Photoperiod History Modulates Aspartame's Effects on Drosophila Circadian Rhythms

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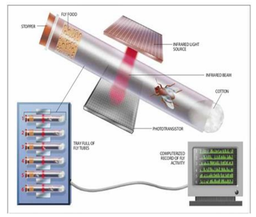
Abstract

Circadian rhythms are 24-hour internal cycles that regulate sleep, activity, metabolism, and other essential biological processes. Disruption of these rhythms can alter both physiology and behavior. As part of our ongoing research program examining how dietary chemicals interact with the circadian system, we previously observed that the non-caloric sweetener aspartame shifts circadian timing in Drosophila melanogaster, with distinct effects in males and females. However, when these experiments were repeated at different times of the year, the results varied, suggesting that environmental history, particularly the light conditions experienced during development, may influence how aspartame acts on the circadian clock.

Hypothesis: Aspartame's effects on circadian rhythms are shaped by both sex and developmental photoperiod, with prior light-exposure history determining how sensitive the circadian clock is to dietary inputs.

To investigate this emerging pattern, we examined flies raised under seasonally relevant photoperiods: long-day (16:8), short-day (8:16), and equinox (12:12). All flies were later maintained under 12:12 light:dark conditions, and their sleep and locomotor activity were assessed using the Drosophila Activity Monitoring system. Our findings indicate that aspartame's circadian effects depend not only on sex but also on the developmental photoperiod, revealing a key interaction between dietary cues and environmental light history. Together, these results highlight that environmental context is a critical, and often overlooked, factor shaping how diet-related chemicals influence circadian organization, and they advance our broader goal of understanding how external and internal signals combine to regulate temporal biology.

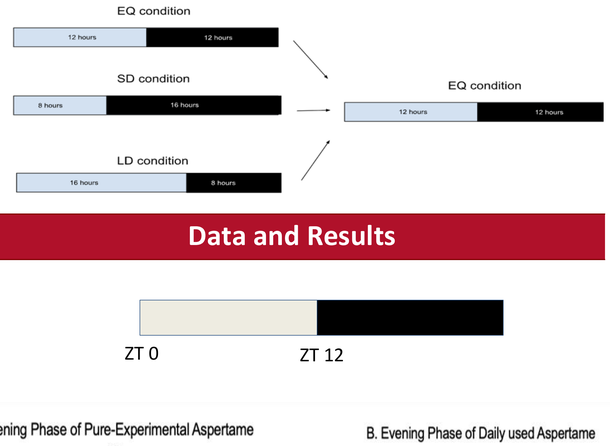
Material and Methods



Drosophila melanogaster (Oregon R strain) were reared for two weeks under one of the following photoperiod conditions:

- **Equinox (EQ):** 12 h light : 12 h dark
- **Long-day (LD):** 16 h light : 8 h dark
- **Short-day (SD):** 8 h light : 16 h dark

After rearing, flies were transferred to a standardized equinox chamber (LD12:12) for behavioral testing. Experimental groups received either standard control food or food supplemented with aspartame. Locomotor behavior was recorded using the Drosophila Activity Monitoring (DAM) system, which tracks infrared beam crossings to generate measures of activity and sleep.



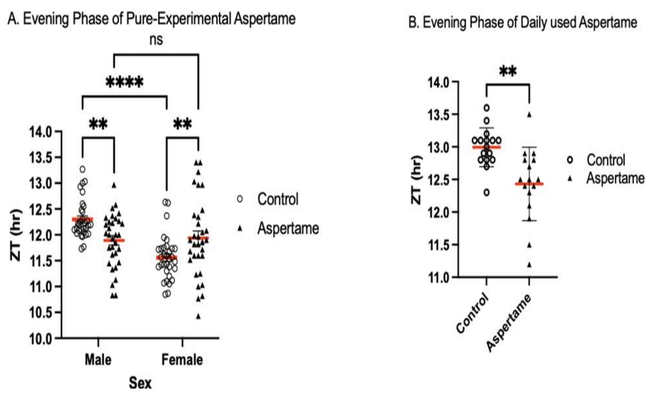


Figure 1. Aspartame's effect on evening-phase (E-phase) activity in *Drosophila melanogaster*.(A) **Pure Aspartame:** Evening-phase activity data for male and female flies treated with pure aspartame (n = 32 per group). Pure aspartame advanced the E-phase in males by approximately 26 minutes but delayed it in females, effectively removing the strong baseline sex difference observed in controls.(B) **Commercial Aspartame:** Evening-phase activity data for male flies treated with commercial aspartame (n = 32; 16 control and 16 treated). Commercial aspartame produced a similar advancing effect on males but required a higher concentration to achieve significance, suggesting reduced potency compared to pure aspartame. **Statistical significance:** ****p < 0.0001, ***p < 0.001, *$p < 0.01$, $p < 0.05$. Error bars represent mean ± SEM.

Data and Results

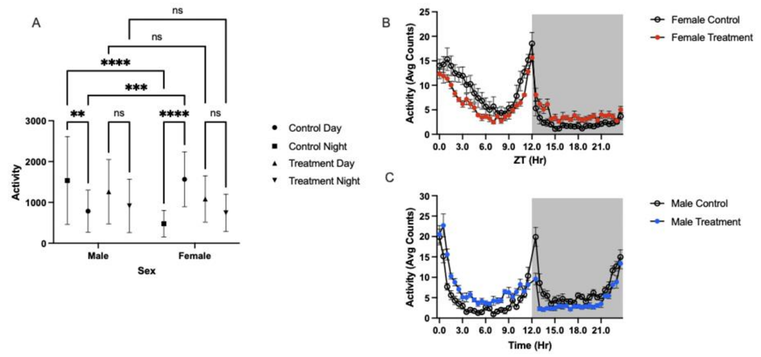


Figure 2. Aspartame's effect on motor activity in *Drosophila melanogaster*.(A) **Day and night activity:** Average activity counts for male and female flies during day and night periods (n = 32 per group). Significant differences are indicated.(B) **Female activity:** Average locomotor activity patterns for control and aspartame-treated females (n = 32 per group).(C) **Male activity:** Average locomotor activity patterns for control and aspartame-treated males (n = 32 per group). Error bars represent ± SEM.Control females showed higher activity during the morning phase, while males were more active in the evening. Aspartame treatment reduced this sex-specific difference, producing more balanced morning and evening activity across both sexes. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, ns = not significant.

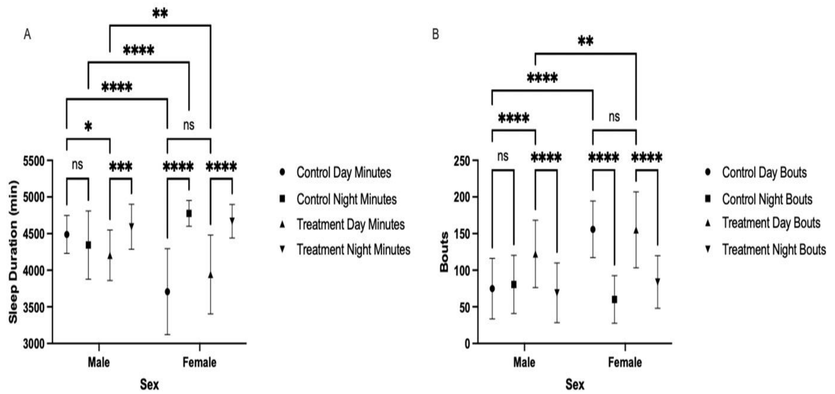


Figure 3. Aspartame's effect on sleep behavior in *Drosophila melanogaster*.(A) **Sleep Duration:** Average sleep duration for male and female flies during day and night (n = 32 per group). (B) **Sleep Bouts:** Average number of sleep bouts for male and female flies (n = 32 per group). Error bars represent ± SEM. Control females exhibited longer and more consolidated evening sleep compared to males. Following aspartame treatment, males showed increased sleep duration and reduced sleep fragmentation, whereas females remained largely unchanged. These results suggest that aspartame modulates sleep architecture in a sex-specific manner, primarily enhancing male sleep stability.****p < 0.0001. ***p < 0.001, **p < 0.01, *p < 0.05, ns = not significant.

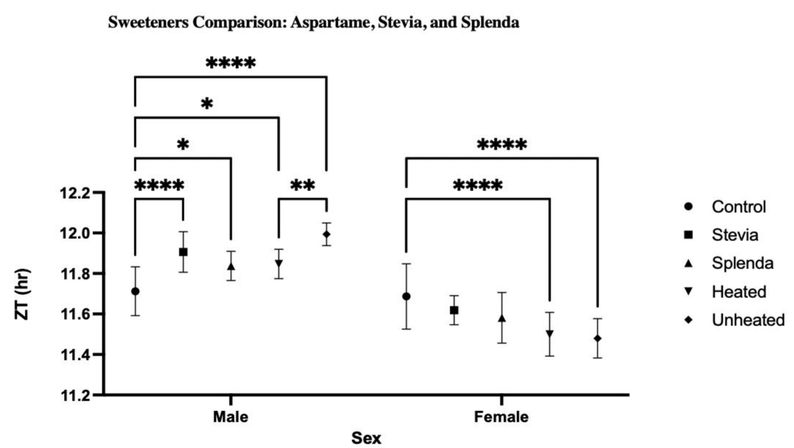


Figure 4. Effects of different non-caloric sweeteners on evening-phase (ZT) timing in male and female *Drosophila melanogaster* under equinox (LD12:12) conditions. Flies were fed standard control food or food supplemented with **Stevia, Splenda, or Aspartame (heated/unheated)**, and locomotor activity was measured using the **Drosophila Activity Monitoring (DAM)** system. Data represent mean ± SEM. All sweeteners induced significant evening-phase shifts in males, though the magnitude varied among treatments. Aspartame produced the strongest phase shift, while females exhibited smaller or non-significant responses across all treatments, indicating a clear **sex-specific sensitivity** to non-caloric sweeteners. ****p < 0.0001, **p < 0.01, *p < 0.05.

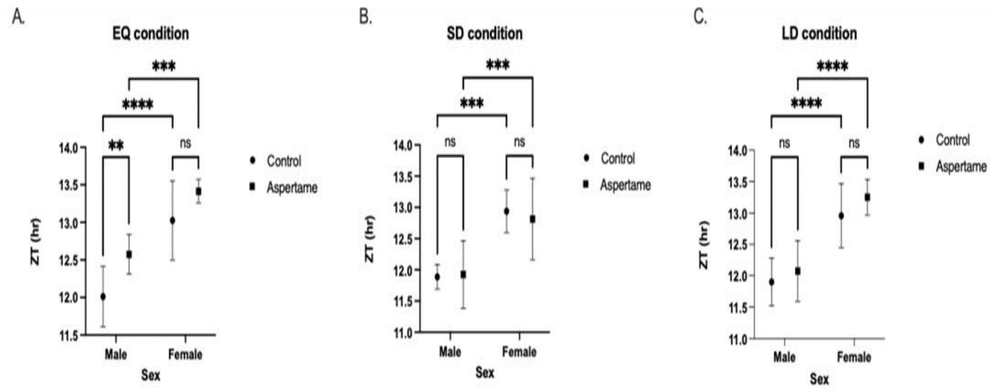


Figure 5. Photoperiod history modulates aspartame's effects on circadian phase in *Drosophila melanogaster*. (A) **Equinox (EQ) condition (n = 8):** Males showed a significant phase difference between control and aspartame groups, while females showed no treatment effect. Significant sex differences were also present between control and aspartame-treated groups. (B) **Short-day (SD) condition (n = 8):** No significant treatment effect was observed within each sex; however, significant sex differences were found between males and females in both control and treatment groups. (C) **Long-day (LD) condition (n = 8):** Neither sex showed a treatment effect, but significant sex differences were present in both control and aspartame conditions.These results indicate that **photoperiod history influences the sex-specific response to aspartame**, with males being more sensitive under equinox conditions. In contrast, long-day and short-day light histories reduced or masked treatment effects, suggesting that prior light exposure modulates how diet interacts with the circadian system.****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05; ns = not significant.Error bars represent mean ± SEM.

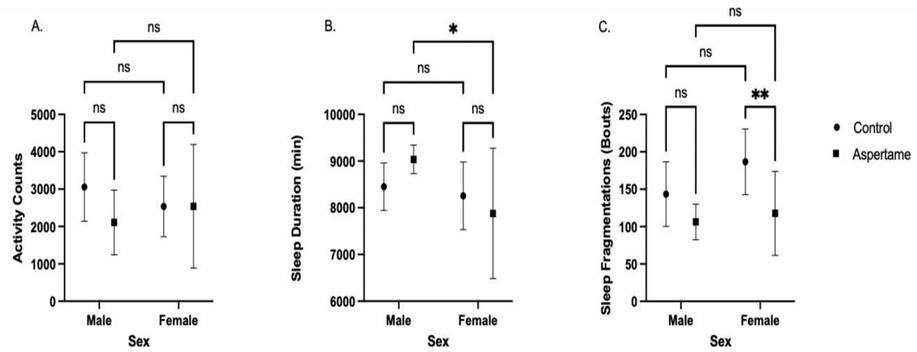


Figure 6. Activity, Sleep, and Sleep Fragmentation in EQ→EQ Flies Treated With Aspartame. Panels A–C show total activity counts (A), total sleep duration (B), and sleep fragmentation (bouts) (C) for male and female flies reared and tested under 12:12 (EQ→EQ) conditions. Circles represent control groups; squares represent aspartame-treated groups. Error bars indicate SEM.
No significant differences were observed between treatment groups for activity, sleep duration, or fragmentation, indicating that **aspartame does not alter overall locomotor activity or sleep structure in EQ→EQ flies**.
These results suggest that aspartame's primary effect is on circadian phase timing, rather than general behavior or sleep architecture.

Discussion, Conclusion and Future Direction

Our results support the hypothesis that aspartame's circadian effects depend on both sex and the photoperiodic environment the flies experienced during development. Across experiments, aspartame consistently shifted circadian phase, with males showing stronger advances, but these effects were not always stable throughout the year. This variability suggests that environmental light history changes how sensitive the circadian system is to dietary sweeteners. Flies reared under equinox (12:12) photoperiods showed the clearest and most reliable phase shifts, while long-day and short-day rearing often weakened or altered the response, indicating that extreme photoperiods may tune or buffer the circadian system differently.

Importantly, under the EQ→EQ condition, aspartame did **not** alter total activity, sleep duration, or sleep fragmentation (Figure 6). This shows that aspartame's influence is **specific to circadian phase**, rather than causing broad behavioral or sleep disturbances. The ability to shift timing without altering overall sleep or activity strengthens the idea that sweeteners act on the circadian clock itself rather than indirectly through general behavior changes.

Overall, these findings highlight that dietary sweeteners can influence circadian timing, but their effects depend on both sex and environmental conditions. This underscores the importance of considering photoperiod history when interpreting diet–circadian interactions.

For future directions, we plan to investigate the **mechanisms** behind these shifts, particularly whether photoperiod-dependent sensitivity arises from changes in **dopamine signaling**, **metabolic state**, or **feeding behavior**. We also aim to study whether aspartame works through the **sweetness pathway** or through a separate neural or metabolic pathway. These mechanistic studies will help explain why sweeteners change circadian timing and why the effect varies with season and light exposure.

Acknowledgements

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Reference

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