"Singing" Fish Rely on Circadian Rhythm and Melatonin for the Timing of Nocturnal Courtship Vocalization
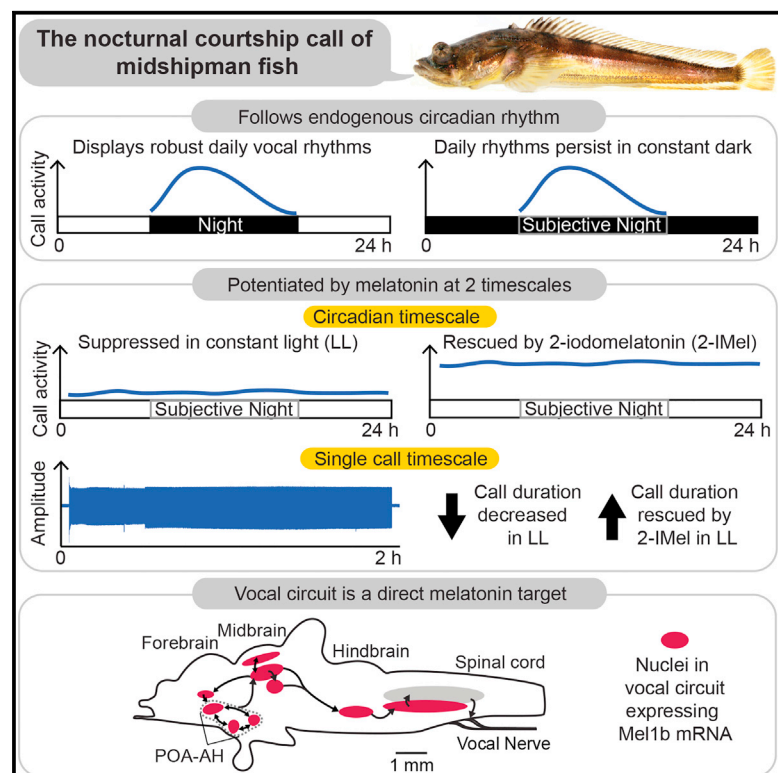
Graphical Abstract

Authors

Ni Y. Feng, Andrew H. Bass

Correspondence

ahb3@cornell.edu

In Brief

Feng and Bass reveal a circadian rhythm in fish mating calls. These nocturnal calls are suppressed by light but rescued by melatonin, the major vertebrate timekeeping hormone. Neural distribution of melatonin receptor supports melatonin action in neural circuits controlling vocalization and in neuroendocrine centers controlling reproduction.

Highlights

- Nocturnal fish vocalizations follow a circadian rhythm under constant darkness

- Calling is suppressed under constant light but rescued by melatonin

- Melatonin receptor 1b is expressed in vocal and neuroendocrine circuits

- Results provide insights into regulation of diurnal versus nocturnal vocal behavior

Accession Numbers

KT878765

CellPress

CellPress

"Singing" Fish Rely on Circadian Rhythm and Melatonin for the Timing of Nocturnal Courtship Vocalization

Ni Y. Feng[1,2] and Andrew H. Bass[1,3,*]
[1]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA
[2]Present address: Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT 06520, USA
[3]Lead Contact
*Correspondence: ahb3@cornell.edu
http://dx.doi.org/10.1016/j.cub.2016.07.079

SUMMARY

The patterning of social acoustic signaling at multiple timescales, from day-night rhythms to acoustic temporal properties, enhances sender-receiver coupling and reproductive success [1–8]. In diurnal birds, the nocturnal production of melatonin, considered the major vertebrate timekeeping hormone [9, 10], suppresses vocal activity but increases song syllable duration over circadian and millisecond timescales, respectively [11, 12]. Comparable studies are lacking for nocturnal vertebrates, including many teleost fish species that are also highly vocal during periods of reproduction [4, 13–20]. Utilizing continuous sound recordings, light cycle manipulations, hormone implants, and in situ hybridization, we demonstrate in a nocturnally breeding teleost fish that (1) courtship vocalization exhibits an endogenous circadian rhythm under constant dark conditions that is suppressed under constant light, (2) exogenous delivery of a melatonin analog under inhibitory constant light conditions rescues courtship vocal activity as well as the duration of single calls, and (3) melatonin receptor 1b is highly expressed in evolutionarily conserved neuroendocrine and vocal-acoustic networks crucial for patterning reproductive and vocal behaviors in fishes and tetrapods. Our findings, together with those in birds, show melatonin's remarkable versatility as a timing signal in distantly related lineages. It exerts opposing effects on vocalization in nocturnal versus diurnal species at the circadian timescale but comparable effects at the finer timescale of acoustic features. We propose that melatonin's separable effects at different timescales depends on its actions within distinct neural networks that control circadian rhythms, reproduction, and vocalization, which may be selected upon over evolutionary time as dissociable modules to pattern and coordinate social behaviors.

RESULTS

Vocal behavior is a prominent component of vertebrate social communication [21]. Except in birds, few studies have examined endogenous circadian regulation of daily rhythms in vocalization. Furthermore, underlying neural and hormone control mechanisms remain largely unexplored in nocturnally active species, as diurnal birds have been the predominant models for studying vocalizations.

Robust nocturnal elevation in acoustic signaling is documented in a number of sonic fish species [15–19], including the plainfin midshipman (*Porichthys notatus*) and closely related toadfishes [20, 22–24]. During the breeding season, nest-guarding midshipman males produce "hum" vocalizations in nocturnal choruses (Figure 1A; Movie S1) [20, 22, 25]. Within a larger repertoire of social context-dependent calls, hums function during courtship to attract females for spawning, advertising a male's motivation to reproduce [3, 22]. Hums can last up to hours per call and are produced repetitively at night by contracting swim bladder muscles at ~100 Hz in 14°C–16°C seawater (Figure 1A). The temporal features of midshipman calls are controlled by a neural network whose excitability is elevated at night, inhibited by constant light, and rescued by exogenous melatonin [26–30].

By manipulating external light cycles and internal melatonin levels, we tested the hypothesis that nocturnal fish vocalizations are under circadian control and are stimulated by melatonin. Our study addresses the general hypothesis that melatonin conveys the permissive time period for vocalization irrespective of daily activity pattern, which to date has been supported by its suppressive effects on singing in diurnal species of birds [8, 11] (Figure 1B).

Circadian Rhythm in Fish Courtship Vocalization

Hums were continuously recorded from individual males with sole access to artificial nests in aquaria housed in a temperature-controlled room (Figure 1C). To test for endogenous circadian rhythmicity, the normal 15:9 hr light:dark (LD) regimes were changed to constant dark (DD) or constant light (LL) (Figure 1D).

Midshipman courtship hums exhibited daily rhythms (Figures 2A–2C) with a period of 23.93 ± 0.05 hr under LD (n = 10; Figure 2C). After removal of environmental cues under
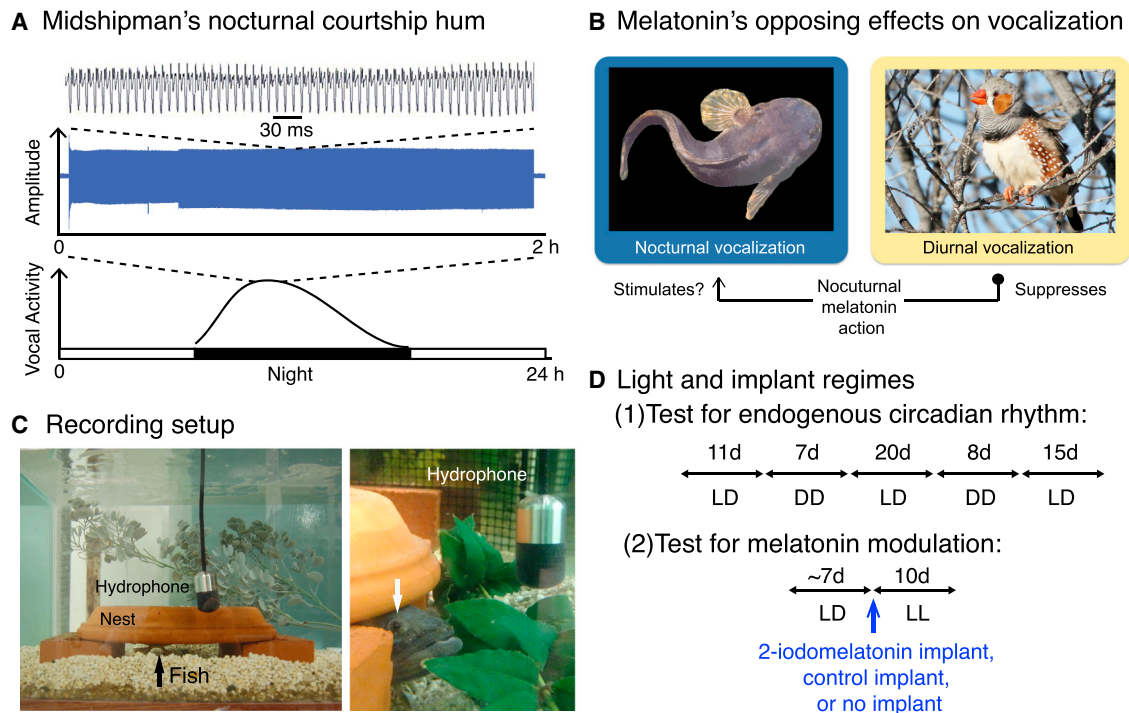
Figure 1. Schematics of Midshipman Vocal Behavior, Broad Hypothesis, and Experimental Setup

(A) The courtship vocalizations of male midshipman fish, known as "hums," are produced almost exclusively at night during the summer breeding season. Continuous hums can last from minutes to more than an hour, shown by the 1.85 hr hum recorded from a captive male (blue trace), and are produced repetitively throughout a night of courtship activity. See also Movie S1.

(B) Levels of melatonin, the predominant time-keeping hormone, typically increase at night in vertebrates regardless of the daily activity pattern (e.g., diurnal versus nocturnal) of a species. We tested the hypothesis that melatonin stimulates nocturnal vocalization in our model organism, the plainfin midshipman fish (left; photo by Margaret A. Marchaterre), opposing its inhibitory action in diurnal songbirds like the zebra finch (right; photo by Nicole M. Baran).

(C) In our captive recording setup, each fish is provided with an artificial nest and is recorded by a hydrophone. Hour-long recordings were continuously written to a computer each day. The black arrow points to a resident fish under his nest. Note that during the day, fish sometimes expose their eyes to room lighting (white arrow).

(D) Light and treatment regimes used in this study. In the first experiment, fish were held under normal light:dark (LD) and then constant dark (DD) to test for an endogenous hum rhythm. In the second experiment, fish were held first in LD and then transitioned to constant light (LL). On the first day of LL, fish were implanted with 2-iodomelatonin or vehicle control, or were non-implanted. Duration spent under each light regime is denoted in days (d).

DD, cyclic humming activity persisted and free-ran with a phase delay (Figures 2A and S1), indicating an endogenous circadian rhythm [31]. Circadian rhythmicity was confirmed by correlating each fish's humming activity with itself (autocorrelation [32]) at successive 5 min lags, revealing cyclical peaks in autocorrelation values at periods near 24 hr (Figure 2B). The endogenous free-running period under DD was 25.0 ± 0.4 (n = 6), significantly longer than the entrained cycle under LD ($t_{(5)} = 3.42$, p = 0.019) (Figure 2C). The strength of the free-running rhythm was significantly weaker under DD than LD, shown by lower autocorrelation values ($t_{(5)} = 6.19$, p = 0.0016) (Figure 2D). Unlike under DD, the circadian rhythm was abolished under LL with no circadian autocorrelation peaks (Figure 2B). Light regime (LD versus DD) had no effect on mean duration hummed per day ($F_{(1,191.1)} = 0.004$, p = 0.95) (Figure 2E), number of hums produced per day ($F_{(1,191.1)} = 0.004$, p = 0.95) (Figure 2F), or duration of single hums ($F_{(1,10)} = 0.34$, p = 0.57) (Figure 2G), consistent with our previous study showing no significant effects of DD on neural excitability of the vocal circuit [30].

Melatonin Potentiates Nocturnal Fish Courtship Vocalization

Melatonin is primarily produced by the pineal gland at night, translating the external light-dark cycle into an internal hormonal message [9, 10, 33]. Constant light is an effective, non-invasive method for decreasing melatonin synthesis [33] and suppresses midshipman vocal circuit excitability [30]. To test the hypothesis that melatonin stimulates nocturnal vocalization, a group of fish maintained under LL were implanted with 2-iodomelatonin (2-IMel), a potent melatonin analog [34]. We predicted that LL would suppress hum activity and that exogenous melatonin would rescue it to LD levels.

On the first day of LL, fish were implanted in the abdominal cavity with 2-IMel (n = 6), implanted with vehicle alone (coconut oil; n = 6), or non-implanted (n = 2; see Figure 1D and Supplemental Experimental Procedures). As predicted, LL suppressed hum duration in control-implanted and non-implanted fish that had been humming under LD (Figures 3A and S2A). Implantation with 2-IMel rescued humming activity under LL (Figures 3A, 3B, and S2B; Movie S2), supporting the hypothesis that melatonin
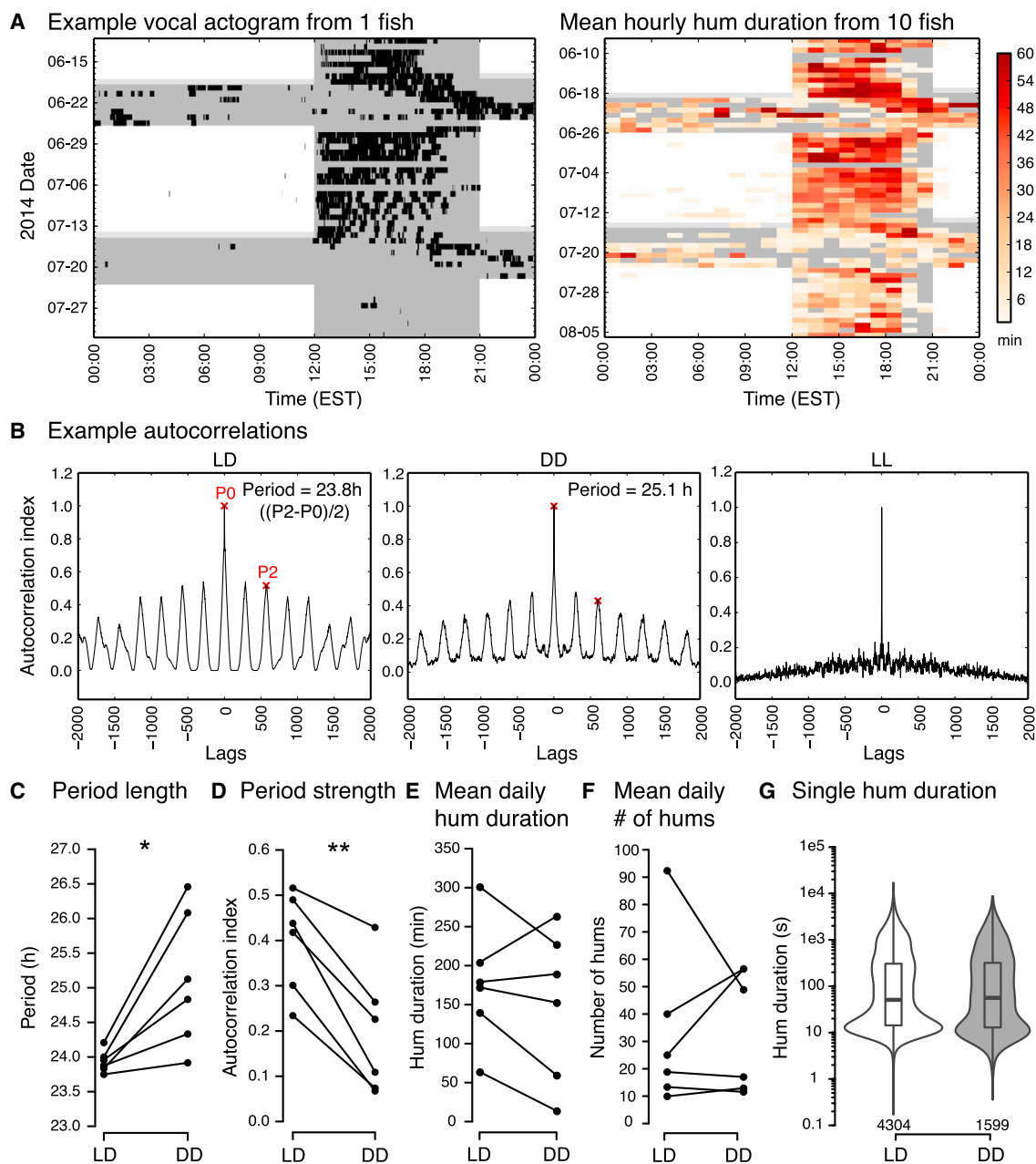
Figure 2. Constant Darkness Reveals Circadian Rhythm of Courtship Vocalization

(A) Left: vocal actogram of one fish. Right: mean hourly hum durations from ten fish. Black bars indicate when hums occurred, dark gray indicates lights-off, and white indicates lights-on. Light gray bars one day before each constant dark (DD) period indicate that main room lights were turned off and only floodlights illuminated the tanks.

(B) Example autocorrelation plots used to estimate the period and strength of daily/circadian humming activity under light:dark (LD), DD, and constant light (LL). Left two panels are autocorrelation plots from the same fish whose vocal actogram is shown in left panel of (A). Red "x"s denote peaks (P0 and P2) used to calculate the period length and strength. No circadian autocorrelation peaks were observed under LL (right panel).

(C) Period length was significantly increased under DD compared to LD. Lines connect data from the same fish.

(D) Strength of the free-running circadian rhythm was weaker compared to LD.

(E and F) Mean daily hum duration (E) and number of hums produced per day (F) were not significantly different across LD versus DD.

(G) The duration of single hums (pooled from six fish) also did not differ between LD and DD. Numbers under violin plots denote number of hums. Dark middle lines in boxplots are medians, box boundaries represent the first and third quartiles, and whiskers extend to a maximum of 1.5*(third quartile − first quartile) beyond the box.

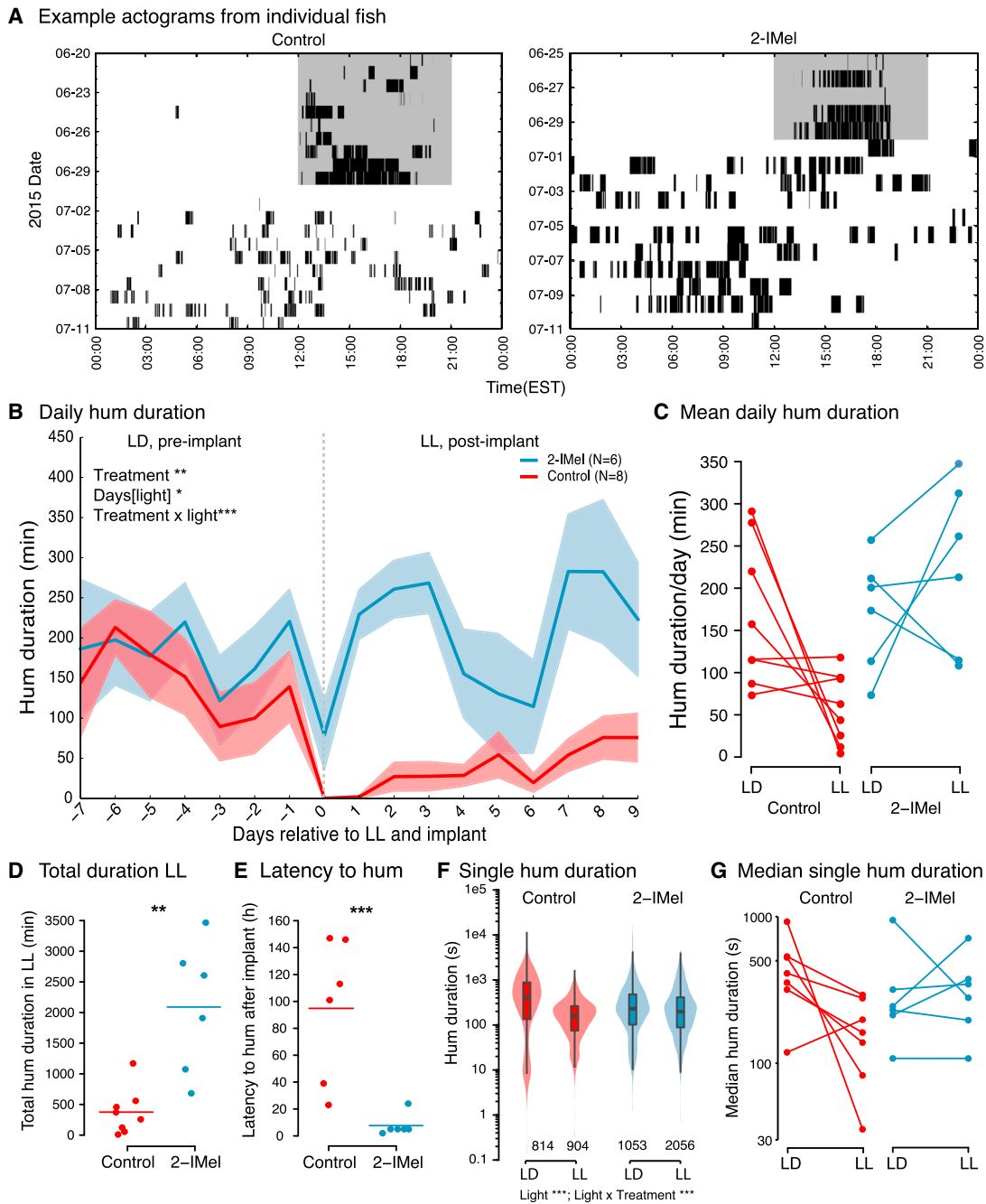*p = 0.019; **p = 0.0016. See also Figure S1.

Figure 3. Melatonin Action Rescues Nocturnal Courtship Vocal Activity under Constant Light

(A) Actograms of humming activity of a control (left) and a 2-iodomelatonin (2-IMel)-implanted fish (right). Black bars indicate when hums occurred, dark gray indicates lights-off, and white indicates lights-on.

(B) Hum duration per day aligned to the first day of constant light (LL) and implantation (shown here as 0). Mean daily hum duration is shown as colored lines, and shaded regions represent SEM. Humming activity was inhibited by LL in control fish but was rescued in 2-IMel-implanted fish (see also C). All main effects were significant except for light regime.

(C) Mean daily hum duration of each fish by light regime and treatment.

(D) 2-IMel fish hummed for a significantly longer duration than control fish under the entire LL period.

(E) 2-IMel fish showed shorter latency to resume humming after implantation than control fish.

(F) The duration of single hums decreased under LL in control fish but was maintained in 2-IMel fish. Numbers below violin plots denote number of hums in each group pooled from control (n = 8) and 2-IMel (n = 6) fish. Dark middle lines in boxplots are medians, box boundaries represent the first and third quartiles, and whiskers extend to a maximum of 1.5*(third quartile − first quartile) beyond the box.

(G) Median duration of single hums for each fish by light regime and treatment.

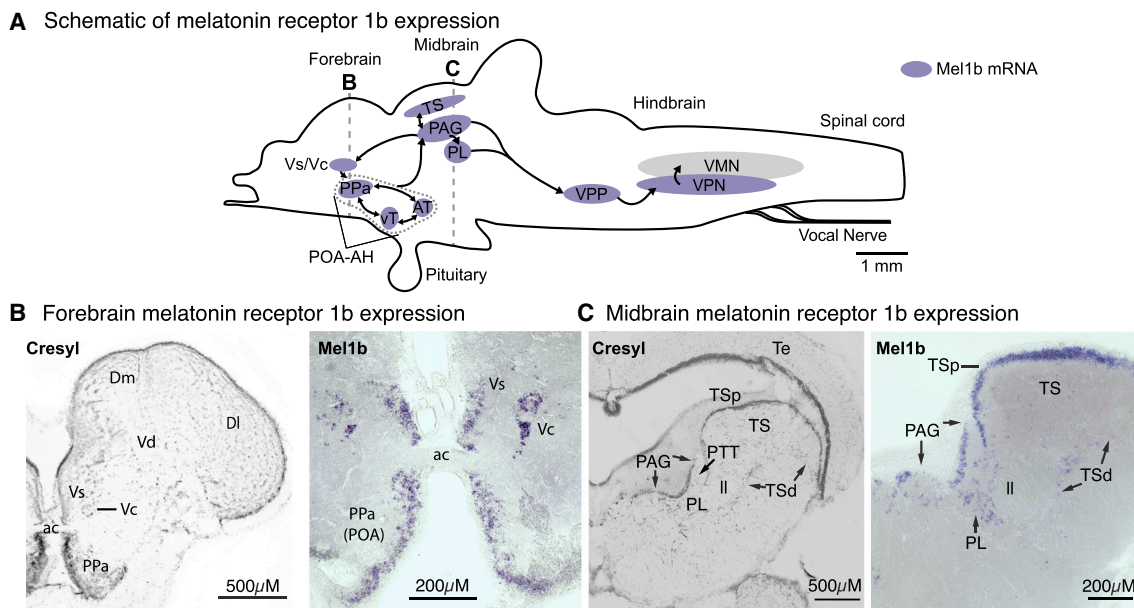*p < 0.05; **p < 0.01; ***p < 0.001. See also Figure S2 and Movie S2.

Figure 4. Melatonin Receptor 1b Expression Supports Melatonin Action in Neuroendocrine and Vocal Circuits

(A) Schematic of the side view of a midshipman brain, showing the distribution of melatonin receptor 1b (Mel1b) mRNA (purple) within critical nodes of the descending vocal-acoustic network.

(B and C) Example transverse sections of reference brain (cresyl violet stain) showing cytoarchitecture (left) and Mel1b expression (right) at forebrain (B) and midbrain (C) levels. Note differences in magnification. Abbreviations: ac, anterior commissure; AT, anterior tuberal nucleus; Dl, dorsolateral zone of dorsal telencephalon; Dm, medial zone of dorsal telencephalon; ll, lateral lemniscus; nMLF, nucleus of the medial longitudinal fasciculus; PAG, periaqueductal gray; PL, paralemniscal midbrain tegmentum; POA-AH, preoptic area-anterior hypothalamus; PPa, anterior parvocellular preoptic nucleus of the POA; PTT, paratoral midbrain tegmentum; Te, optic tectum; TS, torus semicircularis; TSd, deep layer of TS; TSp, periventricular layer of TS; Vc, central nucleus of the ventral telencephalon; Vd, dorsal nucleus of ventral telencephalon; VMN, vocal motor nucleus; VPN, vocal pacemaker nucleus; VPP, vocal pre-pacemaker nucleus; Vs, supracommissural nucleus of V; vT, ventral tuberal nucleus.

potentiates nocturnal vocalization. There were significant fixed effects of hormone treatment (2-IMel versus control; $F_{(1,12.78)} = 16.12$; $p = 0.0015$), days relative to implant nested within light regime ($F_{(2,211.1)} = 3.59$; $p = 0.029$), and treatment × light regime interaction ($F_{(1,212.9)} = 19.37$, $p < 0.0001$) (Figure 3B), but not light regime alone ($F_{(1,210.8)} = 1.23$, $p = 0.27$). Figure 3C summarizes mean daily hum duration across light regimes for each fish, showing a decrease in control-implanted but increase in 2-IMel-implanted fish under LL relative to LD. The total duration hummed under the entire LL period was significantly higher in 2-IMel-implanted fish compared to control-implanted and non-implanted fish ($t_{(12)} = 4.25$, $p = 0.0011$) (Figure 3D). Additionally, the latency to resume humming after implantation was significantly shorter in 2-IMel-implanted fish ($t_{(10)} = 5.86$, $p = 0.0002$) (Figure 3E). Thus, exogenous melatonin treatment rescued vocal suppression by LL.

Constant light also inhibited duration of single hums in control-implanted but not 2-IMel-implanted fish. There were significant effects of light regime ($F_{(1,4778)} = 70.62$, $p < 0.0001$) and light regime × treatment interaction ($F_{(1,4778)} = 128.25$, $p < 0.0001$). Figure 3F shows the distribution of hum durations from all individuals, while Figure 3G shows median hum durations by individuals across light regimes separated by treatment. The number of hums produced per day, however, did not differ across treatment ($F_{(1,12)} = 3.15$, $p = 0.10$) or light regime ($F_{(1,12)} = 0.58$, $p = 0.46$) (data not shown).

Although 2-IMel maintained and even increased humming activity compared to controls under LL in fish that were previously humming under LD conditions (Figure 3), it was not sufficient to induce humming in fish that had not hummed in LD conditions in this environment (n = 3), suggesting a permissive role. In summary, constant darkness (but not constant light) revealed an endogenously generated circadian rhythm controlling the nocturnal courtship vocalization of midshipman fish, while constant light suppression of hum duration was rescued by exogenous melatonin at both daily and single-call timescales.

Melatonin Receptor 1b mRNA Is Expressed in Neuroendocrine and Vocal-Acoustic Circuits

We mapped the distribution of melatonin receptor 1B (Mel1b) mRNA in the midshipman brain to identify targets of melatonin action. Teleost fish possess four subtypes of G protein-coupled melatonin receptors, including Mel1b [35, 36]. We focused on Mel1b because blocking this receptor decreased neural excitability in the midshipman vocal network [30] and song length in a songbird [37]. Mel1b is also the only melatonin receptor subtype highly expressed in the song control system of birds [38, 39].

In situ hybridization localized midshipman-specific Mel1b mRNA to neuroendocrine regions and multiple nodes of the vocal-acoustic network (summarized in Figure 4A). Mel1b was robustly expressed in the highly conserved forebrain preoptic

area-anterior hypothalamus (POA-AH; see Figures 4A and 4B), which controls vertebrate social and reproductive behaviors [40–42], including vocalization in midshipman and other toad-fishes [43, 44]. Robust labeling was also observed in contiguous midbrain regions that comprise a vocal initiation center (PL, PTT, PAG; Figures 4A and 4C) that receives POA input, like comparable regions in tetrapods [30, 44–47]. Lastly, strong labeling was found in regions connected to the POA-AH and/or vocal midbrain [44–46]. This included periventricular and deep layers of the midbrain auditory center, the torus semicircularis (TSp, TSd, TS; Figures 4A and 4C), as well as ventral telencephalic nuclei such as the supracommisural nucleus (Vs; Figure 4B), a vocally active site [43, 44] that has been compared to parts of the amygdala important for social behavior [41, 48, 49].

These results provide relevant support for melatonin's direct action in vocal circuitry. A more complete analysis of Mel1b's robust but discrete expression throughout the brain will be presented elsewhere. This includes olfactory, lateral line, visual, and auditory (see above) pathways, implicating a role for melatonin in modulating multisensory inputs essential to the appropriate timing of behavior.

DISCUSSION

Our results demonstrate in a nocturnal and highly vocal teleost fish that (1) courtship vocalization exhibits an endogenous circadian rhythm under constant darkness, (2) melatonin rescues courtship vocal activity and single call duration under constant light, and (3) melatonin receptor is highly expressed within evolutionarily conserved brain regions that pattern social, reproductive, and vocal behaviors in fish and tetrapods.

Circadian Vocal Rhythms in Vertebrates
Few studies have tested circadian control of daily rhythmic vertebrate vocal behaviors under constant conditions or their regulation by melatonin, with the majority of studies in birds [11, 12] and none to our knowledge in mammals aside from sparse reports of diel vocal rhythms [50, 51]. A circadian rhythm in the pre-dawn crowing of roosters was recently demonstrated, but melatonin modulation was not investigated [52]. The importance of circadian vocal rhythms for reproductive success was causally demonstrated in a songbird, where a delay in dawn chorus singing induced by melatonin reduced male reproductive success [8].

Teleost fish exhibit noisy free-running circadian rhythms (mainly of locomotion and feeding) that dampen over days to weeks with high individual variability in strength [53], similar to midshipman vocal rhythms (Figures 2A–2D). Furthermore, LL masked and/or abolished circadian vocal rhythms in midshipman fish, an effect also observed in zebra finches (*Taeniopygia guttata*) [12]. Our study extends findings in birds to a distant vertebrate group, demonstrating that an endogenous circadian oscillator drives the overt daily rhythm in fish courtship vocalization.

Melatonin Exerts Opposing Effects on Vocalization over Daily Timescales
Our results support the hypothesis that melatonin conveys the appropriate time for vocalization in diurnal and nocturnal species by suppressing and permitting vocalization, respectively. Song-

birds are vocally active during the day when melatonin is low and breed during long days when the duration of nocturnal melatonin release is relatively brief, leading to the prediction that melatonin inhibits vocalization. Indeed, songbirds sing throughout periods of constant light [12], and song and call activity entrain to times of day without melatonin treatment in pinealectomized zebra finches housed under constant dim light [11]. Furthermore, melatonin has been shown to decrease the isolation distress calls of both chickens [54] and ewes [55]. Taking these findings together, sufficient evidence from distantly related diurnal species suggests that melatonin inhibits vocalization.

For nocturnally active vertebrates that are also long-day breeders, it is more difficult to predict whether melatonin would be stimulatory or inhibitory. Some lineages of nocturnally active birds (e.g., owls) have partially atrophied pineal glands and low, arrhythmic plasma melatonin levels [56–58], suggesting an escape from melatonin's suppressive effects. However, this phenomenon is not widespread in birds [56]. Furthermore, robust pineal melatonin rhythms have been reported in nocturnal fish species [59]. The present study shows a robust and permissive role for melatonin in controlling vocalization in a nocturnally active fish.

Melatonin has also been shown to act in opposing directions to regulate general activity levels and arousal in nocturnal versus diurnal mammals, e.g., locomotor activity in nocturnal rats (e.g., [60]) versus sleep duration, efficiency, and onset latency [61] in humans. Melatonin inhibits locomotor activity in diurnal birds [62] and fish [63, 64], while one study in nocturnal fish reported increased locomotor activity during the daytime following melatonin injection [64].

Melatonin Exerts Comparable Effects on Vocalization over Shorter Timescales
Surprisingly, comparable with our data showing LL suppression of single hum duration, LL also shortened single song motifs and syllables in zebra finches, an effect recapitulated by pinealectomy [12]. Like in midshipman (Figures 3F and 3G), exogenous melatonin in zebra finches rescued song motif duration under these inhibitory conditions [12]. In Japanese quail (*Coturnix japonica*), crows produced at night were longer than those produced during the day [65] and were significantly shortened under LL [12], though the effect of melatonin modulation remains to be tested.

Taken together, evidence suggests that regardless of a species' daily activity pattern, melatonin lengthens the duration of single calls, an elemental component of acoustic signals [21]. More generally, melatonin could regulate social signals in other modalities for which timing at both circadian and finer timescales is important. A recent study of gymnotid fish shows that the melatonin antagonist luzindole inhibits the nocturnal rise in the rate of electric organ discharges [66].

Light and Melatonin Modulation of Vocal Neural Circuits
Several lines of evidence from both birds and midshipman fish suggest that environmental light information can be relayed by melatonin directly to the brain's vocal control circuitry, whose output patterns the temporal properties of vocalizations [37–39, 67–72]. Continuous or 14 hr daily melatonin treatment in songbirds mimics winter-like short days by decreasing song control

nuclei volumes [69, 70]. Melatonin application to brain slices leads to decreased levels of spontaneous activity in the forebrain song nucleus, robustus arcopallialis [37]. In midshipman, in vivo measurements of vocal circuit neural excitability increase at night and decrease during the day, likely driving the dramatic daily rhythms in vocal behavior [29]. Constant light suppression of neural excitability in the midshipman vocal circuit is rescued by 2-IMel implantation, while pharmacological blockade of Mel1b inhibits the network's excitability [30], thus providing a neural mechanism underlying the behavioral results presented here. Lastly, expression of melatonin receptors, including Mel1b mRNA, in the vocal network of both midshipman (Figure 4) and songbirds [37–39, 67–72] likely mediates melatonin's modulatory actions in these circuits.

Conclusions

Our results demonstrate that vocalizations in fish, as in birds, follow a circadian rhythm and are sensitive to melatonin. Melatonin is a versatile circadian timing signal, conveying the permissive time period for vocalization in an opposing manner to permit vocalization in nocturnal fish and suppress it in diurnal birds. Elucidating the mechanism (or mechanisms) responsible for interpreting the melatonin signal in opposing ways in diurnal and nocturnal species is an important avenue for future study. Furthermore, investigating whether melatonin permits vocalization in other nocturnal vertebrates will help clarify the phylogenetic history of melatonin regulation of vocalization.

Melatonin lengthens the duration of single calls in both fish and birds, indicating an ability to pattern vocalization at the millisecond-to-minute timescale. Thus, in addition to daily activity patterns, melatonin can also regulate the finer temporal patterning of individual behavioral events. Finally, robust melatonin receptor expression in neuroendocrine and vocal networks suggests that melatonin's timescale-dependent effects are executed by discrete and evolutionarily conserved neural pathways. We propose that melatonin control of vocalization over vastly different timescales is a trait widely shared by ectothermic and endothermic vertebrates.

EXPERIMENTAL PROCEDURES

For more detailed methods, see Supplemental Experimental Procedures.

Animals

Nest-guarding male midshipman fish were housed in large aquaria, with each having sole access to an artificial nest (Figure 1C; Movie S2). All procedures were approved by the Cornell Institutional Animal Care and Use Committee.

Sound Recording and Analyses

Hums from each fish were recorded continuously and written to disk every hour. Raw sound files were analyzed in Raven Pro 1.5; downstream analyses utilized Python.

In Situ Hybridization

Our in situ hybridization protocol largely followed previously reported studies [73] and used midshipman-specific mRNA probes.

Statistics

Linear mixed models used fish as a random variable and appropriate fixed effects for each experiment (e.g., light regime, hormone treatment, and their interactions).

ACCESSION NUMBERS

The accession number for the Mel1b sequence reported in this paper is GenBank: KT878765.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, two movies and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.07.079.

A video abstract is available at http://dx.doi.org/10.1016/j.cub.2016.07.079#mmc5.

AUTHOR CONTRIBUTIONS

N.Y.F. conducted the experiments and statistical analyses. N.Y.F. and A.H.B. planned the experiments, analyzed the data, and wrote the manuscript.

REFERENCES

1. Wells, K.D. (1977). The social behaviour of anuran amphibians. Anim. Behav. 25, 666–693.

2. Rose, G.J. (2014). Time computations in anuran auditory systems. Front. Physiol. 5, 206.

3. McKibben, J.R., and Bass, A.H. (1998). Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. J. Acoust. Soc. Am. 104, 3520–3533.

4. Ruppé, L., Clément, G., Herrel, A., Ballesta, L., Décamps, T., Kéver, L., and Parmentier, E. (2015). Environmental constraints drive the partitioning of the soundscape in fishes. Proc. Natl. Acad. Sci. USA 112, 6092–6097.

5. Danley, P.D., deCarvalho, T.N., Fergus, D.J., and Shaw, K.L. (2007). Reproductive asynchrony and the divergence of Hawaiian crickets. Ethology 113, 1125–1132.

6. Luther, D.A. (2008). Signaller: receiver coordination and the timing of communication in Amazonian birds. Biol. Lett. 4, 651–654.

7. Roth, T., Sprau, P., Schmidt, R., Naguib, M., and Amrhein, V. (2009). Sex-specific timing of mate searching and territory prospecting in the nightingale: nocturnal life of females. Proc. Biol. Sci. 276, 2045–2050.

8. Greives, T.J., Kingma, S.A., Kranstauber, B., Mortega, K., Wikelski, M., van Oers, K., Mateman, A.C., Ferguson, G.A., Beltrami, G., and Hau, M. (2015). Costs of sleeping in: circadian rhythms influence cuckoldry risk in a songbird. Funct. Ecol. 29, 1300–1307.

9. Nelson, R.J. (2011). An Introduction to Behavioral Endocrinology, Second Edition (Sinauer Associates).

10. Reiter, R.J. (1993). The melatonin rhythm: both a clock and a calendar. Experientia 49, 654–664.

11. Wang, G., Harpole, C.E., Trivedi, A.K., and Cassone, V.M. (2012). Circadian regulation of bird song, call, and locomotor behavior by pineal melatonin in the zebra finch. J. Biol. Rhythms 27, 145–155.

12. Derégnaucourt, S., Saar, S., and Gahr, M. (2012). Melatonin affects the temporal pattern of vocal signatures in birds. J. Pineal Res. 53, 245–258.

13. Whang, A., and Janssen, J. (1994). Sound production through the substrate during reproduction in the mottled sculpin, Cottus bairdi (Cottidae). Environ. Biol. Fishes 40, 141–148.

14. Crawford, J.D., Jacob, P., and Bénech, V. (1997). Sound production and reproductive ecology of strongly acoustic fish in Africa: Pollimyrus isidori, Mormyridae. Behaviour 134, 677–725.

15. McCauley, R.D., and Cato, D.H. (2000). Patterns of fish calling in a near-shore environment in the Great Barrier Reef. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355, 1289–1293.

16. Luczkovich, J.J., Pullinger, R.C., Johnson, S.E., and Sprague, M.W. (2008). Identifying Sciaenid critical spawning habitats by the use of passive acoustics. Trans. Am. Fish. Soc. 137, 576–605.

17. Locascio, J.V., and Mann, D.A. (2008). Diel periodicity of fish sound production in Charlotte Harbor, Florida. Trans. Am. Fish. Soc. 137, 606–615.

18. Connaughton, M.A., and Taylor, M.H. (1995). Seasonal and daily cycles in sound production associated with spawning in the weakfish, Cynoscion regalis. Environ. Biol. Fishes 42, 233–240.

19. Lobel, P.S., Kaatz, I.M., and Rice, A.N. (2010). Acoustical behavior of coral reef fishes. In Reproduction and Sexuality in Marine Fishes: Patterns and Processes, K.S. Cole, ed. (University of California Press).

20. Ibara, R.M., Penny, L.T., Ebeling, A.W., van Dykhuizen, G., and Cailliet, G. (1983). The mating call of the plainfin midshipman fish, Porichthys notatus. In Predators and Prey in Fishes (Developments in Environmental Biology of Fishes Volume 2), D.L.G. Noakes, D.G. Lindquist, G.S. Helfman, and J.A. Ward, eds. (Springer Netherlands), pp. 205–212.

21. Bradbury, J.W., and Vehrencamp, S.L. (2011). Principles of Animal Communication, Second Edition (Sinauer Associates).

22. Brantley, R.K., and Bass, A.H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish Porichthys notatus Girard (Teleostei, Batrachoididae). Ethology 96, 213–232.

23. Rice, A.N., and Bass, A.H. (2009). Novel vocal repertoire and paired swimbladders of the three-spined toadfish, Batrachomoeus trispinosus: insights into the diversity of the Batrachoididae. J. Exp. Biol. 212, 1377–1391.

24. Wall, C.C., Simard, P., Lembke, C., and Mann, D.A. (2013). Large-scale passive acoustic monitoring of fish sound production on the West Florida Shelf. Mar. Ecol. Prog. Ser. 484, 173–188.

25. McIver, E.L., Marchaterre, M.A., Rice, A.N., and Bass, A.H. (2014). Novel underwater soundscape: acoustic repertoire of plainfin midshipman fish. J. Exp. Biol. 217, 2377–2389.

26. Bass, A.H., and Baker, R. (1990). Sexual dimorphisms in the vocal control system of a teleost fish: morphology of physiologically identified neurons. J. Neurobiol. 21, 1155–1168.

27. Chagnaud, B.P., Baker, R., and Bass, A.H. (2011). Vocalization frequency and duration are coded in separate hindbrain nuclei. Nat. Commun. 2, 346.

28. Chagnaud, B.P., Zee, M.C., Baker, R., and Bass, A.H. (2012). Innovations in motoneuron synchrony drive rapid temporal modulations in vertebrate acoustic signaling. J. Neurophysiol. 107, 3528–3542.

29. Rubow, T.K., and Bass, A.H. (2009). Reproductive and diurnal rhythms regulate vocal motor plasticity in a teleost fish. J. Exp. Biol. 212, 3252–3262.

30. Feng, N.Y., and Bass, A.H. (2014). Melatonin action in a midbrain vocal-acoustic network. J. Exp. Biol. 217, 1046–1057.

31. Silver, R., and Kriegsfeld, L.J. (2014). Circadian rhythms have broad implications for understanding brain and behavior. Eur. J. Neurosci. 39, 1866–1880.

32. Levine, J.D., Funes, P., Dowse, H.B., and Hall, J.C. (2002). Signal analysis of behavioral and molecular cycles. BMC Neurosci. 3, 1.

33. Ekström, P., and Meissl, H. (1997). The pineal organ of teleost fishes. Rev. Fish Biol. Fish. 7, 199–284.

34. Boutin, J.A., Audinot, V., Ferry, G., and Delagrange, P. (2005). Molecular tools to study melatonin pathways and actions. Trends Pharmacol. Sci. 26, 412–419.

35. Reppert, S.M., Weaver, D.R., Cassone, V.M., Godson, C., and Kolakowski, L.F., Jr. (1995). Melatonin receptors are for the birds: molecular analysis of two receptor subtypes differentially expressed in chick brain. Neuron 15, 1003–1015.

36. Ikegami, T., Motohashi, E., Doi, H., Hattori, A., and Ando, H. (2009). Synchronized diurnal and circadian expressions of four subtypes of melatonin receptor genes in the diencephalon of a puffer fish with lunar-related spawning cycles. Neurosci. Lett. 462, 58–63.

37. Jansen, R., Metzdorf, R., van der Roest, M., Fusani, L., ter Maat, A., and Gahr, M. (2005). Melatonin affects the temporal organization of the song of the zebra finch. FASEB J. 19, 848–850.

38. Fusani, L., and Gahr, M. (2015). Differential expression of melatonin receptor subtypes Mel1a, Mel1b and Mel1c in relation to melatonin binding in the male songbird brain. Brain Behav. Evol. 85, 4–14.

39. Bentley, G.E., Perfito, N., and Calisi, R.M. (2013). Season- and context-dependent sex differences in melatonin receptor activity in a forebrain song control nucleus. Horm. Behav. 63, 829–835.

40. Forlano, P.M., and Bass, A.H. (2011). Neural and hormonal mechanisms of reproductive-related arousal in fishes. Horm. Behav. 59, 616–629.

41. O'Connell, L.A., and Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J. Comp. Neurol. 519, 3599–3639.

42. Goodson, J.L., and Kingsbury, M.A. (2013). What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior networks. Horm. Behav. 64, 103–112.

43. Fine, M.L., and Perini, M.A. (1994). Sound production evoked by electrical stimulation of the forebrain in the oyster toadfish. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 174, 173–185.

44. Goodson, J.L., and Bass, A.H. (2002). Vocal-acoustic circuitry and descending vocal pathways in teleost fish: convergence with terrestrial vertebrates reveals conserved traits. J. Comp. Neurol. 448, 298–322.

45. Kittelberger, J.M., and Bass, A.H. (2013). Vocal-motor and auditory connectivity of the midbrain periaqueductal gray in a teleost fish. J. Comp. Neurol. 521, 791–812.

46. Wild, J.M., and Balthazart, J. (2013). Neural pathways mediating control of reproductive behavior in male Japanese quail. J. Comp. Neurol. 521, 2067–2087.

47. Kittelberger, J.M., Land, B.R., and Bass, A.H. (2006). Midbrain periaqueductal gray and vocal patterning in a teleost fish. J. Neurophysiol. 96, 71–85.

48. Goodson, J.L. (2005). The vertebrate social behavior network: evolutionary themes and variations. Horm. Behav. 48, 11–22.

49. Teles, M.C., Almeida, O., Lopes, J.S., and Oliveira, R.F. (2015). Social interactions elicit rapid shifts in functional connectivity in the social decision-making network of zebrafish. Proc. Biol. Sci. 282, 20151099.

50. Rusak, B. (1981). Vertebrate behavioral rhythms. In Biological Rhythms, J. Aschoff, ed. (Springer), pp. 183–213.

51. Wang, D., Garcia, H., Huang, W., Tran, D.D., Jain, A.D., Yi, D.H., Gong, Z., Jech, J.M., Godø, O.R., Makris, N.C., and Ratilal, P. (2016). Vast assembly of vocal marine mammals from diverse species on fish spawning ground. Nature 531, 366–370.

52. Shimmura, T., and Yoshimura, T. (2013). Circadian clock determines the timing of rooster crowing. Curr. Biol. 23, R231–R233.

53. Zhdanova, I.V., and Reebs, S.G. (2006). Circadian rhythms in fish. In Behaviour and Physiology of Fish, K.A. Sloman, R.W. Wilson, and S. Balshine, eds. (Elsevier), pp. 197–238.

54. Nelson, E., Panksepp, J., and Ikemoto, S. (1994). The effects of melatonin on isolation distress in chickens. Pharmacol. Biochem. Behav. *49*, 327–333.

55. Guesdon, V., Malpaux, B., Delagrange, P., Spedding, M., Cornilleau, F., Chesneau, D., Haller, J., and Chaillou, E. (2013). Rapid effects of melatonin on hormonal and behavioral stressful responses in ewes. Psychoneuroendocrinology *38*, 1426–1434.

56. Quay, W.B. (1972). Infrequency of pineal atrophy among birds and its relation to nocturnality. Condor *74*, 33–45.

57. Taniguchi, M., Murakami, N., Nakamura, H., Nasu, T., Shinohara, S., and Etoh, T. (1993). Melatonin release from pineal cells of diurnal and nocturnal birds. Brain Res. *620*, 297–300.

58. Van't Hof, T.J., Gwinner, E., and Wagner, H. (1998). A highly rudimentary circadian melatonin profile in a nocturnal bird, the barn owl (*Tyto alba*). Naturwissenschaften *85*, 402–404.

59. Oliveira, C., Garcia, E.M., López-Olmeda, J.F., and Sánchez-Vázquez, F.J. (2009). Daily and circadian melatonin release in vitro by the pineal organ of two nocturnal teleost species: Senegal sole (*Solea senegalensis*) and tench (*Tinca tinca*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. *153*, 297–302.

60. Armstrong, S.M. (1989). Melatonin and circadian control in mammals. Experientia *45*, 932–938.

61. Brzezinski, A., Vangel, M.G., Wurtman, R.J., Norrie, G., Zhdanova, I., Ben-Shushan, A., and Ford, I. (2005). Effects of exogenous melatonin on sleep: a meta-analysis. Sleep Med. Rev. *9*, 41–50.

62. Murakami, N., Kawano, T., Nakahara, K., Nasu, T., and Shiota, K. (2001). Effect of melatonin on circadian rhythm, locomotor activity and body temperature in the intact house sparrow, Japanese quail and owl. Brain Res. *889*, 220–224.

63. Zhdanova, I.V., Wang, S.Y., Leclair, O.U., and Danilova, N.P. (2001). Melatonin promotes sleep-like state in zebrafish. Brain Res. *903*, 263–268.

64. López-Olmeda, J.F., Madrid, J.A., and Sánchez-Vázquez, F.J. (2006). Melatonin effects on food intake and activity rhythms in two fish species with different activity patterns: Diurnal (goldfish) and nocturnal (tench). Comp. Biochem. Physiol. A Mol. Integr. Physiol. *144*, 180–187.

65. Derégnaucourt, S., Saar, S., and Gahr, M. (2009). Dynamics of crowing development in the domestic Japanese quail (*Coturnix coturnix japonica*). Proc. Biol. Sci. *276*, 2153–2162.

66. Migliaro, A., and Silva, A. (2016). Melatonin regulates daily variations in electric behavior arousal in two species of weakly electric fish with different social structures. Brain Behav. Evol. Published online May 24, 2016. http://dx.doi.org/10.1159/000445494.

67. Bentley, G.E. (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*, 187–192.

68. Bentley, G.E., and Ball, G.F. (2000). Photoperiod-dependent and -independent regulation of melatonin receptors in the forebrain of songbirds. J. Neuroendocrinol. *12*, 745–752.

69. Bentley, G.E., Van't Hof, T.J., and Ball, G.F. (1999). Seasonal neuroplasticity in the songbird telencephalon: a role for melatonin. Proc. Natl. Acad. Sci. USA *96*, 4674–4679.

70. Cassone, V.M., Bartell, P.A., Earnest, B.J., and 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*, 49–58.

71. Gahr, M., and 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*, 308–318.

72. Whitfield-Rucker, M.G., and Cassone, V.M. (1996). Melatonin binding in the house sparrow song control system: sexual dimorphism and the effect of photoperiod. Horm. Behav. *30*, 528–537.

73. Albersheim-Carter, J., Blubaum, A., Ballagh, I.H., Missaghi, K., Siuda, E.R., McMurray, G., Bass, A.H., Dubuc, R., Kelley, D.B., Schmidt, M.F., et al. (2016). Testing the evolutionary conservation of vocal motoneurons in vertebrates. Respir. Physiol. Neurobiol. *224*, 2–10.