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The Effects of Carbamazepine on Sleep in Altered Light Environments in *Drosophila melanogaster*

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Abstract

Carbamazepine (CBZ) is an anticonvulsant and mood stabilizer that alters sleep and wakefulness. In the fruit fly *Drosophila melanogaster,* CBZ reduced sleep during the dark phase of light/dark (LD) conditions. To determine whether environmental light influences the effects of CBZ, we examined sleep under LD, constant darkness (DD) and constant light (LL). CBZ decreased sleep in all conditions, though its effect was not as restricted to the subjective nighttime in DD and especially LL. Sleep reduction occurred primarily due to an increased likelihood of waking up. Thus, the timing and magnitude of CBZ's wake-promoting mechanisms are influenced by lighting conditions.

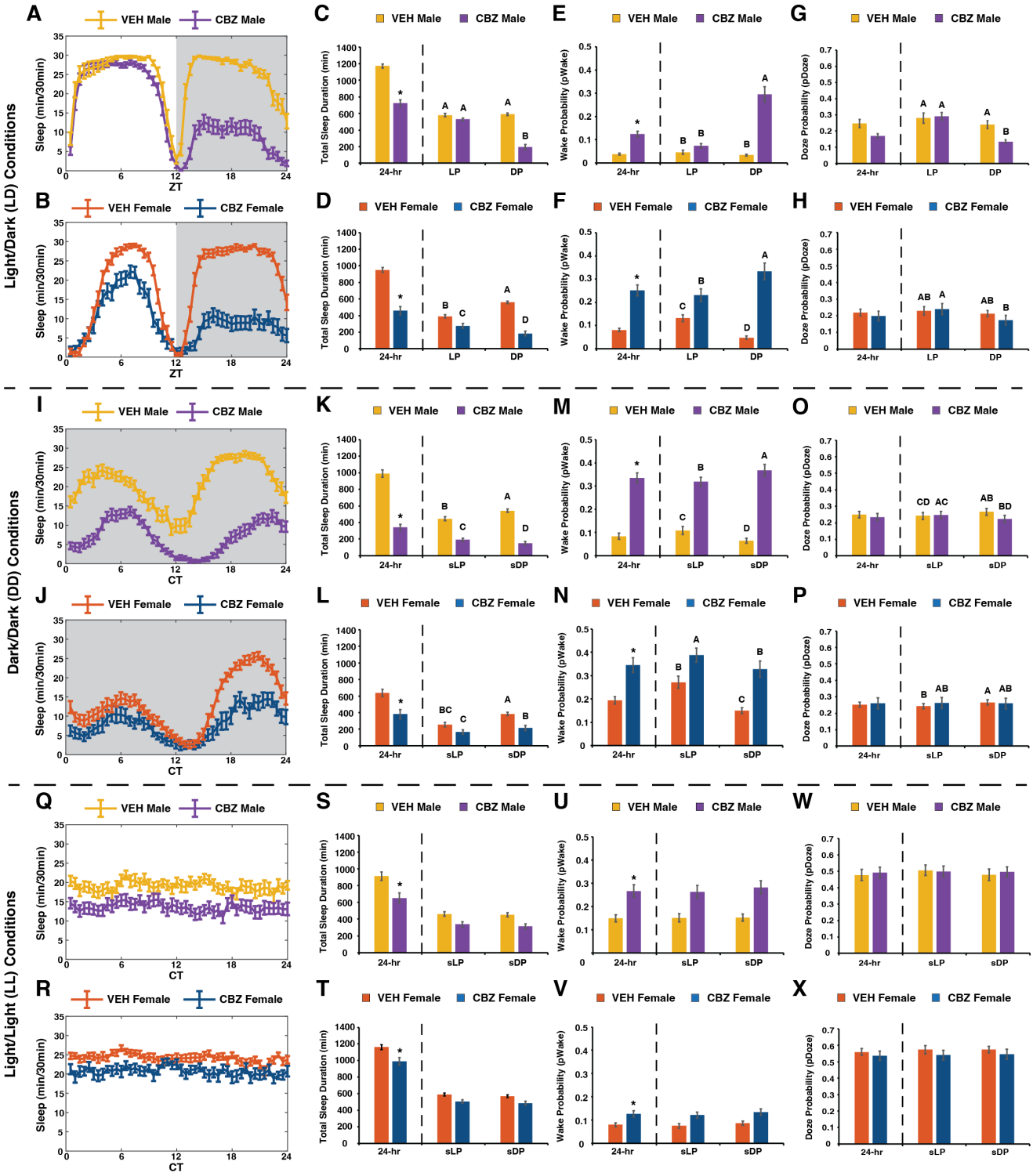


Figure 1. Light conditions affect the wake-promoting effect of carbamazepine (CBZ) in) fruit flies:

Subjects were Canton-S (CS) *Drosophila melanogaster*, and sleep was calculated in 30-minute bins using infrared beam crossings in *Drosophila* Activity Monitors. All data represent 3-day averages. (A-H) **12-hour light and dark (LD) period experiment.** (A,B) Sleep patterns of males and females fed carbamazepine (CBZ) or vehicle control (VEH). (C,D) Total sleep duration (min), (E,F) probability of transitioning to wake (pWake), and (G,H) probability of transitioning to sleep (pDoze) across 24-hour, light phase (LP), and dark phase (DP) periods. (I-P) **24-hour dark (DD) period experiment.** (I,J) Sleep patterns of males and females fed CBZ or VEH. (K,L) Total sleep duration (min), (M,N) probability of transitioning to wake (pWake), and (O,P) probability of transitioning to sleep (pDoze) across 24-hour, subjective light phase (sLP), and subjective dark phase (sDP) periods. (Q-X) **24-hour light (LL) period experiment.** (Q,R) Sleep patterns of males and females fed CBZ or VEH. (S,T) Total sleep duration (min), (U,V) probability of transitioning to wake (pWake), and (W,X) probability of transitioning to sleep (pDoze) across 24-hour, subjective light phase (sLP), and

subjective dark phase (sDP) periods. Sexes were analyzed separately using mixed-model ANOVAs with Treatment as a between-subject factor and Time of Day as a within-subject factor, followed by Tukey post-hoc tests when significant interaction effects were found. Groups that do not share a letter are significantly different from each other. Asterisks represent significant overall effects of treatment. Ns for LD and DD were 31, 24, 21, and 26 for CBZ male, VEH male, CBZ female, and VEH female, respectively. Ns for LL were 28, 27, 23, and 28 for CBZ male, VEH male, CBZ female, and VEH female, respectively.

Description

Sleep is a highly conserved behavior (Hendricks et al., 2000; Shaw et al., 2000) that contributes to our wellbeing in several ways (Perry et al., 2013; van der Kloet et al., 2012). It is therefore important to understand how different medications can influence sleep. Carbamazepine (CBZ), an FDA-approved drug since 1968, is a pharmacological treatment commonly prescribed for individuals with bipolar disorder (BD), trigeminal neuralgia, and tonic-clonic seizures, though can aggravate absence seizures (Liu et al., 2006; Ceron-Litvoc et al., 2009; Gambeta et al., 2020; Nevitt et al., 2019). CBZ is thought to exert its effects by blocking voltage-gated sodium channels and altering gamma-aminobutyric acid (GABA) signaling, thereby reducing neuronal hyperexcitability (Ambrosio et al., 2002; Motohashi et al., 1989). Interestingly, CBZ also seems to influence sleep, though the literature across species is mixed. In humans, meta-analysis has shown that one of the most common side-effects of CBZ treatments is drowsiness, especially in children (Pellock, 1987). However, a study in rats found that CBZ did not increase sleep – in fact, an intermediate dose resulted in increased wakefulness (Bertorelli et al., 1996). CBZ has also been shown to influence thalamocortical spindle generation through $GABA_A$ receptor modulation, which could contribute to CBZ's effects on sleep (Zheng et al., 2014). To further explore the effects of CBZ, researchers have turned to the fruit fly *Drosophila melanogaster*. *Drosophila* is an exceptional system in which to study sleep due to its simplicity, genetic tractability, and evolutionary conservation of sleep-regulating pathways (Dubowy and Sehgal, 2017; Roote and Prokop, 2013). Like mammals, *Drosophila's* sleep-like state is regulated by circadian and homeostatic mechanisms (Hendricks et al., 2000; Shaw et al., 2000).

A previous study found that CBZ altered sleep architecture in *Drosophila* (Agosto et al., 2008). Administration of CBZ to female flies through their food resulted in a dose-dependent decrease in sleep during the dark period under standard 12hr/12hr light/dark (LD) conditions, driven by reductions in both sleep episode number and duration. These effects were at least partially dependent on the function of the GABA-A receptor *Resistant to dieldrin (Rdl)*, with the suggestion that CBZ accelerates *Rdl*'s desensitization. A second study found similar dose-dependent reductions in nighttime sleep, which interestingly were partially mitigated by the presence of a voltage-gated sodium channel mutation modeling generalized epilepsy with febrile seizures (GEFS) (Petruccelli et al., 2015).

It was unclear from these studies why CBZ seemed to affect sleep at night but not during the day. Environmental factors, such as light exposure, can also affect circadian rhythms and sleep patterns in *Drosophila* (Dubowy and Sehgal, 2017), which may disrupt processes like metabolism, hormone release, and drug absorption, ultimately influencing drugs' effectiveness (Dallmann et al., 2016; Zhang et al., 2018; Zhang et al., 2021; Lee et al., 2021). In this study, we therefore investigated whether environmental light cues influenced the wake-promoting effects of CBZ. Specifically, we compared CBZ effects on sleep and the probability of waking and sleeping (Wiggin et al., 2020) under standard LD conditions, dark/dark (DD) or light/light (LL). Based on the greater effect of CBZ during the dark phase in LD conditions, we hypothesized that CBZ would have the greatest effect when light was not present.

Under standard LD conditions (Figure 1A-H), untreated Canton-S flies (CS) exhibited typical locomotor activity peaks at lights on (morning) and lights off (evening) with increased sleep during midday and night (Figure 1A-B). CBZ caused a significant overall decrease in sleep in both female and male flies, with the largest portion of sleep loss (88.7% in males and 76.5% in females) occurring during the nighttime (Figure 1A-C, Extended Data Table 1). The decrease in sleep was due in large part to an increase in the probability of waking (pWake) (Figure 1E-F), with a smaller effect on the probability of transitioning to sleep (pDoze) (Figure 1G-H).

Under constant dark (DD) conditions (Figure 1I-P), CBZ also reduced sleep in both sexes, with the greatest effect in males (Figure 1I-J). Sleep loss again predominantly occurred during the subjective nighttime (sDP), although the distribution of sleep loss was less extreme (60.7% in males and 65.9% in females during sDP) (Figure 1K-L). A significant increase in pWake was found during the subjective light period (sLP) and sDP in both male and female flies (Figure 1M-N), whereas no significant effect on pDoze was observed.

Under constant light (LL) conditions (Figure 1Q-X), all groups of flies became arrhythmic, with a more or less steady sleep pattern across the 24-hr period (Figure 1Q-R). CBZ still reduced sleep under these conditions, but less robustly than in LD (Figure 1S-T). Sleep loss now occurred almost evenly across the day (52.9% in males and 50.1% in females during sDP). Because sleep loss occurred equivalently during sLP and sDP, there were no significant interactions between Treatment and Time of Day (Figure 1S-X, Table 1). Consistent with patterns in LD and DD conditions, CBZ-induced sleep loss in LL was driven by a significant increase in pWake in both sexes (Figure 1U-V), with no significant effect on pDoze (Figure 1W-X). Although CBZ did produce significant reductions in sleep in all three lighting conditions, its effect

was strongest under DD and weakest under LL conditions. Combined across male and female flies, CBZ reduced sleep by 55.6% in DD conditions and by 41.5% in LD conditions, but only by 23.2% in LL conditions.

This study investigated how light condition manipulations influenced the effects of CBZ on sleep in *Drosophila melanogaster*. Under standard LD conditions, CBZ significantly reduced sleep, with a stronger wake-promoting effect during the dark period. This suggests that, in the absence of light, sleep may rely more heavily on internal neurochemical signals altered by CBZ. We also show for the first time that the effects of CBZ on sleep are mediated specifically by a change in the likelihood of transitioning from sleep to wake (pWake), with a much smaller effect on the likelihood of transitioning from wake to sleep (pDoze). This analysis suggests that this dose of CBZ is either promoting mechanisms of arousal or inhibiting sleep maintenance factors. This result is generally consistent with previous findings (Agosto et al., 2008) showing that intermediate doses of CBZ had large effects on sleep episode duration with smaller effects on sleep episode number. In constant darkness (DD), CBZ caused similar sleep reductions compared to in LD, although the magnitude of the effect in males was larger during the subjective daytime (sLP). As expected, constant light (LL) conditions disrupted circadian oscillations in sleep in CS flies. CBZ continued to have a significant wake-promoting effect in LL, but it differed in two ways. First, the effect size was smaller than in LD or DD conditions. And second, the effect was evenly distributed across the subjective day and night, whereas in LD, and to a lesser extent DD, CBZ promoted wake more strongly at night than during the day.

Overall, CBZ produced a wake-promoting effect across altered light environments, indicating that its effects are not strictly dependent on the presence or absence of light. But the more balanced distribution of CBZ's effect across the day in DD and especially LL conditions suggests that normal circadian function is required to restrict CBZ's effects to the nighttime. Additionally, the overall reduction in sleep due to CBZ treatment was qualitatively smaller under LL conditions than in LD and DD, suggesting that light exposure may reduce the wake-promoting effect of CBZ. This could be a factor in why CBZ primarily reduces sleep during the dark period under LD conditions. Future studies should examine the mechanisms through which CBZ produces these effects and whether it interacts directly with components of the circadian clock. Continued studies spanning multiple species should also be carried out to identify the mechanistic differences explaining why CBZ tends to have a somnogenic effect in humans, milder effects in rodents, and a strong wake-promoting effect in *Drosophila*.

Methods

Fly Husbandry

Canton-S flies (sourced from the lab of Dr. Leslie Griffith) were raised in a Percival incubator at 25°C on a 12hr/12hr white light schedule and were fed a cornmeal/yeast food mixture as previously described (Juneau et al., 2019). Recent offspring were sorted by sex, and female flies were combined with a few males for about three days to ensure all females would be mated, because virgin female flies have increased sleep relative to mated females (Isaac, 2019; Dove et al., 2017).

Sleep Testing

All experiments were conducted on flies aged for approximately 1 week after eclosion. Flies were anesthetized on CO_2 and added to polycarbonate tubes (5 mm diameter x 65 mm length) with food for sleep experiments, and the tubes were capped with Parafilm with a single needle hole for air and placed into *Drosophila* Activity Monitors (DAMs) (Trikinetics, Inc.) as previously described (Bond et al., 2024). Sleep was defined as a period of five consecutive minutes without any infrared beam crossings (Hendricks et al., 2000). Monitors were placed in an incubator with light exposure from white LED strips. Temperature and humidity were maintained at 25°C and >50%, respectively.

Sleep Food Preparation

Standard sleep food consisted of 5% sucrose w/v, 2% Bacto agar (Carolina Biological Supply) w/v in deionized water. To provide a dose of CBZ shown to cause near-maximal behavioral effects on sleep (Agosto et al., 2008), 0.8 mg/mL of CBZ was added to the food mixture, which was then heated to boiling in a microwave until all ingredients were fully dissolved. This general process is described in more detail in **Juneau et al. (2019).**

Light Exposure

We examined three different types of light exposure: 12hr/12hr light/dark (LD), dark/dark (DD), and light/light (LL) conditions. For the DD and LL conditions, the flies were first exposed to LD conditions before light conditions were switched to either 24-hour light or 24-hour dark. In both cases, the first day after switching light conditions was excluded from analysis.

Data Analysis

DAM data were sorted from the raw data into 1- and 30-minute bins using DAMFileScan113x (Trikinetics, Inc.). Further analysis was then performed using v2 and v4 of the Sleep and Circadian Analysis MATLAB Program (SCAMP)

(Donelson et al., 2012; Vecsey et al., 2024). Individual flies that did not survive for the entire experiment run were removed from the analysis.

Mathematical Equations

Percent reduction in total sleep duration during the (subjective) dark period was calculated as:

[(mean sleep in (subjective) dark period in control group – mean sleep in (subjective) dark period in CBZ group) / (mean total sleep in control group - mean total sleep in CBZ group)] x 100.

Statistical Analysis

Sexes were analyzed separately. Two-way mixed-model ANOVAs were run using JMP Pro 15 (SAS Institute) to compare experimental and control groups to obtain statistical significance measures, with Treatment as a between-subject factor and Time of Day as a within-subject factor. Tukey post-*hoc* tests were used to ascertain which specific comparisons had significant differences. A p-value of < 0.05 was considered significant for ANOVA and Tukey tests.

Reagents

Reagents

- Canton-S *Drosophila melanogaster*
- Carbamazepine (CBZ), 0.8 mg/mL (Sigma-Aldrich)
- Sucrose (Fisher Scientific)
- Difco Bacto Agar (Carolina Biological Supply)

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Extended Data

Description: Extended Data Table 1 contains the statistical results from all ANOVA tests performed to analyze sleep metrics.. Resource Type: Dataset. File: Waldron et al (2026) Extended Data Table 1.pdf. DOI: 10.22002/tk2v5-zk658

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