy Impedes Social but Not Photic Reentrainment of Circadian Rhythms in Female Octodon degus. J Biol Rhythms. 12(4):362–370. doi:10.1177/074873049701200408.

Goldman B, Hall V, Hollister C, Roychoudhury P, Tamarkin L, Westrom W. 1979. Effects of melatonin on the reproductive system in intact and pinealectomized male

hamster maintained under various photoperiods. Endocrinology. 104(1):82–8. doi:10.1210/endo-104-1-82.

Goldman SA, Nottebohm F. 1983. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci U S A. 80(8):2390–4. doi:10.1073/pnas.80.8.2390.

Gould PD, Locke JCW, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, Putterill J, Millar AJ, et al. 2006. The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell. 18(5):1177–1187. doi:10.1105/tpc.105.039990.

Governale MM, Lee TM. 2001. Olfactory cues accelerate reentrainment following phase shifts and entrain free-running rhythms in female Octodon degus (Rodentia). J Biol Rhythms. 16(5):489–501. doi:10.1177/074873001129002169.

Gulledge CC, Deviche P. 1998. Photoperiod and testosterone independently affect vocal control region volumes in adolescent male songbirds. J Neurobiol. 36(4):550–558. doi:10.1002/(SICI)1097-4695(19980915)36:4<550::AID-NEU8>3.0.CO;2-V.

Guo H, Brewer JMK, Champhekar A, Harris RBSS, Bittman EL. 2005. Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. Proc Natl Acad Sci U S A. 102(8):3111–3116. doi:10.1073/pnas.0409734102.

Guo H, Brewer JMK, Lehman MN, Bittman EL. 2006. Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: Effects of transplanting the pacemaker. J Neurosci. 26(24):6406–6412. doi:10.1523/JNEUROSCI.4676-05.2006.

Gwinner E. 1967. Periodicity of a circadian rhythm in birds by species-specific song cycles (Aves, Fringillidae: Carduelis spinus, Serinus serinus). Experientia. 23(2):160–160. doi:10.1007/BF02135982.

Gwinner E. 1978. Effects of pinealectomy on circadian locomotor activity rhythms in european starlings,Sturnus vulgaris. J Comp Physiol ? A. 126(2):123–129. doi:10.1007/BF00666364.

Gwinner E. 1989a. Melatonin in the circadian system of birds: model of internal resonance. In: Circadian clocks and ecology. Hokkaido Univ. Press. p. 127–153.

Gwinner E. 1989b. Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. J Biol Rhythms. 4(2):237–50. doi:10.1177/074873048900400210.

Gwinner E. 2003. Circannual rhythms in birds. Curr Opin Neurobiol. 13(6):770–8.

Gwinner E, Brandstätter R. 2001. Complex bird clocks. Philos Trans R Soc Lond B Biol Sci. 356(1415):1801–10. doi:10.1098/rstb.2001.0959.

Gwinner E, Hau M, Heigl S. 1997. Melatonin: generation and modulation of avian circadian rhythms. Brain Res Bull. 44(4):439–44.

Gwinner E, König S, Zeman M. 1995. Endogenous gonadal, LH and molt rhythms in tropical stonechats: effect of pair bond on period, amplitude, and pattern of circannual cycles. J Comp Physiol A. 177(1):73–79. doi:10.1007/BF00243399.

Gwinner E, Scheuerlein A. 1998. Seasonal changes in day-light intensity as a potential zeitgeber of circannual rhythms in equatorial stonechats. J fur Ornithol. 139(4):407–412. doi:10.1007/BF01653467.

Gwinner E, Subbaraj R, Bluhm CK, Gerkema M. 1987. Differential Effects of Pinealectomy on Circadian Rhythms of Feeding and Perch Hopping in the European Starling. J Biol Rhythms. 2(2):109–120. doi:10.1177/074873048700200203.

Hahn TP, Brazeal KR, Schultz EM, Chmura HE, Cornelius JM, Watts HE, MacDougall-Shackleton SA. 2015. Annual Schedules. In: Scanes CG, editor. Sturkie's Avian Physiology (Sixth Edition). Sixth Edit. San Diego: Academic Press. p. 847–867.

Hahnloser RHR, Kozhevnikov A a, Fee MS. 2002. An ultra-sparse code underliesthe generation of neural sequences in a songbird. Nature. 419(6902):65–70. doi:10.1038/nature00974.

Halberg F. 1969. Chronobiology. Annu Rev Physiol. 31(1):675–726. doi:10.1146/annurev.ph.31.030169.003331.

Halfwerk W, Varkevisser J, Simon R, Mendoza E, Scharff C, Riebel K. 2019. Toward Testing for Multimodal Perception of Mating Signals. Front Ecol Evol. 7(Pt 20):3497–506. doi:10.3389/fevo.2019.00124.

Hamilton KS, King AP, Sengelaub DR, West MJ. 1997. A brain of her own: A neural correlate of song assessment in a female songbird. Neurobiol Learn Mem. 68(3):325–332. doi:10.1006/nlme.1997.3781.

Hamner WM. 1963. Diurnal Rhythm and Photoperiodism in Testicular Recrudescence of the House Finch. Science. 142(3597):1294–5. doi:10.1126/science.142.3597.1294.

Hanon EA, Lincoln GA, Fustin J-M, Dardente H, Masson-Pévet M, Morgan PJ, Hazlerigg DG. 2008. Ancestral TSH Mechanism Signals Summer in a Photoperiodic Mammal. Curr Biol. 18(15):1147–1152. doi:10.1016/j.cub.2008.06.076.

Harker JE. 1958. Diurnal Rhythms in the Animal Kingdom. Biol Rev. 33(1):1–52. doi:10.1111/j.1469-185X.1958.tb01407.x.

Harpole CE, Miles MD, Cassone VM. 2020. Melatonin duration gates photoperiodic vocal state change in a songbird. J Pineal Res. 68(2):1–37. doi:10.1111/jpi.12625.

Hastings MH, Maywood ES, Brancaccio M. 2018. Generation of circadian rhythms in the suprachiasmatic nucleus. Nat Rev Neurosci. 19(8):453–469. doi:10.1038/s41583-018-0026-z.

Hastings MH, Maywood ES, Brancaccio M. 2019. The Mammalian Circadian Timing System and the Suprachiasmatic Nucleus as Its Pacemaker. Biology (Basel).

8(1):13. doi:10.3390/biology8010013.

Hau M, Gwinner E. 1996. Food as a Circadian Zeitgeber for House Sparrows: The Effect of Different Food Access Durations. J Biol Rhythms. 11(3):196–207. doi:10.1177/074873049601100302.

Hayashi E. 2001. Seasonal changes in sleep and behavioral problems in a pubescent case with autism. Psychiatry Clin Neurosci. 55(3):223–224. doi:10.1046/j.1440-1819.2001.00833.x.

Hayden P, Lindberg RG. 1969. Circadian rhythm in mammalian body temperature entrained by cyclic pressure changes. Science. 164(3885):1288–9. doi:10.1126/science.164.3885.1288.

Hazlerigg D. 2012. The evolutionary physiology of photoperiodism in vertebrates. In: Kalsbeek A, Merrow M, Roenneberg T, Foster RG, editors. Progress in Brain Research. Vol. 199. 1st ed. Elsevier B.V. p. 413–422.

Hedley RW, Denton KK, Weiss RE. 2017. Accounting for syntax in analyses of countersinging reveals hidden vocal dynamics in a songbird with a large repertoire. Anim Behav. 131:23–32. doi:10.1016/j.anbehav.2017.06.021.

Heigl S, Gwinner E. 1994. Periodic Melatonin in the Drinking Water Synchronizes Circadian Rhythms in Sparrows. Naturwissenschaften. 81(2):83–85. doi:10.1007/s001140050035.

Herbert J, Thorpe PA, Klinowska M. 1975. Light and the pineal gland in the control of the breeding season in the female ferret. Int J Biometeorol. 19(4):280–281. doi:10.1007/BF01451038.

Hessler NA, Doupe AJ. 1999. Singing-Related Neural Activity in a Dorsal Forebrain–Basal Ganglia Circuit of Adult Zebra Finches. J Neurosci. 19(23):10461–10481. doi:10.1523/JNEUROSCI.19-23-10461.1999.

Hill SD. 2014. Influences of environmental and biological factors on song complexity in songbirds. Massey University.

Hill SD, Amiot C, Ludbrook MR, Ji W. 2015. Seasonal variation in the song structure of tui (Prosthemadera novaeseelandiae). N Z J Ecol. 39(1):110–115.

Hill SD, Pawley MDM, Anderson MG, Ji W. 2018. Higher song complexity and intruder pressure at dawn in a vocally complex songbird. Emu - Austral Ornithol. 118(2):147–157. doi:10.1080/01584197.2017.1380503.

Hoffmann K. 1960. Experimental Manipulation of the Orientational Clock in Birds. Cold Spring Harb Symp Quant Biol. 25:379–387. doi:10.1101/SQB.1960.025.01.040.

Honma K-I, Honma S, Hiroshige T. 1987. Activity rhythms in the circadian domain appear in suprachiasmatic nuclei lesioned rats given methamphetamine. Physiol Behav. 40(6):767–774. doi:10.1016/0031-9384(87)90281-2.

Honomichl RD, Goodlin-Jones BL, Burnham M, Gaylor E, Anders TF. 2002. Sleep patterns of children with pervasive developmental disorders. J Autism Dev Disord. 32(6):553–61. doi:10.1023/a:1021254914276.

Horvath K, Perman JA. 2002. Autistic disorder and gastrointestinal disease. Curr Opin Pediatr. 14(5):583–7. doi:10.1097/00008480-200210000-00004.

Hoshino Y, Watanabe H, Yashima Y, Kaneko M, Kumashiro H. 1984. An Investigation on Sleep Disturbance of Autistic Children. Psychiatry Clin Neurosci. 38(1):45–51. doi:10.1111/j.1440-1819.1984.tb00353.x.

Van Hout AJ-M, Eens M, Balthazart J, Pinxten R. 2009. Complex modulation of singing behavior by testosterone in an open-ended learner, the European Starling. Horm Behav. 56(5):564–73. doi:10.1016/j.yhbeh.2009.09.010.

Van Hout AJ-M, Pinxten R, Darras VM, Eens M. 2012. Testosterone increases repertoire size in an open-ended learner: an experimental study using adult male European starlings (Sturnus vulgaris). Horm Behav. 62(5):563–8. doi:10.1016/j.yhbeh.2012.09.008.

Hu VW, Sarachana T, Kim KS, Nguyen A, Kulkarni S, Steinberg ME, Luu T, Lai Y, Lee NH. 2009. Gene expression profiling differentiates autism case-controls and phenotypic variants of autism spectrum disorders: evidence for circadian rhythm dysfunction in severe autism. Autism Res. 2(2):78–97. doi:10.1002/aur.73.

Hutchinson JMC. 2002. Two explanations of the dawn chorus compared: how monotonically changing light levels favour a short break from singing. Anim Behav. 64(4):527–539. doi:10.1006/anbe.2002.3091.

Ikegami K, Atsumi Y, Yorinaga E, Ono H, Murayama I, Nakane Y, Ota W, Arai N, Tega A, Iigo M, et al. 2015. Low temperature-induced circulating triiodothyronine accelerates seasonal testicular regression. Endocrinology. 156(2):647–59. doi:10.1210/en.2014-1741.

Ikegami K, Yoshimura T. 2012. Circadian clocks and the measurement of daylength in seasonal reproduction. Mol Cell Endocrinol. 349(1):76–81. doi:10.1016/j.mce.2011.06.040.

Immelmann K. 1969. Song development in the zebra finch and other estrildid finches. In: Bird vocalizations. Cambridge University Press. p. 61–77.

Izumo M, Pejchal M, Schook AC, Lange RP, Walisser JA, Sato TR, Wang X, Bradfield CA, Takahashi JS. 2014. Differential effects of light and feeding on circadian organization of peripheral clocks in a forebrain Bmal1 mutant. Elife. 3:1–27. doi:10.7554/eLife.04617.

Janik D, Dittami J, Gwinner E. 1992. The Effect of Pinealectomy on Circadian Plasma Melatonin Levels in House Sparrows and European Starlings. J Biol Rhythms. 7(4):277–286. doi:10.1177/074873049200700402.

Jansen R, Metzdorf R, van der Roest M, Fusani L, ter Maat A, Gahr M. 2005. Melatonin affects the temporal organization of the song of the zebra finch. Faseb J.

19(7):848–50. doi:10.1096/fj.04-2874fje.

Jarvis ED, Nottebohm F. 1997. Motor-driven gene expression. Proc Natl Acad Sci U S A. 94(8):4097–102. doi:10.1073/pnas.94.8.4097.

Jawor JM, MacDougall-Shackleton SA. 2008. Seasonal and sex-related variation in song control nuclei in a species with near-monomorphic song, the northern cardinal. Neurosci Lett. 443(3):169–173. doi:10.1016/j.neulet.2008.07.085.

Jay TM, Jouvet M, Des Rosiers MH. 1985. Local cerebral glucose utilization in the free moving mouse: a comparison during two stages of the activity-rest cycle. Brain Res. 342(2):297–306. doi:10.1016/0006-8993(85)91129-1.

Jechura TJ, Walsh JM, Lee TM. 2003. Testosterone suppresses circadian responsiveness to social cues in the diurnal rodent Octodon degus. J Biol Rhythms. 18(1):43–50. doi:10.1177/0748730402239675.

Johnson CH, Elliott JA, Foster R. 2003. Entrainment of Circadian Programs. Chronobiol Int. 20(5):741–774. doi:10.1081/CBI-120024211.

Johnson CR. 1996. Sleep Problems in Children with Mental Retardation and Autism. Child Adolesc Psychiatr Clin N Am. 5(3):673–684. doi:10.1016/S1056-4993(18)30355-9.

Johnson F, Bottjer SW. 1993. Hormone-induced changes in identified cell populations of the higher vocal center in male canaries. J Neurobiol. 24(3):400–418. doi:10.1002/neu.480240311.

Johnson F, Soderstrom K, Whitney O. 2002. Quantifying song bout production during zebra finch sensory-motor learning suggests a sensitive period for vocal practice. Behav Brain Res. 131(1–2):57–65. doi:10.1016/S0166-4328(01)00374-6.

Jolley CC, Ukai-Tadenuma M, Perrin D, Ueda HR. 2014. A mammalian circadian clock model incorporating daytime expression elements. Biophys J. 107(6):1462–73. doi:10.1016/j.bpj.2014.07.022.

Jones SG, Paletz EM, Obermeyer WH, Hannan CT, Benca RM. 2010. Seasonal influences on sleep and executive function in the migratory White-crowned Sparrow (Zonotrichia leucophrys gambelii). BMC Neurosci. 11:87. doi:10.1186/1471-2202-11-87.

Juss RS, Davies IR, Follet BK, Mason R. 1994. Circadian rhythm in neuronal discharge activity in the quail lateral hypothalamic retinorecipient nucleus (LHRN) recorded in vitro. J Physiol. 475:132.

Karaganis SP, Bartell P a, Shende VR, Moore AF, Cassone VM. 2009. Modulation of metabolic and clock gene mRNA rhythms by pineal and retinal circadian oscillators. Gen Comp Endocrinol. 161(2):179–92. doi:10.1016/j.ygcen.2008.12.015.

Karaganis SP, Kumar V, Beremand PD, Bailey MJ, Thomas TL, Cassone VM. 2008. Circadian genomics of the chick pineal gland in vitro. BMC Genomics. 9:206. doi:10.1186/1471-2164-9-206.

Kidd PB, Young MW, Siggia ED. 2015. Temperature compensation and temperature sensation in the circadian clock. Proc Natl Acad Sci U S A. 112(46):E6284–E6292. doi:10.1073/pnas.1511215112.

Kiesel A. 1894. Untersuchungen zur Physiologie des facettierten Auges.

King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, Steeves TD., Vitaterna MH, Kornhauser JM, Lowrey PL, et al. 1997. Positional Cloning of the Mouse Circadian Clock Gene. Cell. 89(4):641–653. doi:10.1016/S0092-8674(00)80245-7.

King JR, Follett BK, Farner DS, Morton ML. 1966. Annual Gonadal Cycles and Pituitary Gonadotropins in Zonotrichia leucophrys gambelii. Condor. 68(5):476–487. doi:10.2307/1365320.

Kirn JR, Clower RP, Kroodsma DE, Devoogd TJ. 1989. Song-related brain regions in the red-winged blackbird are affected by sex and season but not repertoire size. J Neurobiol. 20(3):139–163. doi:10.1002/neu.480200304.

Kleiman GH, Barnea A, Gozes I. 2015. ADNP: A major autism mutated gene is differentially distributed (age and gender) in the songbird brain. Peptides. doi:10.1016/j.peptides.2015.04.008.

Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Rodriguez IR, Bégay V, et al. 1997. The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. Recent Prog Horm Res. 52:307–57; discussion 357-8.

Klein M, Michel F, Jouvet M. 1964. [POLYGRAPHIC STUDY OF SLEEP IN BIRDS]. C R Seances Soc Biol Fil. 158:99–103.

Ko GYP, Shi L, Ko ML. 2009. Circadian regulation of ion channels and their functions. J Neurochem. 110(4):1150–1169. doi:10.1111/j.1471-4159.2009.06223.x.

Kollmorgen S, Hahnloser RHR, Mante V. 2020. Nearest neighbours reveal fast and slow components of motor learning. Nature. 577(7791):526–530. doi:10.1038/s41586-019-1892-x.

Konopka RJ, Benzer S. 1971. Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci U S A. 68(9):2112–6.

Kramer G. 1952. EXPERIMENTS ON BIRD ORIENTATION *. Ibis (Lond 1859). 94(2):265–285. doi:10.1111/j.1474-919X.1952.tb01817.x.

Kräuchi K, Cajochen C, Pache M, Flammer J, Wirz-Justice A. 2006. Thermoregulatory effects of melatonin in relation to sleepiness. Chronobiol Int. 23(1–2):475–484. doi:10.1080/07420520500545854.

Kräuchi K, Cajochen C, Wirz-Justice A. 1997. A relationship between heat loss and sleepiness: effects of postural change and melatonin administration. J Appl Physiol. 83(1):134–139. doi:10.1152/jappl.1997.83.1.134.

Kuhlman SJ, McMahon DG. 2006. Encoding the Ins and Outs of Circadian Pacemaking. J Biol Rhythms. 21(6):470–481. doi:10.1177/0748730406294316.

Kulman G, Lissoni P, Rovelli F, Roselli MG, Brivio F, Sequeri P. 2000. Evidence of pineal endocrine hypofunction in autistic children. Neuro Endocrinol Lett. 21(1):31–34.

Kumar V, Singh S, Misra M, Malik S, Rani S. 2002. Role of melatonin in photoperiodic time measurement in the migratory redheaded bunting (Emberiza bruniceps) and the nonmigratory Indian weaver bird (Ploceus philippinus). J Exp Zool. 292(3):277–286. doi:10.1002/jez.10079.

Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature. 413(6855):519–23. doi:10.1038/35097076.

Lam F, Farner DS. 1976. The ultrastructure of the cells of Leydig in the white-crowned sparrow (Zonotrichia leucophrys gambelii) in relation to plasma levels of luteinizing hormone and testosterone. Cell Tissue Res. 169(1):93–109. doi:10.1007/BF00219310.

Langmore NE. 1998. Functions of duet and solo songs of female birds. Trends Ecol Evol. 13(4):136–140. doi:10.1016/S0169-5347(97)01241-X.

Larkin P, Baehr W, Semple-Rowland SL. 1999. Circadian regulation of iodopsin and clock is altered in the retinal degeneration chicken retina. Mol Brain Res. 70(2):253–263. doi:10.1016/S0169-328X(99)00154-0.

Laucht S, Kempenaers B, Dale J. 2010. Bill color, not badge size, indicates testosterone-related information in house sparrows. Behav Ecol Sociobiol. 64(9):1461–1471. doi:10.1007/s00265-010-0961-9.

De Leersnyder H, Zisapel N, Laudon M. 2011. Prolonged-Release Melatonin for Children With Neurodevelopmental Disorders. Pediatr Neurol. 45(1):23–26. doi:10.1016/j.pediatrneurol.2011.02.001.

Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, Bittman EL. 1987. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J Neurosci. 7(6):1626–38. doi:10.1523/JNEUROSCI.07-06-01626.1987.

Lein MR. 2007. Patterns of dawn singing by Buff-breasted Flycatchers. J F Ornithol. 78(4):343–351. doi:10.1111/j.1557-9263.2007.00122.x.

Leitner S, Catchpole CK. 2004. Syllable repertoire and the size of the song control system in captive canaries (Serinus canaria). J Neurobiol. 60(1):21–7. doi:10.1002/neu.10331.

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. 1958. Isolation of Melatonin, the Pineal Gland Factor That Lightens Melanocytes. J Am Chem Soc. 80(10):2587–2587. doi:10.1021/ja01543a060.

Lesku JA, Rattenborg NC, Valcu M, Vyssotski AL, Kuhn S, Kuemmeth F, Heidrich W, Kempenaers B. 2012. Adaptive Sleep Loss in Polygynous Pectoral Sandpipers. Science. 388(6639):235. doi:10.1126/science.1220939.

Levine JD, Funes P, Dowse HB, Hall JC. 2002. Resetting the circadian clock by social experience in Drosophila melanogaster. Science. 298(5600):2010–2. doi:10.1126/science.1076008.

Li XC, Jarvis ED, Alvarez-Borda B, Lim DA, Nottebohm F. 2000. A relationship between behavior, neurotrophin expression, and new neuron survival. Proc Natl Acad Sci U S A. 97(15):8584–8589. doi:10.1073/pnas.140222497.

Li Y, Cassone VM. 2015. Clock-Controlled Regulation of the Acute Effects of Norepinephrine on Chick Pineal Melatonin Rhythms. J Biol Rhythms. 30(6):519–532. doi:10.1177/0748730415607060.

Lindsay WR, Houck JT, Giuliano CE, Day LB. 2015. Acrobatic courtship display coevolves with brain size in manakins (Pipridae). Brain Behav Evol. 85(1):29–36. doi:10.1159/000369244.

Liu W, Kroodsma DE. 2007. Dawn and Daytime Singing Behavior of the Chipping Sparrows (Spizella Passerina). Auk. 124(1):44. doi:10.1642/0004-8038(2007)124[44:dadsbo]2.0.co;2.

Liu WC. 2004. The effect of neighbours and females on dawn and daytime singing behaviours by male chipping sparrows. Anim Behav. 68(1):39–44. doi:10.1016/j.anbehav.2003.06.022.

Logan RW, McClung CA. 2019. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat Rev Neurosci. 20(1):49–65. doi:10.1038/s41583-018-0088-y.

Lohmann M, Enright JT. 1967. The influence of mechanical noise on the activity rhythms of finches. Comp Biochem Physiol. 22(1):289–296. doi:10.1016/0010-406X(67)90188-0.

Lowrey PL, Shimomura K, Antoch MP, Yamazaki S, Zemenides PD, Ralph MR, Menaker M, Takahashi JS. 2000. Positional syntenic cloning and functional characterization of the mammalian circadian mutation tau. Science. 288(5465):483–92. doi:10.1126/science.288.5465.483.

Lu G-D, Needham J. 1980. Celestial Lancets: A History and Rationale of Acupuncture and Moxa. New York, NY: Routledge.

Lu J, Cassone VM. 1993a. Daily melatonin administration synchronizes circadian patterns of brain metabolism and behavior in pinealectomized house sparrows,Passer domesticus. J Comp Physiol A. 173(6):775–782. doi:10.1007/BF02451908.

Lu J, Cassone VM. 1993b. Pineal regulation of circadian rhythms of 2-deoxy[14C]glucose uptake and 2[125I]iodomelatonin binding in the visual system of the house sparrow,Passer domesticus. J Comp Physiol A. 173(6):765–774. doi:10.1007/BF02451907.

Lu J, Cassone VM. 1993c. Pineal regulation of circadian rhythms of 2-deoxyl14C] glucose uptake and 2112sI] iodomelatonin binding in the visual system of the house sparrow , Passer domesticus. :765–774.

Lu J, Zoran MJ, Cassone VM. 1995. Daily and circadian variation in the electroretinogram of the domestic fowl: effects of melatonin. J Comp Physiol A. 177(3):299–306.

M//oller AP. 1988. Badge size in the house sparrow Passer domesticus - Effects of intra- and intersexual selection. Behav Ecol Sociobiol. 22(5):373–378. doi:10.1007/BF00295107.

Magoolagan L, Mawby PJ, Whitehead FA, Sharp SP. 2019. The structure and context of male and female song in White-throated Dippers. J Ornithol. 160(1):195–205. doi:10.1007/s10336-018-1599-z.

Malow B, Adkins KW, McGrew SG, Wang L, Goldman SE, Fawkes D, Burnette C. 2012. Melatonin for sleep in children with autism: a controlled trial examining dose, tolerability, and outcomes. J Autism Dev Disord. 42(8):1729–37; author reply 1738. doi:10.1007/s10803-011-1418-3.

Malow BA. 2004. Sleep disorders, epilepsy, and autism. Ment Retard Dev Disabil Res Rev. 10(2):122–5. doi:10.1002/mrdd.20023.

Maney D, Pinaud R. 2011. Estradiol-dependent modulation of auditory processing and selectivity in songbirds. Front Neuroendocrinol. 32(3):287–302. doi:10.1016/j.yfrne.2010.12.002.

Maquet P. 2001. The role of sleep in learning and memory. Science. 294(5544):1048–52. doi:10.1126/science.1062856.

de Marain J-J d'Ortous. 1729. Observation Botanique. Hist l'Academie R des Sci.:35–36.

Marimuthu G, Rajan S, Chandrashekaran MK. 1981. Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat Hipposideros speoris. Behav Ecol Sociobiol. 8(2):147–150. doi:10.1007/BF00300827.

Marimuthu G, Subbaraj R, Chandrashekaran MK. 1978. Social synchronization of the activity rhythm in a cave-dwelling insectivorous bat. Naturwissenschaften. 65(11):600. doi:10.1007/BF00364921.

Martin M, Macias M, Escames G, Reiter RJ, Agapito MT, Ortiz GG, Acuna-Castroviejo D. 2000. Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J Pineal Res. 28(4):242–248. doi:10.1034/j.1600-079X.2000.280407.x.

Martín M, Macías M, León J, Escames G, Khaldy H, Acuña-Castroviejo D. 2002. Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. Int J Biochem Cell Biol. 34(4):348–57. doi:10.1016/s1357-2725(01)00138-8.

Mazuski C, Abel JH, Chen SP, Hermanstyne TO, Jones JR, Simon T, Doyle FJ, Herzog ED. 2018. Entrainment of Circadian Rhythms Depends on Firing Rates and Neuropeptide Release of VIP SCN Neurons. Neuron. 99(3):555-563.e5. doi:10.1016/j.neuron.2018.06.029.

McCasland JS. 1987. Neuronal control of bird song production. J Neurosci. 7(1):23–39. doi:10.1523/JNEUROSCI.07-01-00023.1987.

McClung CR. 2006. Plant Circadian Rhythms. Plant Cell. 18(4):792–803. doi:10.1105/tpc.106.040980.

McCord CP, Allen FP. 1917. Evidences associating pineal gland function with alterations in pigmentation. J Exp Zool. 23(1):207–224. doi:10.1002/jez.1400230108.

McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, Crawley JN. 2008. Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes, Brain Behav. 7(2):152–163. doi:10.1111/j.1601-183X.2007.00330.x.

McGoogan JM, Cassone VM. 1999. Circadian regulation of chick electroretinogram: effects of pinealectomy and exogenous melatonin. Am J Physiol. 277(5 Pt 2):R1418-27.

McMillan JP. 1972. Pinealectomy abolishes the circadian rhythm of migratory restlessness. J Comp Physiol. 79(2):105–112. doi:10.1007/BF00697766.

McNeil R, McSween A, Lachapelle P. 2005. Comparison of the Retinal Structure and Function in Four Bird Species as a Function of the Time They Start Singing in the Morning. Brain Behav Evol. 65(3):202–214. doi:10.1159/000083881.

Meitzen J, Moore IT, Lent K, Brenowitz EA, Perkel DJ. 2007. Steroid hormones act transsynaptically within the forebrain to regulate neuronal phenotype and song stereotypy. J Neurosci. 27(44):12045–57. doi:10.1523/JNEUROSCI.3289-07.2007.

Meitzen J, Perkel DJ, Brenowitz EA. 2007. Seasonal changes in intrinsic electrophysiological activity of song control neurons in wild song sparrows. J Comp Physiol A. 193(6):677–683. doi:10.1007/s00359-007-0222-1.

Meitzen J, Thompson CK, Choi H, Perkel DJ, Brenowitz EA. 2009. Time course of changes in Gambel's white-crowned sparrow song behavior following transitions in breeding condition. Horm Behav. 55(1):217–227. doi:10.1016/j.yhbeh.2008.10.006.

Melke J, Goubran Botros H, Chaste P, Betancur C, Nygren G, Anckarsäter H, Rastam M, Ståhlberg O, Gillberg IC, Delorme R, et al. 2008. Abnormal melatonin synthesis in autism spectrum disorders. Mol Psychiatry. 13(1):90–8. doi:10.1038/sj.mp.4002016.

Mello C V. 2004. Gene regulation by song in the auditory telencephalon of songbirds. Front Biosci. 9(1–3):63. doi:10.2741/1201.

Mello C V., Clayton DF. 1994. Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. J Neurosci. 14(11 I):6652–6666. doi:10.1523/jneurosci.14-11-06652.1994.

Menaker M. 1968. Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. Proc Natl Acad Sci U S A. 59(2):414–21. doi:10.1073/pnas.59.2.414.

Menaker M. 1971. Rhythms, Reproduction, and Photoreception1. Biol Reprod. 4(3):295–308. doi:10.1093/biolreprod/4.3.295.

Menaker M, Eskin A. 1966. Entrainment of circadian rhythms by sound in Passer domesticus. Science. 154(3756):1579–81.

Menaker M, Eskin A. 1967. Circadian clock in photoperiodic time measurement: a test of the Bünning hypothesis. Science. 157(3793):1182–5.

Menaker M, Roberts R, Elliott J, Underwood H. 1970. Extraretinal light perception in the sparrow. 3. The eyes do not participate in photoperiodic photoreception. Proc Natl Acad Sci U S A. 67(1):320–5.

Millar-Craig MW, Bishop CN, Raftery EB. 1978. Circadian variation of blood-pressure. Lancet (London, England). 1(8068):795–7. doi:10.1016/s0140-6736(78)92998-7.

Mintz EM, Phillips NH, Berger RJ. 1998. Daytime melatonin infusions induce sleep in pigeons without altering subsequent amounts of nocturnal sleep. Neurosci Lett. 258(2):61–64. doi:10.1016/S0304-3940(98)00849-0.

Mistlberger RE, Skene DJ. 2004. Social influences on mammalian circadian rhythms: animal and human studies. Biol Rev Camb Philos Soc. 79(3):533–56. doi:10.1017/S1464793103006353.

Mooney R. 2014. Auditory-vocal mirroring in songbirds. Philos Trans R Soc B Biol Sci. 369(1644). doi:10.1098/rstb.2013.0179.

Moore RY. 1969. Visual pathways controlling neuroendocrine function. In: Progress in Endocrinology. Excerpta Medica Amsterdam. p. 490–494.

Moore RY. 1973. Retinohypothalamic projection in mammals: a comparative study. Brain Res. 49(2):403–409. doi:10.1016/0006-8993(73)90431-9.

Moore RY. 1996a. Entrainment pathways and the functional organization of the circadian system. Prog Brain Res. 111:103–19. doi:10.1016/s0079-6123(08)60403-3.

Moore RY. 1996b. Neural control of the pineal gland. Behav Brain Res. 73(1–2):125–30.

Moore RY, Eichler VB. 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 42(1):201–6. doi:10.1016/0006-8993(72)90054-6.

Moore RY, Lenn NJ. 1972. A retinohypothalamic projection in the rat. J Comp Neurol. 146(1):1–14. doi:10.1002/cne.901460102.

Morgan PJ, Barrett P, Howell HE, Helliwell R. 1994. Melatonin receptors: Localization, molecular pharmacology and physiological significance. Neurochem Int.

24(2):101–146. doi:10.1016/0197-0186(94)90100-7.

Naylor E. 1996. Crab Clockwork: The Case for Interactive Circatidal and Circadian Oscillators Controlling Rhythmic Locomotor Activity of Carcinus Maenas. Chronobiol Int. 13(3):153–161. doi:10.3109/07420529609012649.

Nelson DA, Croner LJ. 1991. Song Categories and Their Functions in the Field Sparrow (Spizella pusilla). Auk. 108(1):42–52. doi:10.1093/auk/108.1.42.

Newbury DF, Bonora E, Lamb JA, Fisher SE, Lai CSL, Baird G, Jannoun L, Slonims V, Stott CM, Merricks MJ, et al. 2002. FOXP2 Is Not a Major Susceptibility Gene for Autism or Specific Language Impairment. Am J Hum Genet. 70(5):1318–1327. doi:10.1086/339931.

Nicholas B, Rudrasingham V, Nash S, Kirov G, Owen MJ, Wimpory DC. 2007. Association of Per1 and Npas2 with autistic disorder: Support for the clock genes/social timing hypothesis. Mol Psychiatry. 12(6):581–592. doi:10.1038/sj.mp.4001953.

Nir I, Meir D, Zilber N, Knobler H, Hadjez J, Lerner Y. 1995. Brief report: Circadian melatonin, thyroid-stimulating hormone, prolactin, and cortisol levels in serum of young adults with autism. J Autism Dev Disord. 25(6):641–654. doi:10.1007/BF02178193.

Nivison JJ. 1978. The vocal behavior and displays of the house sparrow, Passer domesticus L., in the United States. Wayne State University.

Nixdorf-Bergweiler BE, Bischof H-J. 2007. A stereotaxic atlas of the brain of the zebra finch, Taeniopygia guttata, with special emphasis on telencephalic visual and song system nuclei in transverse and sagittal sections. Bethesda, MD: National Center for Biotechnology Information (US).

Noakes MA, Campbell MT, Van Hest BJ. 2000. The chicken CLOCK gene maps to chromosome 4. Anim Genet. 31(5):344–344. doi:10.1111/j.1365-2052.2000.00666.pp.x.

Notes and News. 1874. Academy. vi:262.

Nottebohm F. 1981. A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. Science. 214(4527):1368–70.

Nottebohm F, Arnold AP. 1976. Sexual dimorphism in vocal control areas of the songbird brain. Science. 194(4261):211–3. doi:10.1126/science.959852.

Nottebohm F, Nottebohm ME, Crane L. 1986. Developmental and seasonal changes in canary song and their relation to changes in the anatomy of song-control nuclei. Behav Neural Biol. 46(3):445–71. doi:10.1016/s0163-1047(86)90485-1.

Nottebohm F, Nottebohm ME, Crane L a, Wingfield JC. 1987. Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. Behav Neural Biol. 47(2):197–211. doi:10.1016/s0163-1047(87)90327-x.

Nottebohm F, Stokes TM, Leonard CM. 1976. Central control of song in the

canary, Serinus canarius. J Comp Neurol. 165(4):457–86. doi:10.1002/cne.901650405.

Nyce J, Binkley S. 1977. Extraretinal Photoreception in Chickens: Entrainment of the Circadian Locomotor Activity Rhythm. Photochem Photobiol. 25(6):529–531. doi:10.1111/j.1751-1097.1977.tb09123.x.

Odom KJ, Hall ML, Riebel K, Omland KE, Langmore NE. 2014. Female song is widespread and ancestral in songbirds. Nat Commun. 5:1–6. doi:10.1038/ncomms4379.

Odom KJ, Riebel K, Webster M. 2017. Female Bird Song. [accessed 2018 May 4]. http://femalebirdsong.org/.

Ohgushi E, Mori C, Wada K. 2015. Diurnal oscillation of vocal development associated with clustered singing by juvenile songbirds. J Exp Biol. 218(14):2260–2268. doi:10.1242/jeb.115105.

Oishi T, Konishi T. 1978. Effects of photoperiod and temperature on testicular and thyroid activity of the Japanese quail. Gen Comp Endocrinol. 36(2):250–254. doi:10.1016/0016-6480(78)90031-X.

Okano T, Fukada Y. 2001. Photoreception and circadian clock system of the chicken pineal gland. Microsc Res Tech. 53(1):72–80. doi:10.1002/jemt.1070.

Ollason JC, Slater PJB. 1973. Changes in the behaviour of the male zebra finch during a 12-hr day. Anim Behav. 21(1):191–6. doi:10.1016/s0003-3472(73)80059-4.

Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, Ebihara S, Korf H-W, Yoshimura T. 2008. Involvement of thyrotropin in photoperiodic signal transduction in mice. Proc Natl Acad Sci. 105(47):18238–18242. doi:10.1073/pnas.0808952105.

van Oosterhout F, Lucassen EA, Houben T, VanderLeest HT, Antle MC, Meijer JH. 2012. Amplitude of the SCN clock enhanced by the behavioral activity rhythm. PLoS One. 7(6):e39693. doi:10.1371/journal.pone.0039693.

Ord TJ. 2008. Dawn and Dusk "Chorus" in Visually Communicating Jamaican Anole Lizards. Am Nat. 172(4):585–592. doi:10.1086/590960.

Oshima I, Yamada H, Goto M, Sato K, Ebihara S. 1989. Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. J Comp Physiol A. 166(2). doi:10.1007/BF00193466.

Ota N, Gahr M, Soma M. 2015. Tap dancing birds: the multimodal mutual courtship display of males and females in a socially monogamous songbird. Sci Rep. 5(1):16614. doi:10.1038/srep16614.

Paavonen EJ, Nieminen-von Wendt T, Vanhala R, Aronen ET, von Wendt L. 2003. Effectiveness of Melatonin in the Treatment of Sleep Disturbances in Children with Asperger Disorder. J Child Adolesc Psychopharmacol. 13(1):83–95. doi:10.1089/104454603321666225.

Panaitof SC. 2012. A songbird animal model for dissecting the genetic bases of

autism spectrum disorder. Dis Markers. 33(5):241–249. doi:10.3233/DMA-2012-0918.

Partch CL, Green CB, Takahashi JS. 2014. Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 24(2):90–9. doi:10.1016/j.tcb.2013.07.002.

Patrick GTW, Gilbert JA. 1896. Studies from the psychological laboratory of the University of Iowa: On the effects of loss of sleep. Psychol Rev. 3(5):469–483. doi:10.1037/h0075739.

Patterson TL, Petrinovich L. 1979. Field studies of habituation: II. Effect of massed stimulus presentation. J Comp Physiol Psychol. 93(2):351–359. doi:10.1037/h0077546.

Patzold LM, Richdale AL, Tonge BJ. 1998. An investigation into sleep characteristics of children with autism and Asperger's Disorder. J Paediatr Child Health. 34(6):528–33. doi:10.1046/j.1440-1754.1998.00291.x.

Paulose JK, Peters JL, Karaganis SP, Cassone VM. 2009. Pineal melatonin acts as a circadian zeitgeber and growth factor in chick astrocytes. J Pineal Res. 46(3):286–94. doi:10.1111/j.1600-079X.2008.00659.x.

Pavlova D, Pinxten R, Eens M. 2007. Seasonal singing patterns and individual consistency in song activity in female European starlings (Sturnus vulgaris). Behaviour. 144(6):663–680. doi:10.1163/156853907781347835.

Peça J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, Lascola CD, Fu Z, Feng G. 2011. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature. 472(7344):437–442. doi:10.1038/nature09965.

Pendergast JS, Yamazaki S. 2018. The Mysterious Food-Entrainable Oscillator: Insights from Mutant and Engineered Mouse Models. J Biol Rhythms. 33(5):458–474. doi:10.1177/0748730418789043.

Pengra IGG, Marchaterre MA, Bass AH. 2018. FoxP2 Expression in a Highly Vocal Teleost Fish with Comparisons to Tetrapods. Brain Behav Evol. 91(2). doi:10.1159/000487793.

Pérez-Granados C, Osiejuk TS, López-Iborra GM. 2018. Dawn chorus interpretation differs when using songs or calls: the Dupont's Lark Chersophilus duponti case. PeerJ. 6:e5241. doi:10.7717/peerj.5241.

Perfito N. 2010. The reproductive and stress physiology of Zebra Finches in context: Integrating field and laboratory studies. Emu. 110(3):199–208. doi:10.1071/MU09091.

Perfito N, Kwong JMY, Bentley GE, Hau M. 2008. Cue hierarchies and testicular development: is food a more potent stimulus than day length in an opportunistic breeder (Taeniopygia g. guttata)? Horm Behav. 53(4):567–72. doi:10.1016/j.yhbeh.2008.01.002.

Perfito N, Zann RA, Bentley GE, Hau M. 2007. Opportunism at work: Habitat predictability affects reproductive readiness in free-living zebra finches. Funct Ecol. 21(2):291–301. doi:10.1111/j.1365-2435.2006.01237.x.

Perfito N, Zann RA, Hau M, Bentley GE. 2015. Seasonal-like variation in song control system volume of wild zebra finches. J Exp Zool A Ecol Genet Physiol. 323(8):586–91. doi:10.1002/jez.1956.

Petrinovich L, Patterson TL. 1979. Field studies of habituation: I. Effect of reproductive condition, number of trials, and different delay intervals on responses of the white-crowned sparrow. J Comp Physiol Psychol. 93(2):337–350. doi:10.1037/h0077547.

Petrinovich L, Patterson TL. 1980. Field studies of habituation: III. Playback contingent on the response of the white-crowned sparrow. Anim Behav. 28(3):742–751. doi:10.1016/S0003-3472(80)80134-5.

Petrinovich L, Patterson TL. 1981. Field studies of habituation: IV. Sensitization as a function of the distribution and novelty of song playback to white-crowned sparrows. J Comp Physiol Psychol. 95(5):805–812. doi:10.1037/h0077810.

Petrinovich L, Patterson TL. 1982. Field studies of habituation: V. Evidence for a two-factor, dual-process system. J Comp Physiol Psychol. 96(2):284–296. doi:10.1037/h0077868.

Pett JP, Kondoff M, Bordyugov G, Kramer A, Herzel H. 2018. Co-existing feedback loops generate tissue-specific circadian rhythms. Life Sci alliance. 1(3):e201800078. doi:10.26508/lsa.201800078.

Pfenning AR, Hara E, Whitney O, Rivas M V., Wang R, Roulhac PL, Howard JT, Wirthlin M, Lovell P V., Ganapathy G, et al. 2014. Convergent transcriptional specializations in the brains of humans and song-learning birds. Science (80-). 346(6215). doi:10.1126/science.1256846.

Phillips NH, Berger RJ. 1992. Melatonin infusions restore sleep suppressed by continuous bright light in pigeons. Neurosci Lett. 145(2):217–20.

Piersma T, Jukema J. 2002. Contrast in adaptive mass gains: Eurasian golden plovers store fat before midwinter and protein before prebreeding flight. Proc R Soc London Ser B Biol Sci. 269(1496):1101–1105. doi:10.1098/rspb.2002.1990.

Pittendrigh CS. 1950. The ecotopic specialisation of Anopheles homunculus; and its relation to competition with A.bellator. Evolution (N Y). 4(1):64–78. doi:10.1111/j.1558-5646.1950.tb01383.x.

Pittendrigh CS. 1954. On Temperature Independence in the Clock System Controllnig Emergence Time in Drosophila. Proc Natl Acad Sci. 40(10):1018–1029. doi:10.1073/pnas.40.10.1018.

Pittendrigh CS. 1960. Circadian Rhythms and the Circadian Organization of Living Systems. Cold Spring Harb Symp Quant Biol. 25:159–184. doi:10.1101/SQB.1960.025.01.015.

Pittendrigh CS. 1981. Circadian Systems: Entrainment. In: Biological Rhythms. Vol. 174. Boston, MA: Springer US. p. 95–124.

Pittendrigh CS. 1993. Temporal organization: reflections of a Darwinian clock-

watcher. Annu Rev Physiol. 55:16–54. doi:10.1146/annurev.ph.55.030193.000313.

Pittendrigh CS, Daan S. 1976a. A functional analysis of circadian pacemakers in nocturnal rodents. I. The stability and lability of spontaneous frequency. J Comp Physiol ? A. 106(3):223–252. doi:10.1007/BF01417856.

Pittendrigh CS, Daan S. 1976b. A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock. J Comp Physiol ? A. 106(3):291–331. doi:10.1007/BF01417859.

Pittendrigh CS, Minis DH. 1964. The Entrainment of Circadian Oscillations by Light and Their Role as Photoperiodic Clocks. Am Nat. 98(902):261. doi:10.1086/282327.

Poesel A, Kunc HP, Foerster K, Johnsen A, Kempenaers B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in blue tits, Cyanistes (formerly Parus) caeruleus. Anim Behav. 72(3):531–538. doi:10.1016/j.anbehav.2005.10.022.

Poirel V., Boggio V, Dardente H, Pevet P, Masson-Pevet M, Gauer F. 2003. Contrary to other non-photic cues, acute melatonin injection does not induce immediate changes of clock gene mrna expression in the rat suprachiasmatic nuclei. Neuroscience. 120(3):745–755. doi:10.1016/S0306-4522(03)00344-0.

Pradhan DS, Newman AEM, Wacker DW, Wingfield JC, Schlinger BA, Soma KK. 2010. Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. Horm Behav. 57(4–5):381–389. doi:10.1016/j.yhbeh.2010.01.008.

Ralph CL, Binkley S, MacBride SE, Klein DC. 1975. Regulation of pineal rhythms in chickens: effects of blinding, constant light, constant dark, and superior cervical ganglionectomy. Endocrinology. 97(6):1373–8. doi:10.1210/endo-97-6-1373.

Ralph M, Foster R, Davis F, Menaker M. 1990. Transplanted suprachiasmatic nucleus determines circadian period. Science (80-). 247(4945):975–978. doi:10.1126/science.2305266.

Ralph MR, Menaker M. 1988. A mutation of the circadian system in golden hamsters. Science. 241(4870):1225–7.

Rani S, Kumar V. 2013. Avian circannual systems: persistence and sex differences. Gen Comp Endocrinol. 190(2013):61–7. doi:10.1016/j.ygcen.2013.04.013.

Rashotte ME, Sedunova E V, Johnson F, Pastukhov IF. 2001. Influence of food and water availability on undirected singing and energetic status in adult male zebra finches (Taeniopygia guttata). Physiol Behav. 74(4–5):533–41. doi:10.1016/s0031-9384(01)00600-x.

Rasika S, Nottebohm F, Alvarez-Buylla A. 1994. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. Proc Natl Acad Sci U S A. 91(17):7854–8. doi:10.1073/pnas.91.17.7854.

Rattenborg NC, Amlaner CJ, Lima SL. 2000. Behavioral, neurophysiological and

evolutionary perspectives on unihemispheric sleep. Neurosci Biobehav Rev. 24(8):817–42.

Rattenborg NC, Mandt BH, Obermeyer WH, Winsauer PJ, Huber R, Wikelski M, Benca RM. 2004. Migratory sleeplessness in the white-crowned sparrow (Zonotrichia leucophrys gambelii). PLoS Biol. 2(7):E212. doi:10.1371/journal.pbio.0020212.

Rattenborg NC, Voirin B, Cruz SM, Tisdale R, Dell'Omo G, Lipp H-P, Wikelski M, Vyssotski AL. 2016. Evidence that birds sleep in mid-flight. Nat Commun. 7(1):12468. doi:10.1038/ncomms12468.

Reebs SG. 1989. Acoustical Entrainment of Circadian Activity Rhythms in House Sparrows: Constant Light is not Necessary. Ethology. 80(1–4):172–181. doi:10.1111/j.1439-0310.1989.tb00737.x.

Refinetti R. 2010. The circadian rhythm of body temperature. Front Biosci (Landmark Ed. 15(1):564–94. doi:10.2741/3634.

Reiter RJ. 1974. Influence of pinealectomy on the breeding capability of hamsters maintained under natural photoperiodic and temperature conditions. Neuroendocrinology. 13(6):366–70. doi:10.1159/000122222.

Reiter RJ. 1975. Exogenous and endogenous control of the annual reproductive cycle in the male golden hamster: participation of the pineal gland. J Exp Zool. 191(1):111–20. doi:10.1002/jez.1401910111.

Reppert SM. 1997. Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. J Biol Rhythms. 12(6):528–31. doi:10.1177/074873049701200606.

Reppert SM, Weaver DR, Cassone VM, Godson C, Kolakowski LF. 1995. Melatonin receptors are for the birds: Molecular analysis of two receptor subtypes differentially expressed in chick brain. Neuron. 15(5):1003–1015. doi:10.1016/0896-6273(95)90090-X.

Richdale AL. 1999. Sleep problems in autism: prevalence, cause, and intervention. Dev Med Child Neurol. 41(1):60–6. doi:10.1017/s0012162299000122.

Richdale AL, Prior MR. 1995. The sleep/wake rhythm in children with autism. Eur Child Adolesc Psychiatry. 4(3):175–86. doi:10.1007/BF01980456.

Richter CP. 1922. A behavioristic study of the activity of the rat. In: Hunter WS, editor. Comparative Psychology Monographs. Baltimore, MD: Williams & Wilkins Company. p. 1–55.

Richter CP. 1965. Biological Clocks in Medicine and Psychiatry. Springfield, IL: Charles C Thomas.

Riebel K. 2003. The "Mute" Sex Revisited: Vocal Production and Perception Learning in Female Songbirds. In: Slater PJB, Rosenblatt JS, Snowdon CT, Roper TJ, Naguib M, editors. Advances in the Study of Behavior. Vol. 33. Elsevier Academic Press. p. 49–86.

Riebel K, Odom KJ, Langmore NE, Hall ML. 2019. New insights from female bird song: towards an integrated approach to studying male and female communication roles. Biol Lett. 15(4):20190059. doi:10.1098/rsbl.2019.0059.

Rijo-Ferreira F, Takahashi JS. 2019. Genomics of circadian rhythms in health and disease. Genome Med. 11(1):1–16. doi:10.1186/s13073-019-0704-0.

Rivkees SA, Cassone VM, Weaver DR, Reppert SM. 1989. Melatonin receptors in chick brain: characterization and localization. Endocrinology. 125(1):363–8. doi:10.1210/endo-125-1-363.

Rivkees SA, Fox CA, Jacobson CD, Reppert SM. 1988. Anatomic and functional development of the suprachiasmatic nuclei in the gray short-tailed opossum. J Neurosci. 8(11):4269–76. doi:10.1523/JNEUROSCI.08-11-04269.1988.

Rowan W. 1925. Relation of Light to Bird Migration and Developmental Changes. Nature. 115(2892):494–495. doi:10.1038/115494b0.

Rowan W. 1938. Light and Seasonal Reproduction in Animals. Biol Rev. 13(4):374–401. doi:10.1111/j.1469-185X.1938.tb00523.x.

Saarela S, Reiter RJ. 1994. Function of melatonin in thermoregulatory processes. Life Sci. 54(5):295–311. doi:10.1016/0024-3205(94)00786-1.

Saini C, Liani A, Curie T, Gos P, Kreppel F, Emmenegger Y, Bonacina L, Wolf J-P, Poget Y-A, Franken P, et al. 2013. Real-time recording of circadian liver gene expression in freely moving mice reveals the phase-setting behavior of hepatocyte clocks. Genes Dev. 27(13):1526–1536. doi:10.1101/gad.221374.113.

Sakata JT, Vehrencamp SL. 2012. Integrating perspectives on vocal performance and consistency. J Exp Biol. 215(2):201–209. doi:10.1242/jeb.056911.

Sartor JJ, Balthazart J, Ball GF. 2005. Coordinated and dissociated effects of testosterone on singing behavior and song control nuclei in canaries (Serinus canaria). Horm Behav. 47(4):467–76. doi:10.1016/j.yhbeh.2004.12.004.

Schaap J, Pennartz CMA, Meijer JH. 2003. Electrophysiology of the circadian pacemaker in mammals. Chronobiol Int. 20(2):171–88. doi:10.1081/cbi-120019311.

Scharff C, White SA. 2004. Genetic components of vocal learning. Ann N Y Acad Sci. 1016:325–47. doi:10.1196/annals.1298.032.

Schmidt KL, Pradhan DS, Shah AH, Charlier TD, Chin EH, Soma KK. 2008. Neurosteroids, immunosteroids, and the Balkanization of endocrinology. Gen Comp Endocrinol. 157(3):266–274. doi:10.1016/j.ygcen.2008.03.025.

Schreck KA, Mulick JA. 2000. Parental report of sleep problems in children with autism. J Autism Dev Disord. 30(2):127–35. doi:10.1023/a:1005407622050.

Schwartz WJ, Davidsen LC, Smith CB. 1980. In vivo metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. J Comp Neurol. 189(1):157–167. doi:10.1002/cne.901890109.

Schwartz WJ, Reppert SM, Eagan SM, Moore-Ede MC. 1983. In vivo metabolic activity of the suprachiasmatic nuclei: a comparative study. Brain Res. 274(1):184–187. doi:10.1016/0006-8993(83)90538-3.

Seebohm H. 1888. The geographical distribution of the family Charadriidae, or the plovers, sandpipers, snipes, and their allies. London: H. Sotheran & Co.

Segall LA, Perrin JS, Walker C-D, Stewart J, Amir S. 2006. Glucocorticoid rhythms control the rhythm of expression of the clock protein, Period2, in oval nucleus of the bed nucleus of the stria terminalis and central nucleus of the amygdala in rats. Neuroscience. 140(3):753–7. doi:10.1016/j.neuroscience.2006.03.037.

Shank SS, Margoliash D. 2009. Sleep and sensorimotor integration during early vocal learning in a songbird. Nature. 458(7234):73–7. doi:10.1038/nature07615.

Sharp PJ. 2005. Photoperiodic regulation of seasonal breeding in birds. Ann N Y Acad Sci. 1040(44):189–199. doi:10.1196/annals.1327.024.

Shaw BK. 2000. Involvement of a midbrain vocal nucleus in the production of both the acoustic and postural components of crowing behavior in Japanese quail. J Comp Physiol A. 186(7–8):747–757. doi:10.1007/s003590000128.

Shevchouk OT, Ghorbanpoor S, Ball GF, Cornil CA, Balthazart J. 2017. Testosterone-induced neuroendocrine changes in the medial preoptic area precede song activation and plasticity in song control nuclei of female canaries. Silver R, editor. Eur J Neurosci. 45(7):886–900. doi:10.1111/ejn.13530.

Shibata S. 2004. Neural regulation of the hepatic Circadian rhythm. Anat Rec. 280A(1):901–909. doi:10.1002/ar.a.20095.

Shibata S, Cassone VM, Moore RY. 1989. Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus in vitro. Neurosci Lett. 97(1–2):140–4.

Shimmura T, Nakayama T, Shinomiya A, Fukamachi S, Yasugi M, Watanabe E, Shimo T, Senga T, Nishimura T, Tanaka M, et al. 2017. Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat Commun. 8(1):412. doi:10.1038/s41467-017-00432-8.

Shimmura T, Ohashi S, Yoshimura T. 2015. The highest-ranking rooster has priority to announce the break of dawn. Sci Rep. 5:11683. doi:10.1038/srep11683.

Shimmura T, Tamura M, Ohashi S, Sasaki A, Yamanaka T. 2019. Cholecystokinin induces crowing in chickens. Sci Rep.(February):1–7. doi:10.1038/s41598-019-40746-9.

Shimmura T, Yoshimura T. 2013. Circadian clock determines the timing of rooster crowing. Curr Biol. 23(6):R231–R233. doi:10.1016/j.cub.2013.02.015.

Silverman JL, Yang M, Lord C, Crawley JN. 2010. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci. 11(7):490–502. doi:10.1038/nrn2851.

Simpson HB, Vicario DS. 1990. Brain pathways for learned and unlearned vocalizations differ in zebra finches. J Neurosci. 10(5):1541–56. doi:10.1523/JNEUROSCI.10-05-01541.1990.

Singh D, Rani S, Kumar V. 2013. Daily expression of six clock genes in central and peripheral tissues of a night-migratory songbird: evidence for tissue-specific circadian timing. Chronobiol Int. 30(10):1208–17. doi:10.3109/07420528.2013.810632.

Siopes TD. 1983. Effect of Pinealectomy on Broodiness of Turkey Hens. Poult Sci. 62(11):2245–2248. doi:10.3382/ps.0622245.

Smith GT, Brenowitz E a, Wingfield JC, Baptista LF. 1995. Seasonal changes in song nuclei and song behavior in Gambel's white-crowned sparrows. J Neurobiol. 28(1):114–25. doi:10.1002/neu.480280110.

Smith G Troy, Brenowitz EA, Beecher MD, Wingfield JC. 1997. Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. J Neurosci. 17(15):6001–10. doi:10.1523/JNEUROSCI.17-15-06001.1997.

Smith GT, Brenowitz EA, Prins GS. 1996. Use of PG-21 immunocytochemistry to detect androgen receptors in the songbird brain. J Histochem Cytochem. 44(9):1075–1080. doi:10.1177/44.9.8773574.

Smith G. Troy, Brenowitz EA, Wingfield JC. 1997. Roles of photoperiod and testosterone in seasonal plasticity of the avian song control system. J Neurobiol. 32(4):426–42. doi:10.1002/(sici)1097-4695(199704)32:4<426::aid-neu6>3.0.co;2-8.

Sohrabji F, Nordeen EJ, Nordeen KW. 1990. Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav Neural Biol. 53(1):51–63. doi:10.1016/0163-1047(90)90797-a.

Soma KK, Hartman VN, Wingfield JC, Brenowitz EA. 1999. Seasonal changes in androgen receptor immunoreactivity in the song nucleus HVc of a wild bird. J Comp Neurol. 409(2):224–236. doi:10.1002/(SICI)1096-9861(19990628)409:2<224::AID-CNE4>3.0.CO;2-V.

Soma KK, Sullivan K, Wingfield J. 1999. Combined Aromatase Inhibitor and Antiandrogen Treatment Decreases Territorial Aggression in a Wild Songbird during the Nonbreeding Season. Gen Comp Endocrinol. 115(3):442–453. doi:10.1006/gcen.1999.7334.

Soma M, Mori C. 2015. The Songbird as a Percussionist: Syntactic Rules for Non-Vocal Sound and Song Production in Java Sparrows. Cooper BG, editor. PLoS One. 10(5):e0124876. doi:10.1371/journal.pone.0124876.

Staicer CA, Spector DA, Horn AG. 1996. The dawn chorus and other diel patterns of acoustic signaling. In: Kroodsma DE, Miller EH, editors. Ecology and evolution of acoustic communication in birds. Ithaca, NY: Cornell University Press. p. 426–453.

Stephan FK, Swann JM, Sisk CL. 1979. Anticipation of 24-hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. Behav Neural Biol. 25(3):346–63.

doi:10.1016/s0163-1047(79)90415-1.

Stephan FK, Zucker I. 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A. 69(6):1583–6. doi:10.1073/pnas.69.6.1583.

Stripling R, Milewski L, Kruse AA, Clayton DF. 2003. Rapidly learned song-discrimination without behavioral reinforcement in adult male zebra finches (Taeniopygia guttata). Neurobiol Learn Mem. 79(1):41–50. doi:10.1016/S1074-7427(02)00005-9.

Sturdy CB, Wild JM, Mooney R. 2003. Respiratory and telencephalic modulation of vocal motor neurons in the zebra finch. J Neurosci. 23(3):1072–1086. doi:10.1523/jneurosci.23-03-01072.2003.

Summers-Smith JD. 2009. House Sparrow. In: del Hoyo J, Elliott A, Christie DA, editors. Handbook of the Birds of the Worlds. Vol. 14. Bush-shrikes to Old World Sparrows. Barcelona: Lynx Edicions. p. 793–794.

Surbhi, Kumari Y, Rani S, Tsutsui K, Kumar V. 2015. Duration of melatonin regulates seasonal plasticity in subtropical Indian weaver bird, Ploceus philippinus. Gen Comp Endocrinol. 220:46–54. doi:10.1016/j.ygcen.2014.06.004.

Szymanski JS. 1918. Die Verteilung von Ruhe- und Aktivitätsperioden bei einigen Tierarten. Pflugers Arch Gesamte Physiol Menschen Tiere. 172(1):430–448. doi:10.1007/BF01722010.

Tahara Y, Kuroda H, Saito K, Nakajima Y, Kubo Y, Ohnishi N, Seo Y, Otsuka M, Fuse Y, Ohura Y, et al. 2012. In vivo monitoring of peripheral circadian clocks in the mouse. Curr Biol. 22(11):1029–34. doi:10.1016/j.cub.2012.04.009.

Takahashi JS, Menaker M. 1982. Role of the suprachiasmatic nuclei in the circadian system of the house sparrow, Passer domesticus. J Neurosci. 2(6):815–28.

Tamarkin L, Hollister C, Lefebvre N, Goldman B. 1977. Melatonin induction of gonadal quiescence in pinealectomized Syrian hamsters. Science (80-). 198(4320):953–955. doi:10.1126/science.563102.

Tessmar-Raible K, Raible F, Arboleda E. 2011. Another place, another timer: Marine species and the rhythms of life. Bioessays. 33(3):165–72. doi:10.1002/bies.201000096.

Thomas RJ, Székely T, Cuthill IC, Harper DGC, Newson SE, Frayling TD, Wallis PD. 2002. Eye size in birds and the timing of song at dawn. Proc R Soc B Biol Sci. 269(1493):831–837. doi:10.1098/rspb.2001.1941.

Tobari Y, Nakamura KZ, Okanoya K. 2005. Sex differences in the telencephalic song control circuitry in Bengalese finches (Lonchura striata var. domestica). Zoolog Sci. 22(10):1089–1094. doi:10.2108/zsj.22.1089.

Tordjman S, Anderson GM, Pichard N, Charbuy H, Touitou Y. 2005. Nocturnal excretion of 6-sulphatoxymelatonin in children and adolescents with autistic disorder.

Biol Psychiatry. 57(2):134–138. doi:10.1016/j.biopsych.2004.11.003.

Tordjman S, Davlantis KS, Georgieff N, Geoffray M-M, Speranza M, Anderson GM, Xavier J, Botbol M, Oriol C, Bellissant E, et al. 2015. Autism as a Disorder of Biological and Behavioral Rhythms: Toward New Therapeutic Perspectives. Front Pediatr. 3(FEB). doi:10.3389/fped.2015.00001.

Tramontin AD, Brenowitz EA. 1999. A field study of seasonal neuronal incorporation into the song control system of a songbird that lacks adult song learning. J Neurobiol. 40(3):316–326. doi:10.1002/(SICI)1097-4695(19990905)40:3<316::AID-NEU4>3.0.CO;2-S.

Tramontin AD, Hartman VN, Brenowitz EA. 2000. Breeding conditions induce rapid and sequential growth in adult avian song control circuits: a model of seasonal plasticity in the brain. J Neurosci. 20(2):854–61. doi:10.1523/JNEUROSCI.20-02-00854.2000.

Tramontin AD, Perfito N, Wingfield JC, Brenowitz EA. 2001. Seasonal Growth of Song Control Nuclei Precedes Seasonal Reproductive Development in Wild Adult Song Sparrows. Gen Comp Endocrinol. 122(1):1–9. doi:10.1006/gcen.2000.7597.

Tramontin AD, Wingfield JC, Brenowitz EA. 1999. Contributions of social cues and photoperiod to seasonal plasticity in the adult avian song control system. J Neurosci. 19(1):476–83. doi:10.1523/JNEUROSCI.19-01-00476.1999.

Travnickova-Bendova Z, Cermakian N, Reppert SM, Sassone-Corsi P. 2002. Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. Proc Natl Acad Sci U S A. 99(11):7728–33. doi:10.1073/pnas.102075599.

Trivedi AK, Kumar J, Rani S, Kumar V. 2014. Annual life history-dependent gene expression in the hypothalamus and liver of a migratory songbird: insights into the molecular regulation of seasonal metabolism. J Biol Rhythms. 29(5):332–45. doi:10.1177/0748730414549766.

Trivedi AK, Malik S, Rani S, Kumar V. 2016. Pinealectomy abolishes circadian behavior and interferes with circadian clock gene oscillations in brain and liver but not retina in a migratory songbird. Physiol Behav. 156:156–163. doi:10.1016/j.physbeh.2016.01.019.

Tsutsui K, Inoue K, Miyabara H, Suzuki S, Ogura Y, Haraguchi S. 2008. 7Alpha-hydroxypregnenolone mediates melatonin action underlying diurnal locomotor rhythms. J Neurosci. 28(9):2158–67. doi:10.1523/JNEUROSCI.3562-07.2008.

Ullrich R, Norton P, Scharff C. 2016. Waltzing Taeniopygia: Integration of courtship song and dance in the domesticated Australian zebra finch. Anim Behav. 112:285–300. doi:10.1016/j.anbehav.2015.11.012.

Ulltang M. 2018. The effect of foraging ecology on the onset of dawn song for passerine species. University of Bergen.

Underwood H. 1994. The circadian rhythm of thermoregulation in Japanese quail.

J Comp Physiol A. 175(5):639–653. doi:10.1007/BF00199485.

Underwood H, Barrett RK, Siopes T. 1990. The Quail's Eye: A Biological Clock. J Biol Rhythms. 5(3):257–265. doi:10.1177/074873049000500307.

Underwood H, Siopes T. 1984. Circadian organization in Japanese quail. J Exp Zool. 232(3):557–66. doi:10.1002/jez.1402320323.

Underwood H, Steele CT, Zivkovic B. 2001. Circadian organization and the role of the pineal in birds. Microsc Res Tech. 53(1):48–62. doi:10.1002/jemt.1068.

Vanson A, Arnold AP, Schlinger BA. 1996. 3β-Hydroxysteroid Dehydrogenase/Isomerase and Aromatase Activity in Primary Cultures of Developing Zebra Finch Telencephalon: Dehydroepiandrosterone as Substrate for Synthesis of Androstenedione and Estrogens. Gen Comp Endocrinol. 102(3):342–350. doi:10.1006/gcen.1996.0077.

Verner J, Milligan MM. 1971. Responses of Male White-Crowned Sparrows to Playback of Recorded Songs. Condor. 73(1):56–64. doi:10.2307/1366124.

Vitaterna MH, King DP, Chang a M, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS. 1994. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science. 264(5159):719–25.

Voigt C, Leitner S. 2008. Seasonality in song behaviour revisited: Seasonal and annual variants and invariants in the song of the domesticated canary (Serinus canaria). Horm Behav. 54(3):373–378. doi:10.1016/j.yhbeh.2008.05.001.

Voigt C, Leitner S, Gahr M. 2001. Seasonal Changes in the Song Pattern of the Non-Domesticated Island Canary (Serinus Canaria) a Field Study. Behaviour. 138(7):885–904. doi:10.1163/156853901753172700.

Waas JR, Colgan PW, Boag PT. 2005. Playback of colony sound alters the breeding schedule and clutch size in zebra finch (Taeniopygia guttata) colonies. Proc R Soc B Biol Sci. 272(1561):383–388. doi:10.1098/rspb.2004.2949.

Wada M. 1983. Environmental Cycles, Circadian Clock, and Androgen-dependent Behavior in Birds. In: Mikami S, Homma K, Wada M, editors. Avian Endocrinology: Environmental and Ecological Perspectives. Tokyo: Japan Scientific Societies Press Tokyo. p. 191–200.

Wada M. 1993. Low temperature and short days together induce thyroid activation and suppression of LH release in Japanese quail. Gen Comp Endocrinol. 90(3):355–63. doi:10.1006/gcen.1993.1091.

Wagner HO. 1930. Uber Jahres- und Tagesrhythmus bei Zugvogeln. Z Vgl Physiol. 12(3–4):703–724. doi:10.1007/BF00337903.

Wan Q, Man HY, Liu F, Braunton J, Niznik HB, Pang SF, Brown GM, Wang YT. 1999. Differential modulation of GABA(A) receptor function by Mel(1a) and Mel(1b) receptors [1]. Nat Neurosci. 2(5):401–403. doi:10.1038/8062.

Wang G, Harpole CE, Paulose JK, Cassone VM. 2014. The role of the pineal gland in the photoperiodic control of bird song frequency and repertoire in the house sparrow, Passer domesticus. Horm Behav. 65(4):372–9. doi:10.1016/j.yhbeh.2014.02.008.

Wang G, Harpole CE, Trivedi AK, Cassone VM. 2012. Circadian regulation of bird song, call, and locomotor behavior by pineal melatonin in the zebra finch. J Biol Rhythms. 27(2):145–55. doi:10.1177/0748730411435965.

Ward BC, Nordeen EJ, Nordeen KW. 2001. Anatomical and ontogenetic factors producing variation in HVc neuron number in zebra finches. Brain Res. 904(2):318–26. doi:10.1016/s0006-8993(01)02488-x.

Warren WS, Champney TH, Cassone VM. 1994. The suprachiasmatic nucleus controls the circadian rhythm of heart rate via the sympathetic nervous system. Physiol Behav. 55(6):1091–9.

Weaver DR. 1998. The suprachiasmatic nucleus: a 25-year retrospective. J Biol Rhythms. 13(2):100–12. doi:10.1177/074873098128999952.

Whitfield-Rucker M, Cassone VM. 2000. Photoperiodic regulation of the male house sparrow song control system: gonadal dependent and independent mechanisms. Gen Comp Endocrinol. 118(1):173–83. doi:10.1006/gcen.2000.7455.

Whitfield-Rucker MG, Cassone VM. 1996. Melatonin binding in the house sparrow song control system: sexual dimorphism and the effect of photoperiod. Horm Behav. 30(4):528–37. doi:10.1006/hbeh.1996.0056.

Wild JM, Botelho JF. 2015. Involvement of the avian song system in reproductive behaviour. Biol Lett. 11(12):20150773. doi:10.1098/rsbl.2015.0773.

Will RL. 1973. Breeding Success, Numbers, and Movements of House Sparrows at McLeansboro, Illinois. Ornithol Monogr.(14):60–78. doi:10.2307/40168058.

Williams TD. 2012. Hormones, life-history, and phenotypic variation: Opportunities in evolutionary avian endocrinology. Gen Comp Endocrinol. 176(3):286–295. doi:10.1016/j.ygcen.2011.11.028.

Wingfield JC. 2008. Organization of vertebrate annual cycles: implications for control mechanisms. Philos Trans R Soc B Biol Sci. 363(1490):425–441. doi:10.1098/rstb.2007.2149.

Wingfield JC, Farner DS. 1978. The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, Zonotrichia leucophrys gambelii. Biol Reprod. 19(5):1046–56. doi:10.1095/biolreprod19.5.1046.

Wingfield JC, Hahn TP, Wada M, Astheimer LB, Schoech S. 1996. Interrelationship of Day Length and Temperature on the Control of Gonadal Development, Body Mass, and Fat Score in White-Crowned Sparrows,Zonotrichia leucophrys gambelii. Gen Comp Endocrinol. 101(3):242–255. doi:10.1006/gcen.1996.0027.

Wirojanan J, Jacquemont S, Diaz R, Bacalman S, Anders TF, Hagerman RJ, Goodlin-Jones BL. 2009. The Efficacy of Melatonin for Sleep Problems in Children with Autism, Fragile X Syndrome, or Autism and Fragile X Syndrome. J Clin Sleep Med. 05(02):145–150. doi:10.5664/jcsm.27443.

Witschi E. 1935. Seasonal Sex Characters in Birds and Their Hormonal Control. Wilson Bull. 47(3):177–188.

Wolff G, Esser K a. 2012. Scheduled exercise phase shifts the circadian clock in skeletal muscle. Med Sci Sports Exerc. 44(9):1663–70. doi:10.1249/MSS.0b013e318255cf4c.

Woller A, Gonze D. 2013. The Bird Circadian Clock: Insights from a Computational Model. J Biol Rhythms. 28(6):390–402. doi:10.1177/0748730413512454.

Wood WE, Osseward PJ, Roseberry TK, Perkel DJ. 2013. A Daily Oscillation in the Fundamental Frequency and Amplitude of Harmonic Syllables of Zebra Finch Song. White SA, editor. PLoS One. 8(12):e82327. doi:10.1371/journal.pone.0082327.

Wright B, Sims D, Smart S, Alwazeer A, Alderson-Day B, Allgar V, Whitton C, Tomlinson H, Bennett S, Jardine J, et al. 2011. Melatonin Versus Placebo in Children with Autism Spectrum Conditions and Severe Sleep Problems Not Amenable to Behaviour Management Strategies: A Randomised Controlled Crossover Trial. J Autism Dev Disord. 41(2):175–184. doi:10.1007/s10803-010-1036-5.

Wright HW. 1913. Morning Awakening and Even-Song: Second Paper. Auk. 30(4):512–537. doi:10.2307/4072050.

Wu WQ, McGoogan JM, Cassone VM. 2000. Circadian Regulation of Visually Evoked Potentials in the Domestic Pigeon, Columba livia. J Biol Rhythms. 15(4):317–328. doi:10.1177/074873000129001422.

Yamamura T, Hirunagi K, Ebihara S, Yoshimura T. 2004. Seasonal Morphological Changes in the Neuro-Glial Interaction between Gonadotropin-Releasing Hormone Nerve Terminals and Glial Endfeet in Japanese Quail. Endocrinology. 145(9):4264–4267. doi:10.1210/en.2004-0366.

Yasuo S, Ebihara S, Yoshimura T. 2004. Circadian expression of clock gene in the optic tectum of Japanese quail. Brain Res. 1005(1–2):193–6. doi:10.1016/j.brainres.2004.01.031.

Yasuo S, Watanabe M, Nakao N, Takagi T, Follett BK, Ebihara S, Yoshimura T. 2005. The reciprocal switching of two thyroid hormone-activating and -inactivating enzyme genes is involved in the photoperiodic gonadal response of Japanese Quail. Endocrinology. 146(6):2551–2554. doi:10.1210/en.2005-0057.

Yasuo S, Yoshimura T. 2009. Comparative analysis of the molecular basis of photoperiodic signal transduction in vertebrates. Integr Comp Biol. 49(5):507–518. doi:10.1093/icb/icp011.

Yasuo S, Yoshimura T, Bartell PA, Iigo M, Makino E, Okabayashi N, Ebihara S. 2002. Effect of melatonin administration on qPer2, qPer3, and qClock gene expression in

the suprachiasmatic nucleus of Japanese quail. Eur J Neurosci. 16(8):1541–6. doi:10.1046/j.1460-9568.2002.02222.x.

Yasuo S, Yoshimura T, Ebihara S, Korf H-W. 2009. Melatonin Transmits Photoperiodic Signals through the MT1 Melatonin Receptor. J Neurosci. 29(9):2885–2889. doi:10.1523/JNEUROSCI.0145-09.2009.

Yokoyama K, Oksche A, Darden T, Farner D. 1978. The sites of encephalic photoreception in photoperiodic induction of the growth of the testes in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Cell Tissue Res. 189(3):441–467. doi:10.1007/BF00209132.

Yoo S-H, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong H-K, Oh WJ, Yoo OJ, et al. 2004. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A. 101(15):5339–46. doi:10.1073/pnas.0308709101.

Yoo S, Kim H, Lee J, Yoo J. 2020. Seasonal and diurnal patterns of population vocal activity in avian brood parasites. Ibis (Lond 1859). 162(3):1001–1011. doi:10.1111/ibi.12741.

Yoshimura T, Suzuki Y, Makino E, Suzuki T, Kuroiwa A, Matsuda Y, Namikawa T, Ebihara S. 2000. Molecular analysis of avian circadian clock genes. Mol Brain Res. 78(1–2):207–215. doi:10.1016/S0169-328X(00)00091-7.

Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S. 2003. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. Nature. 426(6963):178–81. doi:10.1038/nature02117.

Young AM. 1981. Temporal Selection for Communicatory Optimization: The Dawn-Dusk Chorus as an Adaptation in Tropical Cicadas. Am Nat. 117(5):826–829. doi:10.1086/283770.

Yu AC, Margoliash D. 1996. Temporal hierarchical control of singing in birds. Science. 273(5283):1871–5. doi:10.1126/science.273.5283.1871.

Zann RA. 1996. Vocalisations. In: The Zebra Finch: A Synthesis of Field and Laboratory Studies. Oxford, UK: Oxford University Press. p. 197–247.

Zann RA, Morton SR, Jones KR, Burley NT. 1995. The Timing of Breeding by Zebra Finches in Relation to Rainfall in Central Australia. Emu - Austral Ornithol. 95(3):208–222. doi:10.1071/MU9950208.

Zatz M, Mullen DA. 1988. Norepinephrine, acting via adenylate cyclase, inhibits melatonin output but does not phase-shift the pacemaker in cultured chick pineal cells. Brain Res. 450(1–2):137–143. doi:10.1016/0006-8993(88)91553-3.

Zimmerman NH, Menaker M. 1979. The pineal gland: a pacemaker within the circadian system of the house sparrow. Proc Natl Acad Sci. 76(2):999–1003. doi:10.1073/pnas.76.2.999.

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1. Degrees awarded

    1. 2008   B.S. in Biology, University of Kentucky

2. Scholastic honors

   - Dept. of Biology Ribble Mini-Grant (2013)
   - President, Biology Graduate Student Association (2011-2012)
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3. Professional publications

    1. **Harpole, CE**, Miles, MM, and Cassone, VM. (2020). Melatonin Duration Gates Photoperiodic Vocal State Change in a Songbird. Journal of Pineal Research 68 (2): e12625. https://doi.org/10.1111/jpi.12625
    2. Cassone, VM, Paulose, JK, & **Harpole, CE**. (2017). Aging and the Circadian Control of the Gastrointestinal System: From the Brain to the Gut Microbiome (and Back). In S. M. Jazwinski, V. P. Belancio, & S. M. Hill (Eds.), Circadian Rhythms and Their Impact on Aging (Vol. 7, pp. 83–89). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-64543-8
    3. Cassone, V. M., Paulose, J. K., **Harpole, CE**, Li, Y., Whitfield-Rucker, M. (2017). Avian Circadian Organization. In V. Kumar (Ed.), Biological Timekeeping: Clocks, Rhythms and Behaviour (pp. 241–256). New Delhi: Springer India. https://doi.org/10.1007/978-81-322-3688-7_11
    4. Wang G, **Harpole CE**, Paulose J, Cassone VM. 2014. The role of the pineal gland in the photoperiodic control of bird song frequency and repertoire in the house sparrow, Passer domesticus. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2014.02.008
    5. Wang G, **Harpole CE**, Trivedi AK, Cassone VM. 2012. Circadian regulation of bird song, call, and locomotor behavior by pineal melatonin in the zebra finch. J. Biol. Rhythms 27:145–55. https://doi.org/10.1177/0748730411435965
    6. Esser KA, **Harpole CE**, Prins GS, Diamond AM. 2009. Physical activity reduces prostate carcinogenesis in a transgenic model. Prostate 69:1372–7. https://doi.org/10.1002/pros.20987