Journal of Insect Physiology 107 (2018) 81–88

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Wolbachia affects sleep behavior in *Drosophila melanogaster*

Jie Bi[a,b], Amita Sehgal[b], Julie A. Williams[b], Yu-Feng Wang[a,*]

[a] School of Life Sciences, Hubei Key Laboratory of Genetic Regulation and Integrative Biology, Central China Normal University, Wuhan 430079, PR China
[b] Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

ARTICLE INFO

Keywords:
Wolbachia
Drosophila melanogaster
Sleep
Arousal
Dopamine

ABSTRACT

Wolbachia are endosymbiotic bacteria present in a wide range of insects. Although their dramatic effects on host reproductive biology have been well studied, the effects of *Wolbachia* on sleep behavior of insect hosts are not well documented. In this study, we report that *Wolbachia* infection caused an increase of total sleep time in both male and female *Drosophila melanogaster*. The increase in sleep was associated with an increase in the number of nighttime sleep bouts or episodes, but not in sleep bout duration. Correspondingly, *Wolbachia* infection also reduced the arousal threshold of their fly hosts. However, neither circadian rhythm nor sleep rebound following deprivation was influenced by *Wolbachia* infection. Transcriptional analysis of the dopamine biosynthesis pathway revealed that two essential genes, *Pale* and *Ddc*, were significantly upregulated in *Wolbachia*-infected flies. Together, these results indicate that *Wolbachia* mediates the expression of dopamine related genes, and decreases the sleep quality of their insect hosts. Our findings help better understand the host-endosymbiont interactions and in particular the *Wolbachia*'s impact on behaviors, and thus on ecology and evolution in insect hosts.

1. Introduction

Symbiotic organisms are particularly common in insects, and in some cases they may protect their hosts from pathogenic infections (Goodacre and Martin, 2012) or increase their hosts' biological fitness. *Wolbachia* are gram-negative endosymbiotic bacteria that infect a wide range of arthropods and filarial nematodes. It is estimated that up to 40% of arthropod species are infected with *Wolbachia* (Zug and Hammerstein, 2012). *Wolbachia* are best known for infecting host reproductive tissues and thus manipulating host reproduction, enhancing their transmission through host populations. In addition to gonads, *Wolbachia* are also prevalent in tissues of the nervous system in *Drosophila* (Albertson et al., 2013; Casper-Lindley et al., 2011) as well as digestive and metabolic tissues such as the fat body, gut, salivary glands, and Malpighian tubules of many insect species where they may play a role in mediating host immunity and behavior (Pietri et al., 2016; Rohrscheib et al., 2015). Recently, studies have shown that *Wolbachia* affects male aggression and activity in *Drosophila* hosts (Rohrscheib et al., 2015; Vale and Jardine, 2015). When compared with *Wolbachia*-free flies, *Wolbachia*-infected flies exhibited significantly reduced activity, suggesting an increase of sleep resulted from *Wolbachia* (Vale and Jardine, 2015). Therefore *Drosophila* is an ideal model host to investigate questions at the interface of microbes and host behavior.

The sleep-like state is widely conserved among animal species (Crocker and Sehgal, 2010), and *Drosophila* has been frequently used in studies to identify the genetic basis of sleep/wake regulation (Allada et al., 2017; Dubowy and Sehgal, 2017). Sleep behavior in flies shares cardinal features with mammalian sleep such as prolonged reversible immobility, increased arousal thresholds and homeostatic influence (Shaw et al., 2000; Hendricks et al., 2000). The timing of sleep is controlled by a circadian system, which is seen in most living organisms and allows anticipation of the daily changes of the external world. Although these approximately 24 h rhythms persist in constant conditions, environmental fluctuations such as day: night light or temperature cycles entrain or reset rhythms to precisely 24 h periods and to an appropriate phase (Emery et al., 1998).

Besides this clock system, animals have another sleep regulation system: a homeostatic drive that increases during waking, and dissipates during sleep. Although these two systems can operate independently, recent studies indicate a more intimate relationship (Donlea et al., 2011; Naylor et al., 2000; Sheeba et al., 2008). Among other neuromodulators, dopamine (DA) appears to have an important role in controlling sleep amount, and perhaps even its timing. In the mammalian mesencephalic tegmentum, DA-containing neurons are important for arousal (Jones, 2005). As in mammals, dopamine exerts a wake-promoting function in flies (Liu et al., 2012), indicating that this and other neurotransmitter pathways (Zimmerman et al., 2017) have common functions in sleep in both flies and mammalian species.

* Corresponding author.
E-mail address: yfengw@mail.ccnu.edu.cn (Y.-F. Wang).

https://doi.org/10.1016/j.jinsphys.2018.02.011
Received 3 October 2017; Received in revised form 26 February 2018; Accepted 26 February 2018
Available online 27 February 2018
0022-1910/

To better understand the impact of *Wolbachia* infection on the host's sleep behavior, we investigated the influence of *Wolbachia* on sleep time, sleep homeostasis, circadian rhythm and arousal in *Drosophila melanogaster*.

2. Material and methods

2.1. Fly stocks

The *w*Mel *Wolbachia* infected *D. melanogaster* (Brisbane nuclear background with introgressed *w*Mel from YW), designated as Dmel *w*Mel, was kindly provided by Professor Scott O'Neill (Monash University, Australia). Tetracycline treatments were performed as described previously to generate genetically paired fly lines that were *Wolbachia*-free, here referred as Dmel T (Hoffmann et al., 1986). As tetracycline not only removes *Wolbachia* but also massively shifts the composition of the whole microbiome (Ye et al., 2017), to ensure the specificity of the effect of *Wolbachia* on sleep, we also treated a separate positive control group of flies with penicillin, which does not remove *Wolbachia* but depletes other bacteria. Penicillin treatments were performed as described previously (Gotoh et al., 2007), here referred as Dmel P. Gut flora of flies was reconstituted by standard methods (Chrostek et al., 2013) and all experiments involved in Dmel T or Dmel P flies were conducted at a minimum of six generations post antibiotic treatment. All fly lines were reared on standard cornmeal-yeast-agar medium at 25 °C with a photoperiod of 12 h:12 h LD (light:dark) under non-crowded conditions (200 ± 10 eggs per 50 mL vial of media in 150 mL conical flask).

2.2. Sleep assays

Sleep was monitored using the *Drosophila* Activity Monitoring System (DAMs, TriKinetics, Waltham, MA). This system records activity from individual flies maintained in sealed tubes placed in activity monitors. An infrared beam directed through the midpoint of each tube measures an "activity event" each time a fly crosses the beam. Four day old flies were placed in glass locomotor tubes containing 5% sucrose/2% agarose food. Locomotor activity counts were collected in 1-min bins. Sleep in this assay was defined by zero activity counts for a minimum of five consecutive minutes (Huber et al., 2004). All behavioral experiments were conducted at 25 °C in a 12 h:12 h LD cycle. For sleep deprivation experiments, flies were loaded into activity monitors as described above, and recorded for three days. On the 4th day, flies were subjected to 6 h sleep deprivation from ZT 17–23 (ZT = zeitgeber time, where ZT 0 is lights-on, and ZT 12 is lights off) by mechanical stimulation using an adapted computer-controlled vortexer (TriKinetics, Waltham, MA). Vortexing stimuli were applied for 2 s at random intervals ranging from 20 to 40 s (1–3 times per minute). Net sleep loss and the amount of recovery sleep (or sleep rebound) were calculated for each fly by subtracting the previous corresponding 12 h baseline sleep period from the 12 h nighttime (for sleep loss) or daytime period following sleep deprivation (for sleep gain). Data were processed and analyzed using PySolo (Gilestro and Cirelli, 2009) or custom software, Insomniac 3 (gift of Thomas Coradetti, Gardner et al., 2016). Statistical analyses were performed using GraphPad Prism 6. Differences in daily sleep and other parameters were evaluated with a one-way ANOVA followed by Tukey's post-hoc, or Student's *t*-test, where appropriate. All experiments were conducted a minimum of three times using 16 flies per group per experimental replicate. Flies that did not survive the duration of the experiment were excluded from the analyses.

2.3. Circadian assays

Circadian rhythm assays were performed with the DAMs as described previously (Cavanaugh et al., 2014). Flies were entrained to a 12 h:12 h LD (light:dark) cycle for > 3 days at 25 °C. Four-day-old flies were individually placed into glass tubes with 5% sucrose/2% agar food and monitored in constant darkness (DD) for 7 days at 25 °C. All behavioral experiments were performed at least 2 independent times with at least 16 flies/group each. Circadian rhythm was analyzed with ClockLab software (Actimetrics, Wilmette IL, version: 2.72). Period and rhythm strength were determined for each individual fly using activity data collected from days 2–7 of DD. Period length was determined using χ^2 periodogram analysis, and relative power (or amplitude) of circadian rhythm was determined using fast Fourier transform (FFT). Fly activity was considered rhythmic if the χ^2 periodogram showed a peak above the 95% confidence interval and the FFT value at around a 24 h cycle was > 0.01 (King et al., 2017). Differences in FFT power between groups were considered significant if $p < 0.05$ by Student *t*-test, ns: not significant.

2.4. Arousal assays

For arousal threshold assays, flies were loaded into DAMs monitors (Trikinetics Inc) as described above, and recorded for three days in a 12 h:12 h LD cycle at 25 °C. On the third day, monitors were attached to a computer-controlled vortexer, and single, 0.6 s pulses were applied at nighttime hours at ZT14, ZT16, ZT18, ZT20 and ZT22. The number of sleeping flies that were awakened by the stimulus pulse was determined using Insomniac3 (written in MSVC6, by Thomas Coradetti). This number was divided by the total number of sleeping flies at the time of the stimulus pulse and reported as percent arousal. Sleep latency, or the length of time to the first sleep bout after the arousing stimulus pulse was also measured across all flies, excluding those that did not wake up from the stimulus. Student *t*-test and Mann-Whitney *U* test were used to analyze significance. $p < 0.05$ indicated significant difference.

2.5. Quantitative reverse transcription-PCR (qRT-PCR)

Total RNA was extracted from 3 to 7 day old flies using Trizol reagent (Thermo Fisher Scientific). RNA was reverse transcribed to generate cDNA using a High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). qRT-PCR was performed on a ViiA7 Real-Time PCR System (Applied Biosystems) using SYBR Green PCR master mix (Thermo Fisher Scientific). Gene specific primers for *Ddc* and *Pale* were as follows: *Ddc*-F ACACAAATGGATGCTGGTGA; *Ddc*-R AAGAGG GTCCACATTGAACG; and *Pale*-F AGTTCTCGCAGGAGATTGGA; *Pale*-R TTCCTTGCAGAGACCGAACT. Ribosomal protein gene *rp49* (primers are: *rp49*-F CTAAGCTGTCGCACAAATGG; *rp49*-R TAAACGCGGTTCTG CATGAG) was used as an internal control. The relative expression of each gene was calibrated against the reference gene using $2^{-\Delta Ct}$ [ΔCt = Ct(target gene) − Ct(reference gene)]. $p < 0.05$ indicated significant difference by Student *t*-test.

3. Results

3.1. Wolbachia infection increases daily sleep

Dmel *w*Mel flies carrying *Wolbachia* were treated with tetracycline to render them free of infection of *Wolbachia*. We demonstrated that the effects of tetracycline treatment on sleep were stable across multiple generations (Table S1). We also treated a separate group of Dmel *w*Mel flies with penicillin for removing other bacteria but *Wolbachia* as a positive control and found that the effects of penicillin treatment on sleep in flies were also stable across at least five generations post treatment (Table S1).

We compared sleep time between *Wolbachia* infected flies, Dmel *w*Mel and Dmel P (penicillin treated *D. melanogaster*), and the tetracycline treated *Wolbachia*-free flies, Dmel T. As shown in Fig. 1A and B, the total sleep time of Dmel *w*Mel was longer than that of Dmel T for both females and males (ANOVA, $p = 0.043$, $p = 0.044$), particularly at nighttime. However, we did not observe any differences in sleep time