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Title: A rebound from nighttime singing suppression as a potential mechanism for the avian dawn chorus

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Abstract

Many birds sing intensely at dawn, a phenomenon known as the dawn chorus, yet its underlying causes remain unclear. In particular, because most research to date has investigated the adaptive functions (i.e., ultimate causes) of the dawn chorus in wild birds, its behavioral and physiological mechanisms (i.e., proximate causes) remain largely unexplored. Here, we investigated the mechanisms of the dawn chorus under controlled laboratory conditions using the zebra finch, a well-established animal model. We found that birds became hormonally aroused in darkness well before dawn, and that the suppression of spontaneous singing by darkness enhanced their intrinsic motivation to sing. This elevated motivation subsequently triggered a rebound of intense singing immediately upon an increase in ambient light. Given the simplicity of this "rebound singing" mechanism and its independence from social interactions and non-light environmental cues, we propose that this mechanism may critically contribute to the dawn chorus in many avian species. Additionally, we found that rebound dawn singing accelerates morning changes in song structure, supporting the existing hypothesis that the dawn chorus functions as intensive vocal exercise to rapidly optimize song performance. Thus, birds may increase their singing motivation before dawn to compensate for the overnight lack of vocal exercise and, ultimately, to enhance their reproductive success. Together, our results provide a mechanistic explanation for the dawn chorus and offer insights into its adaptive function.

Significance statement

The intense singing of many bird species in the early morning is known as the dawn chorus and is widely recognized as a prominent biological indicator of daybreak, yet the underlying mechanisms of this phenomenon remain surprisingly poorly understood. We investigated the behavioral and physiological mechanisms of the dawn chorus in captive songbirds and demonstrated that the suppression of spontaneous singing by pre-dawn darkness induces a rebound of intense singing at dawn. We propose that this rebound singing mechanism may broadly underlie the dawn chorus across bird species. We also obtained experimental evidence supporting the hypothesis that the dawn chorus functions as intensive vocal exercise to rapidly optimize song performance after overnight silence.

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Main Text

Introduction

The dawn chorus, the intense singing of many bird species early in the morning, has been recognized since ancient times as one of the most prominent biological indicators of daybreak. It is observed daily during the breeding season across diverse habitats worldwide[1,2], fascinating researchers, birders, and the general public alike. Despite its ubiquity and prominence, however, the underlying causes of this phenomenon remain surprisingly poorly understood. To date, more than ten different hypotheses have been proposed to explain the dawn chorus, but no consensus has yet been reached[1,2], leaving it a major unresolved mystery in the field of animal behavior.

Importantly, the majority of studies regarding the dawn chorus have focused on its adaptive functions, that is, the ultimate causes. Although a comprehensive understanding of animal behavior requires the elucidation of proximate causes, such as behavioral and physiological mechanisms, as well as ultimate causes[3,4], empirical research directly addressing the proximate mechanisms of the dawn chorus is very scarce and primarily observational. In particular, while it has been reported that the timing and intensity of the dawn chorus are associated with various factors, such as melatonin levels, ambient light levels, and the presence of a female mate[1,2,5], it remains largely unclear to what extent and how these factors interact to elicit the dawn chorus. A recent study showed that daytime darkness during a solar eclipse triggers the dawn chorus in many bird species[6], suggesting a critical role of ambient light changes in driving this phenomenon. However, the inherent limitations of solar eclipses make it difficult to investigate the underlying mechanisms in detail.

One major reason why the mechanisms underlying the dawn chorus remain poorly understood is the difficulty of controlling environmental factors and performing detailed behavioral and physiological measurements and manipulations in field settings. On the other hand, in the laboratory, it is challenging to induce birds to exhibit singing behavior that is regarded as equivalent to the dawn chorus observed in the wild. Here, we report that the zebra finch (*Taeniopygia guttata*), a widely used model organism in life science research[7], exhibits dawn singing behavior under laboratory conditions that shares key features with the natural dawn chorus. We then elucidate the mechanisms by which this dawn chorus-like singing arises from the interaction between ambient light conditions and the circadian rhythm of melatonin, independently of social interaction. Furthermore, we investigate the functional role of this behavior by testing the existing hypothesis that the dawn chorus serves as intensive vocal exercise to rapidly optimize song performance[8–10].

Results

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Captive zebra finches exhibit dawn chorus-like intense singing when morning lights-on time is delayed.

Male zebra finches housed alone in sound recording chambers spontaneously produce hundreds of songs per day (referred to as "undirected singing") under light conditions, but virtually no songs under complete darkness[11]. A previous study demonstrated that temporarily suppressing undirected singing by turning off the lights during the daytime induces intense singing immediately after the lights are turned on and the suppression is lifted[12]. Since this suppression-driven intense singing resembles a typical dawn chorus, we hypothesized that this suppression-driven mechanism may underlie the dawn chorus. As the first step to test this idea, we examined whether suppression-driven intense singing occurs even in the morning following the dark period at night. In particular, given that the magnitude of suppression-driven singing strongly depends on the duration of suppression[12],, we predicted that extending or shortening the night period by delaying or advancing the morning lights-on time (LT) relative to the regular LT would enhance or attenuate morning singing activity, respectively (Fig. 1A; the delay and advancement of LT were randomized on a daily basis). Although birds exhibited slight increases in singing rate relative to baseline even after the regular LT (0h LT in Fig. 1A-D & S1), these increases were markedly enhanced when the LT was delayed by 3 hours (+3 h LT in Fig. 1A–D & S1, Movie S1), resulting in intense singing resembling the dawn chorus. In contrast, almost no transient increases in singing rate were observed when the LT was advanced by 3 hours (−3 h LT in Fig. 1A–D, Movie S2). Moreover, birds began singing sooner after the delayed LT than after the advanced LT (Fig. 1E). These results demonstrate that dawn chorus-like intense singing can be induced in socially-isolated songbirds simply by delaying the morning LT.

Birds begin to sing in dimmer light conditions when LT is delayed.

In addition to intense singing in the morning, the early onset of singing under dim light at dawn is another key feature of the dawn chorus in wild birds[1,2]. We found that such early singing onset under dim light conditions can also be induced by delaying the morning LT. We modified the morning lighting paradigm to mimic natural dawn conditions: Instead of the abrupt lighting used in the previous experiment, the light level was gradually increased over a 2-h period (see Methods), and this "gradual lighting period" was either delayed or advanced by 3 h relative to the regular LT (Fig. 2A-B). When the gradual lighting period was delayed by 3 h (+3h LT), birds began singing earlier within the gradual lighting period compared to when the gradual lighting period was advanced by 3 h (-3h LT) (Fig. 2B-D), indicating that singing initiation occurred under lower light levels when the morning LT was delayed. Taken together, these results suggest that delaying the LT can induce both intense singing at dawn and singing onsets under dim light conditions, which are two major features of the dawn chorus.

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Birds wake up well before the delayed LT and their motivation to sing increases while spontaneous singing is being suppressed by darkness.

Why do birds exhibit dawn chorus-like singing when the LT is delayed? Given that a similar pattern of intense singing occurs following temporary singing suppression by lights-out during the daytime[12], we hypothesized that birds are mostly awake during the dark period before the delayed LT and that their motivation to sing increases while singing is being suppressed by darkness, subsequently producing intense singing as a 'rebound' from the suppression. To test this idea, we monitored the birds' movements in darkness before the LT (with the abrupt lighting paradigm described in Fig. 1A) using infrared cameras mounted above individual cages (Fig. 3A-B). We found that birds moved actively during the 3-h period prior to the delayed LT (+3h LT in Fig. 3C-E, Movie S3), but exhibited little movement before the advanced LT (-3h LT in Fig. 3C-E, Movie S4). These results provide evidence that birds are mostly awake during the dark period before the delayed LT but not before the advanced LT.

Although these results suggest higher arousal levels of the birds before the delayed LT, it does not necessarily indicate that they are highly motivated to sing at that time. To assess the singing motivation in the dark period before the LT more directly, we trained the birds to press a lever that triggered a 10-sec period of lighting (Fig. 3F, Movie S5). Since zebra finches sing almost exclusively under light conditions and not in darkness[11], the frequency of lever presses is likely to reflect, at least to some extent, the levels of singing motivation as well as the arousal levels. When the LT was delayed or advanced by 3 h with the abrupt lighting paradigm, birds frequently pressed the lever and sang before the +3h LT, but only rarely before the -3h LT (Fig. 3G-I). These results support the idea that birds are highly motivated to sing in the dark before the delayed LT but not before the advanced LT. Given that suppression of undirected singing by darkness during the daytime increases intrinsic singing motivation and results in intense rebound singing immediately after the suppression is lifted[12], these results provide evidence that dawn chorus-like singing is driven by a rebound mechanism from dark-induced singing suppression before the delayed LT.

We also investigated the hormonal mechanisms underlying early awakening before the delayed LT. In zebra finches, plasma concentrations of melatonin, a hormone that regulates the sleep-wake cycle in vertebrates[13], are relatively high during the night and drop sharply to daytime levels approximately 0.5–2 hours before the regular LT[14]. This pre-dawn drop in melatonin levels roughly coincides with the early awakening of birds before the delayed LT. Based on these findings, we hypothesized that birds wake up well before the delayed LT due to hormonal regulation including the early melatonin drop but are suppressed from singing by darkness, leading to an increase in singing motivation and eventually dawn chorus-like singing immediately after the LT. To test this hypothesis, we systemically administered the selective melatonin receptor antagonist luzindole 5 h before the regular LT and assessed whether this would induce dawn chorus-like singing. We found that birds began singing earlier after luzindole administration compared to saline administration (paired t-test: $t(7) = 2.79$, $p = 0.0269$; Fig. 3J), although

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the initial singing rates following the LT did not significantly differ between the two treatments ($t(7) = 1.162$, $p = 0.284$). These results suggest a key role of the early drop in plasma melatonin levels prior to the LT in inducing dawn chorus-like singing, thus supporting our hypothesis that the early morning awakening that is responsible for dawn chorus-like singing is caused by melatonin-related hormonal regulation.

Captive zebra finches housed under semi-natural conditions exhibit dawn chorus.

Although the suppression-driven rebound singing observed after the delayed LT in socially isolated zebra finches housed under artificial light conditions resembles the dawn chorus that is generally observed in many bird species in the wild, it is unclear whether these two phenomena are based on the same mechanisms. While zebra finches have been extensively studied in laboratory settings for life science research, to our knowledge, no studies have reported the dawn chorus in wild zebra finches.

To investigate whether the dawn chorus-like singing that we observed is closely related to the dawn chorus that is generally observed in the wild, we recorded the vocal activity of captive zebra finches housed with conspecifics in an aviary exposed to natural light through large windows over a 20-day period from mid-September to early December in 2022. In these birds kept under social and natural light conditions (with almost constant temperature and humidity), the onset of their morning vocal activity appeared to vary depending on sunrise time and weather conditions (Fig. 4A), suggesting that light levels in the aviary critically influence morning vocal behavior. To determine whether these birds exhibited dawn chorus-like vocal activity, we accounted for variations in sunrise time and weather conditions by aligning the vocal activity data to a "dawn reference time (DRT)," defined as the time when light levels in the aviary surpassed a predetermined threshold (see Methods). This analysis revealed a marked increase in vocal activity around the DRT, followed by a gradual decline (Fig. 4B–C), resembling the typical dawn chorus observed in wild birds. Furthermore, detailed analysis of this morning vocal activity showed a relationship between dawn timing and the onset of morning vocalizations consistent with our findings in socially isolated birds under artificial lighting (Fig. 1 & 2). Specifically, during the first 10-day recording period, in which both DRT and sunrise occurred relatively early, the average vocal activity tended to have a peak that was later than that during the subsequent 10-day period (Fig. 4B-C). Also, when the DRT occurred later due to seasonal changes and weather conditions, vocal activity around the DRT tended to be greater (Fig. 4D) and to start earlier (Fig. 4E). These findings in the birds under social and natural light conditions are consistent with the earlier onset and greater amplitude of morning singing after delayed LT observed in the birds under socially isolated and artificial light conditions (Fig. 1 & 2), suggesting similar mechanisms of dawn vocal activity in the two conditions. Given these results, we suggest that zebra finches in the wild may produce the dawn chorus through the suppression-driven rebound mechanisms identified in captive birds.

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Dawn singing accelerates morning changes in song structure.

Our results so far have revealed the behavioral and hormonal mechanisms (i.e., the proximate cause) underlying dawn singing: In the birds that woke up well before LT, suppression of spontaneous singing by pre-dawn darkness increases singing motivation, leading to intense rebound singing shortly after the LT. We further investigated why this pre-dawn singing suppression increases singing motivation and induces rebound singing, which is the adaptive function (i.e., the ultimate cause) of dawn singing. Recent studies in wild songbirds have demonstrated that song performance improves through dawn singing in a rate-dependent manner, leading to the "warm-up hypothesis" to explain the functional role of the dawn chorus[8–10]. This hypothesis posits that high-rate dawn singing serves as an intensive vocal exercise that rapidly optimizes song performance to compensate for the lack of singing at night, providing an advantage in mate attraction and territory defense and ultimately increasing reproductive success. Since daily singing of captive adult finches has been repeatedly shown to serve as vocal exercise (or practice) to optimize and maintain song performance[15–22], we wondered if the warm-up hypothesis might also apply to dawn singing in captive zebra finches. To test this, we analyzed possible changes in song structure during morning singing in the songs recorded under the shifted LT conditions shown in Fig. 1, and examined whether intense dawn singing accelerates such morning song changes.

We examined potential changes in morning song structure by comparing each song produced during the first hour after LT with all songs produced during the second hour in each LT condition (see Methods). Comparisons were made for each song syllable (n = 18 different syllables from 6 birds) by calculating mean acoustic distances in acoustic feature space (cosine distance, see Methods). In both the -3h LT and +3h LT conditions, many syllables showed gradual decreases in acoustic distance during the first hour (i.e., negative slopes of the acoustic distance trajectories; Fig. 5A-B), indicating substantial and monotonic changes in acoustic structure during morning singing. If these song changes were caused by the act of singing rather than simply by the passage of time, the speed of the song changes should depend on the singing rate during that period. Consistent with this prediction, we found that the slopes of song changes over the first 30 min were significantly steeper in the +3h LT condition, where intense dawn singing occurred, than in the -3h LT condition (Fig. 5A-B and Table S1). Moreover, the slopes were significantly correlated with normalized singing rate during the same period (the number of syllable renditions in the first 30-min period normalized to that in the first 2-h period) (Fig. 5C and S2). These results suggest that morning song structure changed as a function of recent singing activity and that such changes were accelerated by intense dawn singing. This conclusion was further supported by the findings that, when acoustic distances were plotted as a function of syllable order instead of time, there was no significant difference in the slopes between the -3h and +3h LT conditions (Fig. 5D-E and Table S2). In

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summary, these findings are consistent with the warm-up hypothesis that dawn singing functions as an intensive vocal exercise that rapidly optimizes song performance following overnight silence.

Discussion

In contrast to the extensive research on the adaptive functions of the dawn chorus, studies on its underlying mechanisms have been limited and primarily observational[1,2,5]. Our findings of suppression-driven rebound singing provide experimental evidence that directly explains the behavioral and physiological mechanisms of the dawn chorus in zebra finches. Specifically, we show that birds wake up in the dark well before dawn, likely mediated by hormonal mechanisms involving melatonin, and their intrinsic motivation to sing increases while spontaneous singing is suppressed by darkness. When ambient light levels increase at dawn, this suppression is lifted, triggering rebound singing characterized by an early singing onset and a high singing rate, the two defining features of the dawn chorus. Importantly, these rebound mechanisms operate independently of social interactions and environmental cues other than ambient light. On this basis, we propose that similar mechanisms may critically contribute to the dawn chorus across many avian species, although its expression is likely to be shaped by species-specific ecological and social factors.

Our findings of suppression-driven rebound singing in captive zebra finches are remarkably consistent with the recent report of dawn chorus-like vocal behavior observed in many species of wild birds after the daytime darkness caused by a solar eclipse[6]. Both behaviors are caused solely by changes in ambient light levels and are likely independent of other factors previously hypothesized to contribute to the dawn chorus, such as non-light environmental conditions, social interactions with conspecifics, predation risk, food availability, and the time of day[1,2,5]. This consistency between the two studies strongly supports the idea that rebound singing is the fundamental mechanism underlying the dawn chorus observed across many bird species. Although the solar eclipse produced relatively short darkness (totality lasted for about 4 minutes) compared to the darkness that induces rebound singing in captive zebra finches[12], this difference is likely attributable to interspecies differences in baseline singing motivation and its enhancement by darkness, as evidenced by the varying effects of solar eclipses on vocalization rates among different species[6].

A key aspect of the rebound singing mechanisms is the time lag between when birds wake up and when light intensity rises (the LT). During this time lag, birds are awake but suppressed from singing by darkness and their intrinsic singing motivation appears to increase depending on the duration of the time lag, just as in birds that are suppressed from singing by lights-out during daytime[12]. This suppression-dependent increase in singing motivation is reminiscent of and consistent with the so-called "Lorentz's psychohydraulic model", in which a reservoir of motivational impulse builds up and is ultimately released

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by appropriate environmental stimuli[23]. Although mechanisms underlying this motivational increase remain largely unclear, a brain region and neuromodulators related to singing motivation have recently been reported[12,24,25]. On the other hand, the mechanisms by which birds awaken before the LT likely involve hormonal regulation including a drop in plasma melatonin levels from early night levels[14], which is caused by a circadian clock even in the absence of light inputs[26,27]. A similar trend of decreased melatonin levels during the late night phase has been reported in songbirds other than zebra finches, although the timing of those changes is unclear[28–31]. Also, in wild songbirds, nighttime melatonin concentrations are positively associated with the onset time of early morning behaviors including dawn singing[30,32]. Thus, it is reasonable to assume that the rebound mechanisms revealed in captive zebra finches are applicable to the dawn chorus in other songbird species. Whereas the circadian secretion of melatonin has been hypothesized to regulate the timing of the dawn chorus[1,2], a number of studies have suggested that the dawn chorus timing is strongly influenced by light inputs to the birds, such as intensity of morning light and the size of birds' eyes[33–36]. Our findings reconcile these seemingly contradictory ideas and provide a clear explanation of the mechanisms by which the circadian regulation of melatonin and light inputs interact to determine the timing of the dawn chorus as well as its magnitude.

In addition to addressing the proximate mechanisms of the dawn chorus, our results provide insight into its potential functional significance. The acceleration of morning song changes by intense dawn singing is consistent with the warm-up hypothesis previously proposed for wild birds[8–10]. This hypothesis posits that dawn singing serves as intensive vocal exercise that rapidly optimizes song performance to compensate for the overnight lack of singing, ultimately enhancing reproductive success. Importantly, our data do not directly address whether such structural changes are functionally adaptive or preferred by females. Since our analysis focused on changes in song structure rather than performance evaluation or reproductive outcomes, further research is necessary to demonstrate whether these changes constitute improvements with functional consequences in zebra finches. Nevertheless, several previous studies support the notion of song improvement. For example, in juvenile zebra finches in the process of song development, song structure deteriorates overnight and then dramatically improves (i.e., approaches to their final adult song) through morning singing[37]. Also, even in adult zebra finches, which normally produce songs with stable overall structure, song performance defined as the degree of female's preference to that song significantly decreases by prolonged suppression of daily singing and gradually recovers after singing is resumed[15,16]. Thus, it is reasonable to suggest that our results of dawn chorus-dependent song changes represent improvement in song structure. Moreover, based on the studies examining the recovery of adult song after suppression-induced deterioration[15,16], it is likely that dawn chorus-dependent song improvement is caused largely by vocal exercise and resulting physiological changes in the muscles of the vocal system, rather than auditory feedback-dependent adjustments of motor circuits. Given that the rebound singing mechanism revealed here also is independent of auditory

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feedback, these functional and mechanistic hypotheses could, in theory, explain the dawn chorus not only in oscine birds, which develop their songs through audition-dependent learning, but also in suboscine birds and other avian species that exhibit the dawn chorus with innate vocalizations.

While the rebound singing hypothesis combined with the warm-up hypothesis offers a novel perspective on the mechanisms and functions of dawn chorus, it does not necessarily contradict the existing hypotheses about dawn chorus. In fact, the rebound singing and warm-up hypotheses can explain many dawn chorus-related findings that support the existing hypotheses. For example, previous studies have reported that the extent of the dawn chorus (e.g., singing duration and timing of the first song) is associated with specific reproductive stages such as the female fertility period and also correlated positively with reproductive success (see[1,2] for review). Based on these findings, it has been proposed that the dawn chorus represents an honest signal of male quality[38–40] and directly regulates female reproductive behavior around dawn[41,42]. The warm-up hypothesis states that the dawn chorus functions to rapidly optimize the quality of song performance, which is critical for mate attraction when produced in courtship context. Thus, it is possible that birds exhibit greater dawn chorus at the female fertility period in order to optimize song performance as early as possible in the morning and to maximize their mating success. The rapid song optimization by the dawn chorus could also be advantageous in territory defense for many territorial species. Although they need to defend their territories throughout the day, they may rapidly optimize their song performance through the dawn chorus early in the morning to prepare for the territory invasion by other birds. This view is consistent with a previous study that the extent of the dawn chorus is affected by territorial intruders[43] and also explains the fact that many songbird species show the dawn chorus even after the female fertility period. Additionally, because birds exhibiting more intense dawn chorus are likely to have higher motivation, not only for singing, but also for other reproductive behaviors, such higher motivation for non-singing behaviors could also contribute to higher reproductive success. Moreover, our rebound singing hypothesis aligns with the unpredictable overnight-condition hypothesis as well, which proposes that birds sing intensely at dawn to expend the surplus of energy stored in preparation for unpredictable overnight conditions[44]. This hypothesis is supported by findings that greater food availability leads to a more pronounced dawn chorus[45–47]. Since food availability positively affects birds' nutritional state and overall singing motivation, as shown in captive zebra finches[24], sufficient food availability could amplify the dawn chorus by elevating overall singing motivation and enhancing suppression-driven rebound singing.

Given that our experiments were conducted under laboratory conditions and considering the unique characteristics of the zebra finch as a non-territorial and opportunistic breeder, further research is needed to determine whether the rebound-singing and warm-up hypotheses apply to the dawn chorus in many other songbird species. In particular, although territorial birds may also exhibit the dawn chorus through similar rebound mechanisms, its primary function may involve re-establishing territory

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boundaries and social relationships with neighbors[48], either in addition to or instead of the rapid optimization of song performance. Because it is often difficult to determine whether wild birds are singing for vocal exercise and/or for other purposes such as territory defense and social interactions, clarifying the specific functional roles of the dawn chorus remains challenging in the field. In contrast, the behavioral mechanisms underlying the dawn chorus, including rebound singing, are more readily testable. Even in field settings, the relationship between the intensity of the dawn chorus and the time of dawn could be examined, as in our experiments with captive zebra finches under natural light conditions (Fig. 4). Furthermore, investigating when birds wake up before dawn and whether this timing correlates with subsequent singing intensity would be essential. Such studies will help elucidate the universal mechanisms driving the dawn chorus and potentially reveal broader principles of avian biology related to singing behavior.

Materials and Methods

Subjects

The subjects used for song recording under socially isolated and artificial light conditions were adult male zebra finches (*Taeniopygia guttata*, 87–260 dph) bred in the songbird facility at the Korea Brain Research Institute (KBRI). They were raised with their parents and siblings until ~60 dph and then housed with their siblings and/or other males conspecifics until the experiments started. Their care and treatment were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the KBRI. The subjects used for recording vocal activity under social and natural light conditions were adult male and female zebra finches bred in the songbird facility at the Teikyo University. Their care and treatment were reviewed and approved by the Animal Care and Use Committee there. All experimental procedures were carried out in compliance with the ARRIVE guidelines[49].

Song recording

For the experiments with song recording under socially isolated and artificial light conditions, each bird was housed in a sound-attenuating and light-proof chamber (MC-050, Muromachi Kikai, or custom-made chambers) throughout the experiment. Songs were recorded using a microphone (PRO35, Audio-Technica) positioned above the cage and a custom-written song recording program (tRec, R. O. Tachibana). Output from the microphone was amplified by a mixer (402-VLZ4, Mackie) and digitized via an audio interface (Octa-Capture UA-1010, Roland) at 44.1-kHz (16-bit). Recorded signals were down-sampled to a sampling rate of 32-kHz. Recording was triggered if the program detected 3 or 4 consecutive sound notes, each of which was defined based on sound amplitude, duration, and intervening gap duration; those parameters for triggering sound recording were optimized for each bird so as to record all

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songs produced. Each recording ended if a silent period lasted longer than 0.5 sec (i.e. each song file contains a single "song bout" that is separated from other bouts by >0.5-sec silent periods). Songs were recorded throughout the day, and all song recordings were of undirected song (i.e. no female was present). Birds with sufficient singing rates (>300 song bouts per day) were used for the experiments. The procedures to record vocal activity of the birds housed under social and natural light conditions and to analyze the recorded vocal activity are described below.

Manipulation of light-dark schedule and illumination level

Birds for the experiments under socially isolated and artificial light conditions (Figs 1-3) were bred and kept on a constant 14:10hr light:dark (LD) cycle (with abrupt light-dark transitions) in the bird colony and after being transferred to the sound-attenuating chambers for > 5 days until the experiments began. During the experiments, we used two different lighting protocols. For the "abrupt lighting" experiment, the time of abrupt dark-light transition (the lights-on time, LT) was shifted daily ±3-h from the original 14:10hr LD cycle; birds underwent the -3hr, 0hr, and +3h LT conditions in a randomized order (neither the -3h LT nor the +3h LT condition was administered on consecutive days to prevent the birds from adapting to the LT condition). Each condition was administered multiple times (ranging from 4 to 8 trials). For the "gradual lighting" experiment, the illumination level was exponentially increased with 12 steps over 2 hr (10-min period for each step) using LED lights with an Arduino-based control unit; the illumination level of the 12^{th} step was approx. 350 lux, and it was followed by a constant illumination level equivalent to the one used for regular light periods (approx. 500 lux). This 2-hr gradual lighting period was shifted daily ±3-h in a randomized order just as for the abrupt lighting experiment. Illuminance was measured at the center of the experimental cages using a light meter (Tenmars, TM-205).

Song analysis

To analyze singing activity recorded in sound-attenuating chambers, we screened all sound files recorded during the time periods of interest to exclude non-song files using a semi-automated method previously described[12]. Briefly, we sorted song files (sound files that include at least one full motif of song) and non-song files by focusing on temporal trajectories of sound amplitude (amplitude envelopes) and those of Weiner entropy, both of which are highly stereotyped across motif renditions and clearly distinct from other sounds including non-song vocalizations. We calculated the maximum correlation coefficients (mCCs) of amplitude envelopes and that of entropy trajectories between a canonical song motif and all sound files recorded, and excluded files that had mCCs for both amplitude envelopes and entropy trajectories below certain thresholds as non-song files. We then visually inspected spectrograms of those excluded files to ensure that no song files were excluded from the song dataset to be analyzed.

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To quantify the magnitude of dawn chorus-like singing activity, we used two measures, the initial singing rate and first song latency[16]. The initial singing rate was measured as the mean singing rate over a 1-hr period starting at the onset of the first song produced in the morning for each trial and averaged across trials with the same LT. The initial singing rate was then normalized to the baseline singing rate, which was cross-trial and cross-LT average of the mean singing rate over the 30-min period starting at 90 min after the morning LT. We also plotted time courses of singing activity by measuring instantaneous singing rates using 3-min bins and averaged across trials with the same LT. The average instantaneous singing rates were then normalized by the baseline singing rates. The first song latency was measured in the abrupt lighting experiments as the time interval from the LT to the onset of the first song recorded in the morning. The latencies were then averaged across trials with the same LT.

To investigate morning changes in song structure, songs recorded in the -3h LT and +3h LT conditions were segmented into syllables using a custom program on Matlab based on the following parameters: amplitude, minimum and maximum syllable duration, and minimum and maximum frequency. These parameters were manually adjusted for each bird. From individual syllables, 8 time-varying acoustic features (centroid, spread, skewness, kurtosis, flatness, slope, pitch, goodness of pitch) and 2 other features (amplitude and duration) were extracted and projected onto a two-dimensional space by dimensionality reduction algorithm t-SNE (t-distributed Stochastic Neighbor Embedding). Syllables were then manually labelled based on clusters formed on the two-dimensional space. All labeled syllables were manually checked and incorrect labels were corrected. For the syllables that had stereotyped temporal structure across renditions, morning changes in acoustic structure were examined in each LT condition by comparing each syllable rendition produced during the first hour after LT with all syllable renditions produced during the second hour (the reference period) and by averaging their acoustic distances. The acoustic distance was quantified as cosine distance computed using the within-syllable mean, standard deviation, and mean absolute derivative of the 8 acoustic features and amplitude described above. The acoustic distances of individual syllable renditions were plotted as a function of time after LT and the local means over a sliding window of ±10 min durations were computed with 1-min increments. Slopes of the local mean changes over the first 30-min period starting at the first rendition were compared between the -3h and +3h LT conditions. The same acoustic distance data were also plotted as a function of syllable order instead of time, and slopes of the local mean changes were compared between the 2 conditions. Additionally, the slopes of acoustic distances were plotted against the number of syllable renditions recorded during the first 30-min period normalized to the number of renditions recorded during the first 2-h period to examine correlations between them.

<u>Recording and analysis of bird movements</u>

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In the birds that underwent the ±3h LT conditions with the abrupt lighting paradigm, their movements in the dark before the LT were monitored using infrared cameras (SQ12, Mrs Win, and UI-3040CP Rev. 2, iDS imaging) positioned on the top of the individual cages. Infrared LED lights were also positioned ~10 cm above the cages and they were on during the night dark period. Each recording session spanned 3 hours, finishing at the LT (-3h, 0h, or +3h LT). To increase the precision of movement tracking, a small fluorescent sticker was affixed on the top of each bird's head. Movements of the birds' heads were tracked using the machine learning software DeepLabCut[50], and their travel distances were calculated over 10-min time bins. Mean travel distances over the 3-h period were calculated for each LT condition and compared across different LT conditions.

<u>Operant lever-pressing task for lighting</u>

To examine the level of motivation in the dark to seek light conditions that allow birds to sing, we trained birds to press a lever that is associated with the activation of ambient light. Birds were kept individually in an experimental cage that was placed in a sound-attenuating and light-proof chamber throughout the experiments. On the ceiling of the chamber, white LED lights were installed in addition to the built-in lights of the chamber. In the cage, a lever switch (Omron Electronics, SS-01GL111-E) was placed on the rear wall near a perch, and a small red LED was also placed on the rear wall above the lever. Throughout the experiment, including day and night, pressing the lever immediately activated the white LED lights for 10 seconds, and the timing of lever pressing by the bird was recorded; the small red LED was always lit so that the lever location could be identified in the dark. Undirected songs were also recorded throughout the experiment using a microphone positioned above the cage. Birds were initially housed in the experimental cage with the lever operational on a regular 14/10 light-dark (LD) cycle for more than 7 days for the purpose of habituating the bird to the experimental apparatus and having them to learn the association between lever pressing and activation of white LED lights. Subsequently, we introduced the behavioral paradigm of shifting the morning LT described above (the "abrupt lighting experiment"): birds underwent -3hr, 0hr, and +3h LT in a randomized order. Each condition was administered multiple times (ranging from 4 to 7 trials). The frequencies of lever pressing and of song bouts over the 3-h period before the LT were averaged across trials and compared across different LT conditions.

<u>Luzindole injections</u>

A potential role of melatonin in morning singing was examined using luzindole (Tocris, 0877), a competitive melatonin MT1/MT2 receptor antagonist[51]. Luzindole was stored as stock solution in DMSO (40 mg/ml) at −20 °C and diluted in PBS before use (2 mg/ml). Birds were kept on a regular 14/10 LD cycle with abrupt light-dark transitions, and injected subcutaneously with luzindole (5.0 mg/kg) or PBS 5

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h before the LT. Each bird received 4 injections for both luzindole and PBS in a randomized order (a single injection per day). Undirected songs were recorded throughout the experiment, and morning singing was analyzed by focusing on the first song latency and initial singing rate. Results were averaged across trials for each bird.

Recording and analysis of vocal activity of the birds under social and natural light conditions

To examine morning vocal activity under social and natural light conditions, audio and video recordings were made in the zebra finch aviary at Teikyo University, which has large glass windows that let in natural light from outdoor, for 20 days intermittently from September 16th to December 2nd, 2022. In the aviary, ~90 zebra finches were housed in 18 cages (8 cages for breeding pairs and 10 cages for adult birds with the same sex) that were placed in a metal rack (4 cages x 3 tiers) near the windows. Audio recordings were made using a microphone (PRO35, Audio-Technica) placed in the middle of the cage rack and connected to an audio interface (Octo-Capture UA-1010, Roland) for digitization at 44.1 kHz (16 bit). To capture vocal sounds, we used a custom-made recording program, which started sound recording when it detected 5 successive sound notes, each of which reached predefined thresholds of amplitude and duration, and ended when it detected a silent period lasted >0.8 sec. This recording system captured mostly vocal sounds (i.e., songs and calls), but non-vocal sounds such as cage noises and wing-flapping sounds were occasionally recorded. To measure vocal activity around dawn, we visually sorted the files that contain vocalizations using Avisoft Saslab (Avisoft Bioacoustics), and calculated the number of the files recorded during 1-min time bins over the time period from 4:00 to 7:30 am. The daily vocal activity data were then aligned relative to the “dawn reference time (DRT)”, which was defined as follows: During the periods of audio recordings, we simultaneously videotaped all cages and windows in the aviary using a wide-angle web camera (BSW200MGK, Buffalo) placed 80 cm away from the cage rack (frame rate, 16 Hz). On the recorded videos, the minute-by-minute changes in luminance for four window locations (10 x 10 pixels) were measured using Fiji (ImageJ, version 1.54f[52]). For each day, the time point when the luminance reached 200 was defined as the DRT, and the vocal activity data were aligned to the DRT. The aligned data were then averaged across days to obtain the average vocal activity patterns. We obtained sunrise times and whether conditions for individual experimental days at the website of the National Astronomical Observatory of Japan (https://eco.mtk.nao.ac.jp/cgi-bin/koyomi/koyomix_en.cgi) and that of the Japan Weather Association (https://tenki.jp/past/2022/09/weather/).

Statistics

Statistical tests were performed using MATLAB (MathWorks) or R (R Core Team and R Foundation for Statistical Computing). To the differences in singing and lever-press activity across

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different LT conditions, we used a Friedman's test followed by Bonferroni corrected Durbin-Conover test. To examine the effects of different LT conditions on birds' travel distances and the effects of luzindole administration on morning singing activity, we used a Wilcoxon signed-rank test and a paired t-test, respectively. To assess the effects of different LT conditions on the slopes of morning song changes, we fitted a linear mixed-effects model predicting each syllable's slope from LT condition (–3h LT vs +3h LT), while accounting for two levels of nesting: each bird has its own baseline (random intercept) and, within each bird, each syllable has its own baseline (another random intercept). A p-value of <0.05 was used as the criterion for statistical significance.

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Competing interests: Authors declare that they have no competing interests.

Supplementary Materials

Materials and Methods

Figs. S1 to S2

Tables S1 to S2

References (50–53)

Movies S1 to S5

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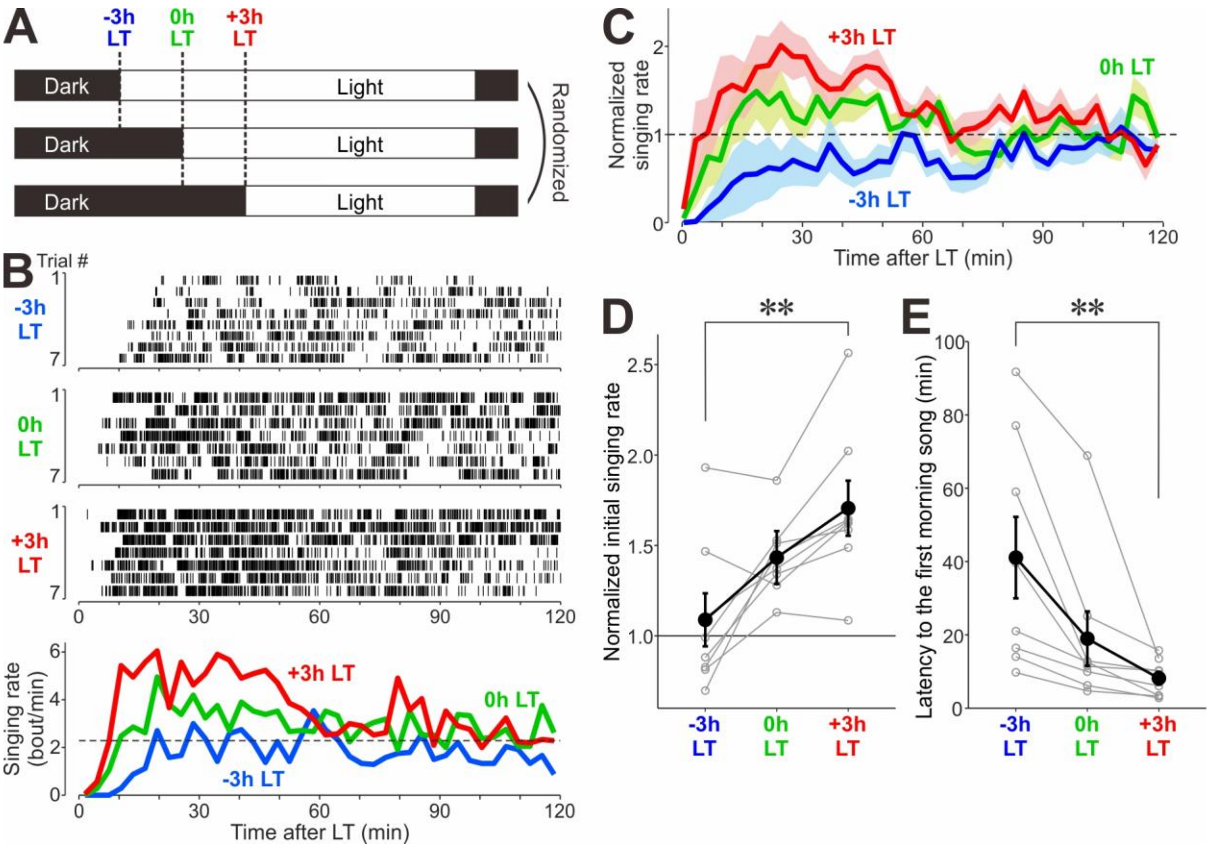

Fig. 1. dawn chorus-like intense singing can be induced in captive zebra finches by delaying the LT in the morning. (A) Daily schedules of light and dark periods with variable LT (-3h LT, 0h LT, and +3h LT). The 3 LT conditions were randomized on a daily basis. **(B)** Raster plots of song bouts (*top*) produced after -3h LT, 0h LT, and +3h LT, and corresponding singing rate histograms (bin size, 3 min) (*bottom*) in a representative bird. **(C)** Time course of instantaneous singing rate in the three different LT conditions (normalized to the baseline rate, mean ± SEM, n = 8 birds). **(D)** Initial singing rate in -3h, 0h, and +3h LT conditions (normalized to baseline singing rate); gray circles with lines indicate the data of individual birds ($p = 0.005$ for 3 conditions, Friedman's test; **$p = 0.005$ for -3h LT vs. +3h LT, Bonferroni corrected Durbin-Conover test). **(E)** Latency to the first morning song in 3 LT conditions ($p = 0.0003$ for 3 conditions; **$p = 0.005$ for -3h LT vs. +3h LT).

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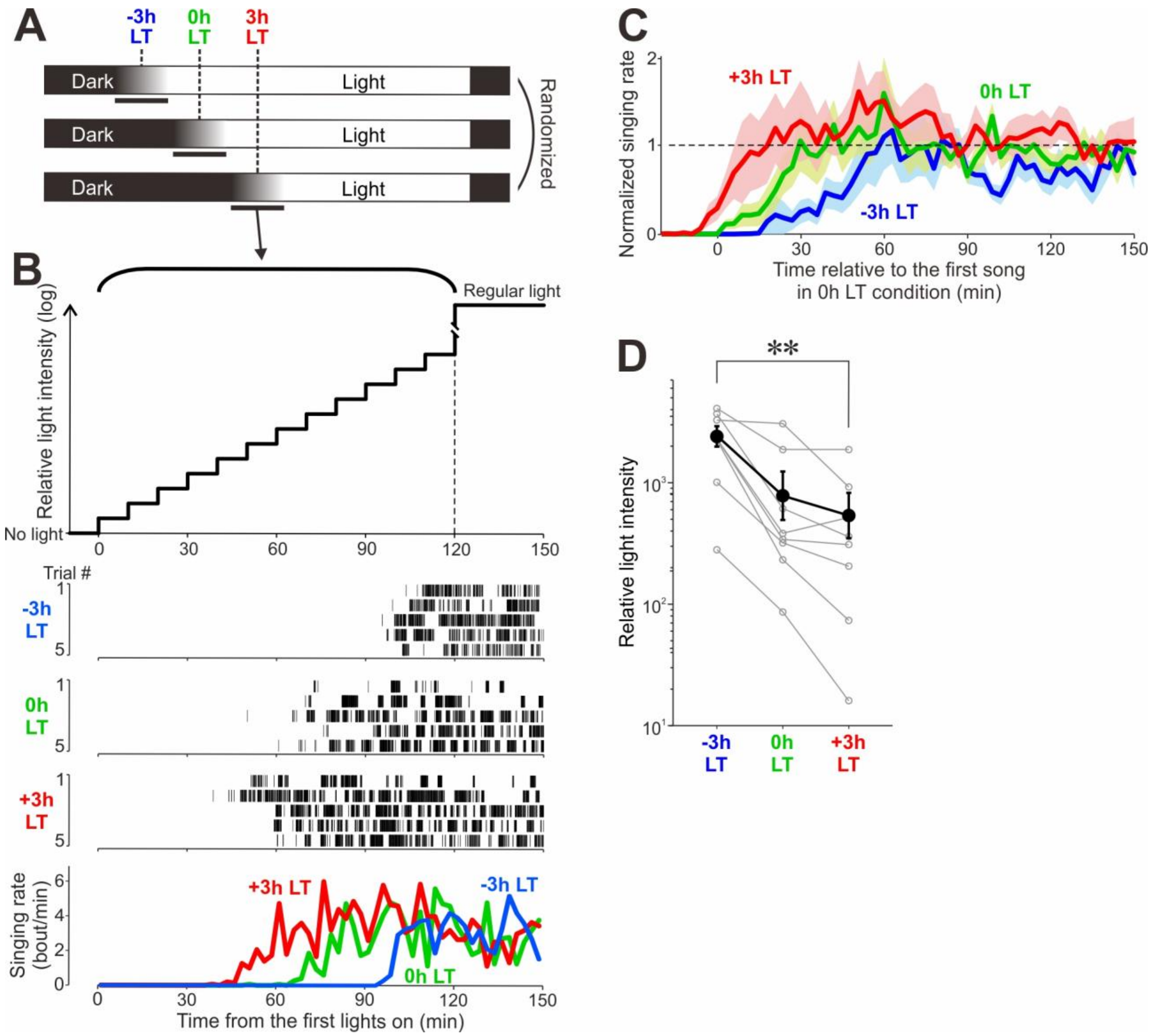

Fig. 2. Birds begin to sing in dimmer light conditions when LT is delayed. (A) Daily schedules of light and dark periods with gradual lighting. The 3 LT conditions were randomized on a daily basis. **(B)** Time course of light level changes during a gradual lighting period (*top*), raster plots of song bouts during gradual lighting periods in -3h LT, 0h LT, and +3h LT conditions (*middle*), and corresponding singing rate histograms (*bottom*) in a representative bird. **(C)** Time course of instantaneous singing rate in the three different LT conditions (normalized to the baseline rate, mean ± SEM, n = 8 birds). The horizontal axis represents the time relative to the first song in the 0h LT condition. **(D)** Relative light intensity of first song in -3h, 0h, and +3h LT conditions (p = 0.0008 for 3 conditions; **p = 0.008 for -3h LT vs. +3h).

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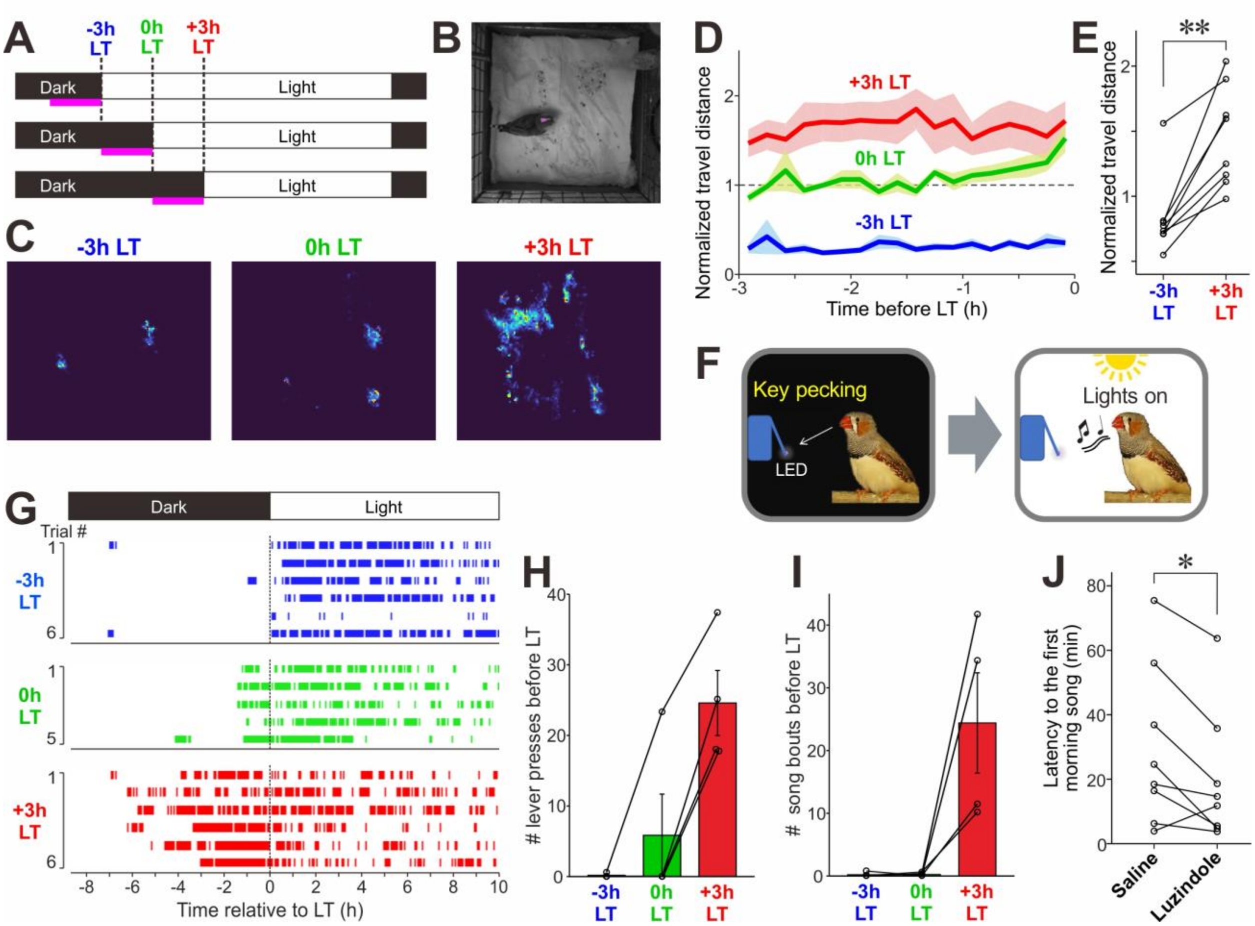

Fig. 3. Birds are awake before the delayed LT and their intrinsic singing motivation increases while singing is being suppressed by darkness. (A) Daily schedule of light/dark periods and the 3-h periods of IR video recording (magenta lines). **(B)** Example IR video image of a bird in the dark. Magenta dot on the bird head indicates the point used to track bird's movement. **(C)** Representative images indicating the positions of a bird during the 3-h dark periods immediately before the -3h, 0h, and +3h LTs (magenta lines in *A*). **(D)** Travel distances over the pre-LT 3-h periods (mean ± SEM across birds, normalized to the mean of 0h LT data for each bird). **(E)** Mean travel distances during the pre-LT 3-h periods, normalized to the 0h LT data, in individual birds (**p = 0.0078, Wilcoxon signed-rank test). **(F)** Schematics of operant conditioning paradigm to associate a lever press with a 10-sec period of lighting. **(G)** Representative plots of lever press activities (vertical bars) before and after the 3 different LTs. (**H & I)** The number of lever presses (H) and song bouts produced (I) before the 3 different LTs; lines represent the data of individual birds (averaged across trials) (n = 4 birds, p = 0.0324 and 0.038 for *H* and *I*, respectively, Friedman's test). **(J)** The effect of the selective melatonin receptor antagonist luzindole on

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first song latency. Note that birds started singing significantly earlier when injected with luzindole compared to saline (*$p = 0.027$, paired t-test).

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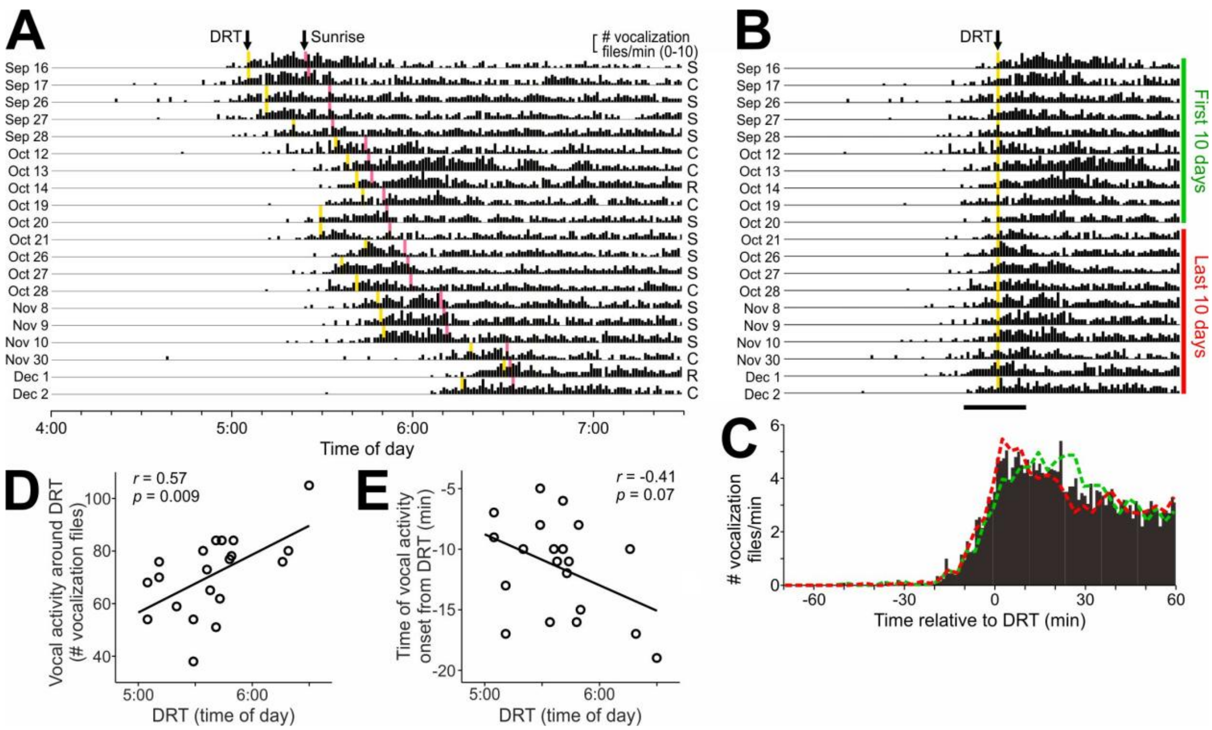

Fig. 4. Captive zebra finches housed under social and natural light conditions exhibit dawn singing. **(A)** Vocal activity (number of vocalization files/min) recorded around dawn for 20 days from mid-September to early December. Yellow lines, dawn reference time (DRT); pink lines, sunrise time. Letters on the right side of the actogram indicate the weather on individual days: S, sunny; C, cloudy; R, rainy. **(B)** Vocal activity aligned to DRT; Green and red vertical lines indicate the first and last 10-day periods analyzed for the data in *C*. Horizontal bar at the bottom indicates the period in which vocal activity on individual days were measured for the plot *D*. **(C)** DRT-aligned vocal activity averaged over the all recording days (black histogram), the first 10-day period (green dashed line) and the last 10-day period (red dashed line). **(D)** Vocal activity during the 20-min period around DRT (horizontal bar in *B*) plotted against DRT; circles represent the data of individual days. Note that vocal activity around DRT was greater on days with later DRTs. **(E)** Time of vocal activity onset relative to DRT plotted against DRT. Note that vocal activity tended to start earlier relative to DRT on days with later DRTs.

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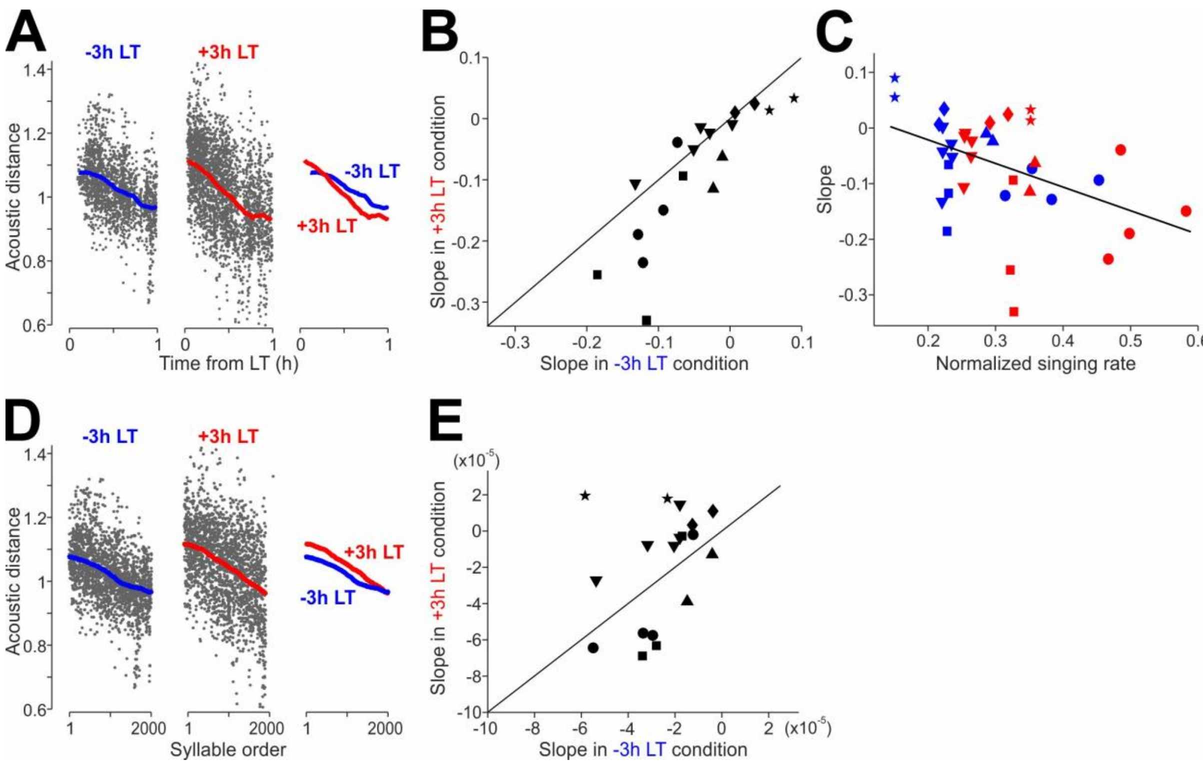

Fig. 5. dawn singing accelerates morning changes in song syllable structure. (A) Changes in acoustic distances of syllable structure in the renditions during the first hour after LT relative to the renditions during the second hour in -3h and +3h LT conditions for an example syllable. Gray dots and thick lines indicate the data of individual syllable renditions and their local mean trajectories, respectively; the local mean trajectories in the 2 conditions are overlaid in the rightmost plot. **(B)** Comparisons of the slopes of acoustic distance changes over the first 30 min between -3h and +3h LT conditions (n = 18 syllable types from 6 birds, t = 2.74, p = 0.0140, a linear mixed-effects model including random intercepts for bird identity and syllable; see Table S1). Each data point indicates a data from individual syllable type; different symbols represent different birds; Diagonal line indicates unity. **(C)** The same slope data in the 2 LT conditions plotted against the singing rate during the first 30-min period (the singing rate was normalized to that in the first 2-h period). Blue and red symbols indicate data in -3h and +3h LT conditions, respectively; symbols are as in *B* (n = 36, pairwise linear correlation, ρ = -0.4445, p = 0.0066). **(D)** Acoustic distances of the same syllable as shown in A, plotted as a function of syllable order instead of time. Conversions are identical to those in A. **(E)** Comparisons of the slopes over the first 1000 syllable renditions between -3h and +3h conditions (t = 0.35, p = 0.3509, linear mixed-effects model with random intercepts for bird identity and syllable; see Table S2).