

Anticonvulsant effects of non-pharmaceutical treatments in bang-sensitive *D. melanogaster* mutants

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SUMMARY

Epilepsy impacts 50 million people worldwide, and approximately 30% of people with epilepsy are unresponsive to treatments. Antiepileptic medications (AEDs) can be costly and may cause harmful side-effects, calling for the development of novel, non-pharmaceutical treatments. We conducted this study to determine whether non-pharmaceutical treatments, including *Annona muricata* (soursop), β -hydroxybutyrate (BHB; representing the ketogenic diet (KD)), and circadian synchronization, are effective in reducing duration of seizure-like behavior in bang-sensitive (bs) *Drosophila melanogaster* mutants, an established model for epilepsy research, and whether circadian synchronization can improve the effectiveness of KD. We hypothesized that non-pharmaceutical treatments, including soursop, KD, and circadian synchronization, either alone or in combination, would decrease recovery time in bs *D. melanogaster* mutants. For soursop treatment, recovery time was measured before *D. melanogaster* mutants were given vortex-induced seizure and measured again after treatment with soursop. For BHB treatment, recovery times of flies given BHB prior to vortex-induced seizure were compared with those of untreated flies. The effect of circadian synchronization (via entrainment to light-dark cycles) was measured alone, as well as in combination with BHB. We observed that treatment with soursop resulted in longer recovery times when compared with no treatment. Non-pharmaceutical interventions such as KD and circadian synchronization both reduced seizure duration in bs *D. melanogaster*. However, the interaction between BHB and circadian synchronization was not statistically significant. Further exploration of the interaction between circadian synchronization and medication effectiveness in fly models and humans is necessary for the development of safe, accessible treatments for epilepsy in humans.

INTRODUCTION

Epilepsy, characterized by repeated, unprovoked seizures, is one of the most common neurological disorders seen in adults and children, affecting approximately 50 million people worldwide (1-3). Seizures are the primary clinical manifestation of epilepsy and occur when there is increased

electrical activity between neurons (1,4). While seizures can be triggered by acute factors such as fever, head injury, or substance withdrawal, epilepsy is the occurrence of repeated, unprovoked seizures, often the result of underlying conditions such as genetic mutations, structural brain abnormalities, or metabolic dysfunction (1,4,5). Epilepsy consists of multiple seizure types and syndromes, each with distinct clinical manifestations and responses to treatment, this variability makes diagnosing and treating epilepsy difficult, since treatments effective for one seizure type may be ineffective or harmful in another (1,5).

Antiepileptic medications (AEDs) are the primary treatment for seizure control in people with epilepsy, and work on a variety of neural pathways and mechanisms. However, approximately 30% of patients still experience seizures despite pharmaceutical treatment (2). Even among individuals whose seizures are controlled by AEDs, AED usage is often associated with side effects including fatigue, dizziness, gastrointestinal distress, and cognitive impairments (2,5). In addition, some AEDs can be expensive, with costs up to \$10,000 per year, making them inaccessible for many patients, specifically those from lower socioeconomic backgrounds (6). Lastly, despite the availability of numerous AEDs for different epilepsy types, the exact mechanisms by which many of these drugs treat seizures remain unknown (1,5). These limitations highlight the need for accessible, low-cost, and low-side-effect non-pharmaceutical or adjunct seizure interventions.

Model organisms are essential for epilepsy research because they allow for controlled investigation of the mechanisms of seizures and timely testing of therapeutic interventions without the constraints of human studies (1). Bang-sensitive (bs) *Drosophila melanogaster* is an established and valuable model for epilepsy. These mutants carry a gain-of-function mutation, p.L1699F, in the voltage-gated sodium channel gene *paralytic*, which causes neuronal hyperexcitability and makes the flies susceptible to seizure-like behavior when mechanically stimulated (7,8). When mutant flies are vortexed, they demonstrate a predictable pattern of convulsing followed by paralysis before returning to a standing position (8,9).

Traditional medicine has historically served as a method of treating neurological and metabolic disorders, yet many plant-based treatments have not been evaluated using epilepsy models. One such non-pharmaceutical remedy, *Annona senegalensis*, a species of plant in the Annonaceae (custard apple) family, was found to be effective at preventing and controlling seizure-like behavior in bs *D. melanogaster* mutants (10). Another species of the same family is *A. muricata* (soursop), which is more common than *A. senegalensis* and often used in traditional medicine to treat a variety of health

conditions (11). It is unknown whether soursop may have similar antiepileptic properties to *A. senegalensis*.

Another non-pharmaceutical treatment includes the ketogenic diet (KD). KD is a high-fat, low-carbohydrate diet that induces ketosis, a metabolic state where the body shifts from using glucose to using ketone bodies for energy (12). KD has been used clinically for the treatment of epilepsy for almost a century, particularly for pediatric and treatment-resistant patients (13). β -hydroxybutyrate (BHB), a metabolite produced during ketosis, is commonly used to mimic KD in experimental studies and has been shown to reduce frequency and severity of seizure-like behavior in both mice and bs *D. melanogaster* mutants (14,15).

Similarly, there has been investigation into the impact of circadian synchronization on seizures. Circadian rhythms are the body's 24-hour biological cycles that regulate behavior and physiology, including sleep-wake patterns, metabolism, and neuronal excitability (16,17). Circadian desynchronization refers to the disruption of the body's internal clock system due to misalignment with environmental cues or between the master and peripheral circadian clocks (17-19). The relationship between epilepsy and circadian rhythm is bidirectional, meaning seizures disrupt sleep and circadian synchronization, and circadian desynchronization can increase seizure activity (16,20). Furthermore, the molecular mechanisms behind circadian clocks are highly conserved between humans and *D. melanogaster*, making flies a useful model for studying circadian influences on epilepsy (16,20,21). Previous studies using bs flies found that circadian synchronization decreases seizure-like behavior and circadian disruption through sleep deprivation increases seizure-like behavior (19,22).

Although these non-pharmaceutical interventions have each been shown to reduce seizure susceptibility in human and animal models, they have been studied independently. There is a lack of research exploring whether circadian synchronization can enhance the effectiveness of dietary therapies such as KD. Addressing this gap is important for identifying low-cost and accessible interventions that may improve seizure management. We aimed to determine whether non-pharmaceutical treatments, including soursop, BHB, and circadian synchronization, individually or in combination, are effective for controlling seizure-like behavior in bs *D. melanogaster* mutants. We hypothesized that each treatment, alone or combined, would reduce recovery time in bs *D. melanogaster* mutants compared to untreated controls. To test this hypothesis, we investigated four experimental parameters: 1) the effect of soursop on recovery time, 2) the effect of consecutive seizures on recovery time, 3) the effect of KD modeled through BHB supplementation on recovery time, and 4) whether circadian synchronization enhances the effectiveness of BHB treatment on recovery time. In this study, BHB supplementation and circadian synchronization each significantly reduced recovery time, while soursop treatment did not significantly reduce recovery time, and no significant interaction was observed between BHB and circadian synchronization.

RESULTS

To investigate the effects of three treatments on duration of seizure-like behavior following vortex stimulation in bs flies, we conducted four experiments. Part I tested soursop treatment, part II examined the effect of repeated vortex

stimulation, part III tested BHB supplementation, and part IV tested circadian synchronization and examined its interaction with BHB treatment.

Part I: Soursop treatment increased recovery time in bs flies

To study the effects of soursop on duration of seizure-like behavior, we fed the bs and wild-type (wt) flies with either soursop-treated (+) or untreated (-) medium and then induced seizure-like behavior via vortex before measuring the recovery time (**Figure 1**). Flies in the wt - ($n = 10$) and bs - ($n = 6$) groups were vortexed once before data collection, while flies in the wt + ($n = 10$) and bs + ($n = 4$) groups were vortexed once during the control trial, followed by soursop treatment, and vortexed a second time before data collection, with a three-day interval between vortexes.

After vortexing, the flies recovered in a staggered manner. Comparison of recovery times determined that there was a statistically significant difference in the recovery times of flies among the four groups (wt +, wt -, bs +, bs -; **Figure 2**; one-way ANOVA, $F(3,26) = 28.08$, $p < 0.001$). Post-hoc comparisons using the Tukey HSD test showed that the bs + flies demonstrated significantly longer recovery times compared to the bs - flies (post-hoc test, mean difference = 5.67, 95% CI [0.94, 10.39]; $p = 0.01$). In contrast, there was no significant difference between the wt - and wt + groups (post-hoc test, mean difference = 0, 95% CI [-3.27, 3.27], $p = 1.00$), as both wt groups demonstrated immediate recovery following vortex. The wt - flies showed statistically significantly shorter recovery times than the bs - group (post-hoc test, mean difference = 6.5833, 95% CI [2.80, 10.36], $p < 0.001$), and the wt + flies showed statistically significantly shorter recovery times than the bs + group (post-hoc test, mean difference = 12.25, 95% CI [7.92, 16.56], $p < 0.001$). These results suggest that soursop treatment increased recovery time in bs flies, indicating a negative effect.

Part II: Consecutive inductions of seizure-like behavior did not significantly affect recovery time

To study whether consecutive inductions of seizure-like behavior affected recovery time, we subjected bs flies to one (Group one; $n = 17$) or two consecutive vortex exposures

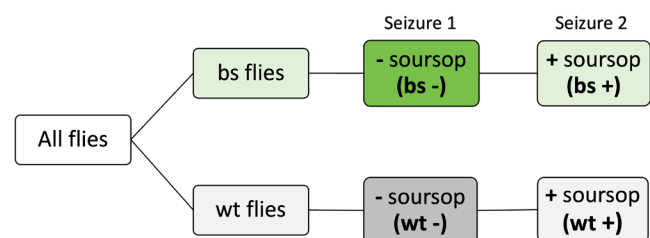


Figure 1: Part I experimental design to examine effects of soursop treatment on recovery time. Each type of fly was divided into two groups (four groups in total). One group of each type of flies was set aside as a control group and did not get vortexed (i.e., no induced seizure behavior). No data was collected on these flies. The second group of each type of flies received mechanical stimulation using a vortex in two sequential trials, first without and then with soursop-infused fly medium. The experimental groups that underwent mechanical induction of seizure-like behavior are named wild-type (wt) without soursop (wt -), wt with soursop (wt +), bang-sensitive (bs) without soursop (bs -), and bs with soursop (bs +).

separated by a three-day interval (Group two; $n = 9$), and recovery times were compared. A three-seizure group was planned, but no flies survived to undergo a third vortex. Flies exposed to one and two consecutive vortices showed similar recovery times (**Figure 3**). There was no statistically significant difference in recovery times between flies undergoing their first vortex exposure and flies undergoing their second vortex exposure (unpaired t-test, $T(24) = 1.21$, 95% CI [-0.46, 1.77], $p = 0.24$). Overall, these results indicate that consecutive inductions of seizure-like behavior did not significantly alter recovery time in bs flies.

Part III: BHB supplementation reduced recovery time in bs flies

To study the effects of KD supplementation, we fed bs flies medium containing BHB ($n = 18$) or untreated medium ($n = 21$) before inducing seizure-like behavior via vortex and measuring recovery time. Following vortex stimulation, BHB-treated flies recovered more quickly than untreated flies (**Figure 4**). Different flies were used for the BHB-treated and untreated groups; therefore, each fly was vortexed once prior to recovery time measurement. Comparison of recovery times showed a statistically significant difference between the recovery times of flies treated with BHB and untreated flies; the BHB-treated flies demonstrated shorter recovery times compared to the untreated flies (unpaired t-test, $T(37) = 3.05$, 95% CI [-2.53, -0.51], $p = 0.004$). Overall, these results indicate that BHB reduced duration of seizure-like behavior in bs flies, suggesting that it was an effective treatment.

Part IV: Circadian synchronization and BHB independently reduced recovery time without significant interaction

To study the effects of circadian synchronization and its interaction with BHB treatment, we entrained bs flies to either a 12-hour light-dark (LD) cycle (synchronization, sync)

or constant darkness (DD) (desynchronization, desync), with or without BHB supplementation, before inducing seizure-like behavior via vortex and measuring recovery time (**Figure 5**). The groups were: LD without BHB (sync -; $n = 12$), DD without BHB (desync -; $n = 21$), LD with BHB (sync +; $n = 50$), and DD with BHB (desync +; $n = 20$) for both bs and wt flies. All wt groups each consisted of more than 30 flies.

Following vortex stimulation, recovery times varied among the four experimental groups (**Figure 6**). Comparison of recovery times determined that there was not a statistically significant interaction between the effects of BHB and synchronization (**Figure 7**; two-way ANOVA, $F(1,99) = 3.22$, $p = 0.076$). Two-way ANOVA revealed significant main effects of BHB on recovery time (two-way ANOVA, $F(1,99) = 4.24$, $p = 0.042$), and of synchronization on recovery time (two-way ANOVA, $F(1,99) = 6.94$, $p < 0.01$). Post-hoc comparisons using the Tukey HSD test showed that desync - flies had significantly longer recovery times than sync - flies (post-hoc test, mean difference = 2.31, 95% CI [0.30, 4.32], $p = 0.018$), sync + flies (post-hoc test, mean difference = 2.41, 95% CI [0.97, 3.85], $p < 0.001$), and desync + flies (post-hoc test, mean difference = 1.81, 95% CI [0.08, 3.54], $p = 0.037$). In contrast, no statistically significant difference was observed between sync - and sync + flies (post-hoc test, mean difference = 0.10, 95% CI [-1.68, 1.89], $p = 0.999$), sync - and desync + flies (post-hoc test, mean difference = 0.50, 95% CI [-1.53, 2.53], $p = 0.918$), or sync + and desync + flies (post-hoc test, mean difference = 0.60, 95% CI [-0.87, 2.07], $p = 0.709$). Overall, these results indicate that both circadian synchronization and BHB independently reduce duration of seizure-like behavior, but do not significantly interact.

DISCUSSION

Our results of this multi-part experiment revealed that non-pharmaceutical treatments can have positive impacts on duration of seizure-like behavior following vortex stimulation

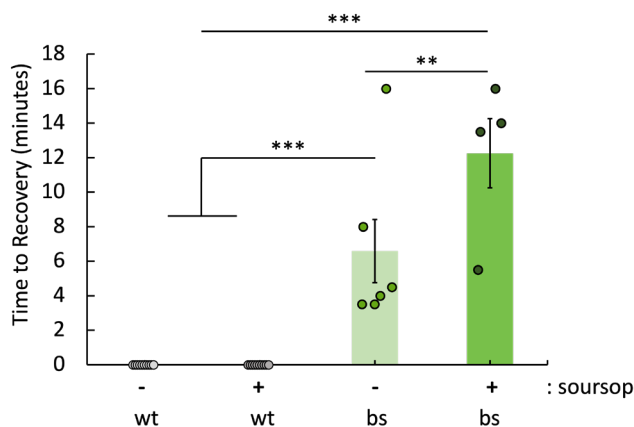


Figure 2: Mean recovery times of soursoop treated and untreated flies. Bang-sensitive (bs) or wild-type (wt) flies were treated with (+) or without (-) soursoop. Data shown as mean $\pm$ standard error of mean. $n = 10$ for wt +, $n = 10$ for wt -, $n = 6$ for bs -, and $n = 4$ for bs +. Non-vortexed control groups each had $n = 10$. One outlier was detected using the Grubbs' Test; the data point was retained because no experimental error was identified, and the removal of the outlier did not significantly change the interpretation of the results. Asterisks denote statistical significance determined by one-way ANOVA and Tukey HSD tests (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

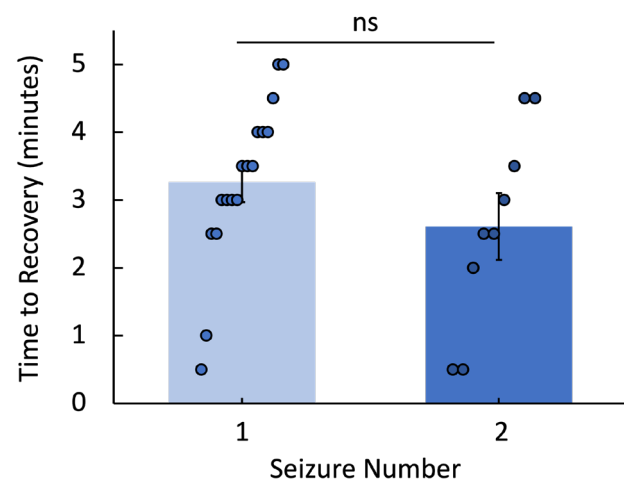


Figure 3: Mean recovery times of bang-sensitive (bs) flies comparing the number of consecutive vortex exposures. Data shown as mean $\pm$ standard error of mean. No outliers were detected using the Grubbs' Test. $n = 17$ for first seizure and $n = 9$ for second seizure. Asterisks denote statistical significance determined by unpaired t-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

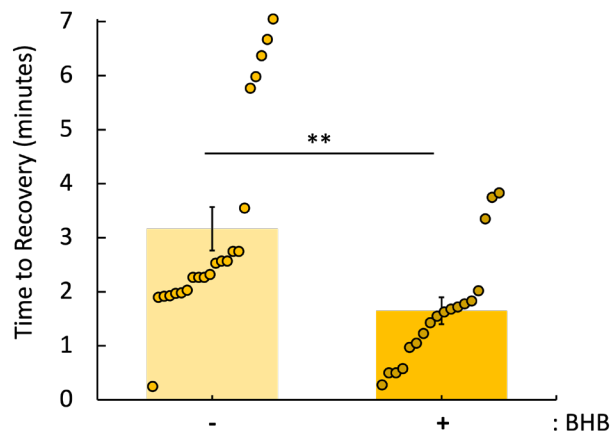


Figure 4: Mean recovery times of β -hydroxybutyrate (BHB) treated and untreated flies. BHB on the graph below indicates whether flies were treated with (+) or without (-) BHB. Data shown as mean $\pm$ standard error of mean. $n = 18$ BHB-treated bang-sensitive (bs) flies and $n = 21$ untreated bs flies. Asterisks denote statistical significance determined by unpaired t-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

in bs *D. melanogaster* mutants. Although the part I experiment did not reveal benefits from soursop treatment, both KD and circadian synchronization independently reduced recovery time. However, circadian synchronization did not improve the effectiveness of BHB treatment, as there was no significant interaction between synchronization and BHB. These findings are in concordance with existing literature and hold promise for development of novel interventions to manage epilepsy in humans (14,15,19).

In part I, when treated with soursop, bs flies demonstrated significantly longer recovery times compared with untreated bs flies. These findings are contrary to the expected results that *D. melanogaster* bs mutants treated with soursop would have shorter durations of seizure-like behavior following mechanical induction. In a previous study, bs flies treated with *A. senegalensis* (wild soursop), a related species from the same Annonaceae family, exhibited significantly shorter recovery times than untreated flies (10). Despite being of the same plant family, differences in phytochemical composition of the two species' leaves, specifically differences in the types and concentrations of alkaloids, acetogenins, and antioxidants, could be a cause for the differing results on the recovery times, as these compounds have been found to affect neural excitability (6,7). The lack of support for the original hypothesis could have also been a result of the reuse of flies from the bs - and wt - (untreated) groups to the bs + and wt + (treated) groups, which was necessary due to the limited availability of flies. The bs + and wt + flies were vortexed twice with two days in between before data collection, while the bs - and wt - flies were only vortexed once.

This reuse of flies raised the concern that consecutive vortex exposure could influence recovery time and potentially confound results. For this reason, we conducted part II to determine whether repeated mechanical stimulation affects duration of seizure-like behavior in bs flies. Bs flies given two consecutive vortex exposures demonstrated recovery times that were not statistically different from bs flies given one

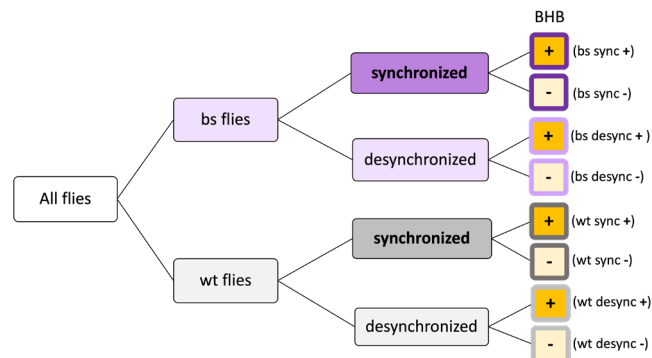


Figure 5: Part IV experimental design to examine effects of circadian synchronization with and without BHB treatment on recovery time. Wild-type (wt) flies were included in this study to act as controls for bang-sensitive (bs) flies. Each type was split into four groups (eight total): synchronized (sync) or desynchronized (desync) with circadian rhythms, and either treated with (+) or without (-) β -hydroxybutyrate (BHB). The resulting groups were bs sync +/-, bs desync +/-, and wt desync +/--. All flies were subjected to mechanical stimulation via vortex.

vortex exposure. These findings indicate that prior seizure experience did not significantly alter sensitivity in the flies, supporting the idea that reusing flies for multiple trials in part I likely did not confound recovery times.

We initially hypothesized that consecutive vortex exposures might lengthen recovery time due to fatigue or cumulative neural stress in the flies. Although the findings of this study did not support this hypothesis, shorter intervals between vortexes may still affect recovery time. We also observed that flies in the third group all died before a third vortex could be given. This may indicate that consecutive vortex exposure caused physiological weakening of the flies rather than actual changes in seizure sensitivity, though it cannot be excluded that the flies died due to another cause.

In part III, when treated with BHB, bs flies demonstrated statistically significantly shorter recovery times compared with untreated bs flies. These findings are consistent with previous studies and further suggest that KD and BHB supplementation are potential treatments for epilepsy in humans (14,15).

In part IV, the final part of this study, we aimed to determine if circadian synchronization could decrease recovery time as a stand-alone intervention, and whether it could improve the effectiveness of BHB for decreasing recovery time in bs flies. Both circadian synchronization and BHB treatment independently reduced recovery time, supporting previous research and suggesting that circadian synchronization and the supplementation of BHB are potential treatments for epilepsy in humans (14,15,19,21,23). However, the interaction between the two factors was not significant, suggesting that synchronization did not conclusively improve the effectiveness of BHB. Across all four experiments, several limitations should be noted. First, experiments were performed with small and unequal numbers of flies and were not independently repeated due to time and resource limitations, which may have reduced the statistical power and generalizability of the results. Environmental and biological variables, particularly temperature

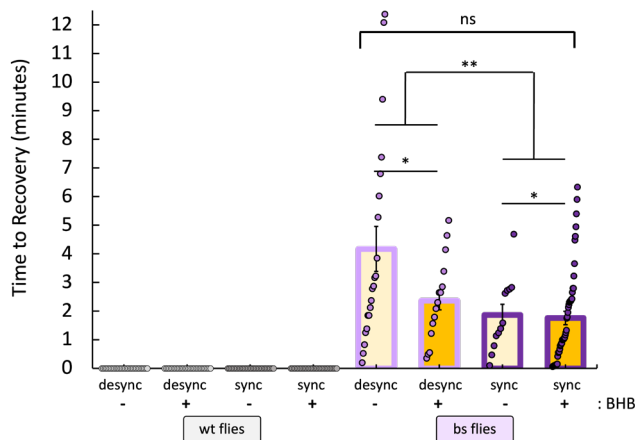


Figure 6: Interaction between synchronization and β -hydroxybutyrate (BHB) treatment on mean recovery time of flies. Wild-type (wt) flies were included in this study to act as controls for bs flies. Data shown as mean $\pm$ standard error of mean. No outliers were detected using the Grubbs' Test. $n = 21$ for bs desync -, $n = 20$ for bs desync +, $n = 12$ for bs sync -, and $n = 50$ for bs sync +. All wt groups had $n > 30$. Asterisks denote statistical significance determined by two-way ANOVA and Tukey HSD tests (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

and age, may have introduced variability in recovery time. Additionally, different bs fly strains were used across the experiments. Part I used mutants of the gene *wpara^{bss1}* (white eyes), while parts II, III, and IV used mutants of the gene *parabss1* (red eyes) because the white eye strain was no longer available. It is unknown whether these genetic differences affect their sensitivity to the treatment. A limitation specific to part I is that untreated bs flies were measured after one vortex exposure, while soursop-treated flies were measured after two. Although part II found no significant effect of consecutive vortex exposure on recovery time, we cannot determine whether it influenced the results of part I. Therefore, the observed increase in recovery time cannot be attributed to soursop treatment alone. Finally, we did not measure food consumption or confirm whether BHB induced ketosis or whether the flies were truly synchronized, such as through ketone tests or measuring molecular markers (22).

Continued evaluation of interventions across models, as well as exploration of accessible therapies, remains necessary. Future studies should test different treatment concentrations, larger sample sizes, sex-specific analyses, comparisons with AEDs, and confirm ketosis and circadian synchronization.

In summary, this study demonstrates that bs *D. melanogaster* can serve as an effective model for investigating how diet and circadian rhythms influence seizure severity. While soursop unexpectedly increased recovery time, both BHB and synchronization significantly reduced it, although synchronization did not have a significant interaction with the effectiveness of BHB. These findings suggest that both metabolic and circadian factors may play important roles in regulating and treating seizures, highlighting potential approaches for safe, accessible, and non-pharmaceutical treatments for epilepsy in humans.

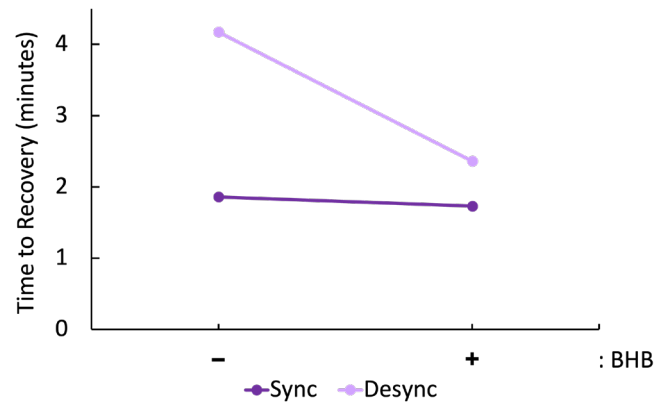


Figure 7: Interaction plot showing the combined effects of synchronization and β -hydroxybutyrate (BHB) treatment on recovery time in flies. Lines represent mean recovery times (minutes) for synchronized (sync) and desynchronized (desync) bang-sensitive (bs) flies, with and without BHB treatment. Parallel lines indicate minimal interaction between the two factors, while any divergence suggests a potential interaction effect. Wt flies were not included as no seizure data was collected.

MATERIALS AND METHODS

Overview

The alpha level for statistical significance used in this study was $p < 0.05$. Statistical analyses were performed using Statistics Kingdom and GraphPad online statistical calculators (24,25). One-way ANOVA with Tukey's HSD post hoc test was used for comparisons between multiple groups, while unpaired two-tailed t-tests were used for comparisons between two groups. A two-way ANOVA was used to examine the interactive and main effects of two treatments. Outlier analysis was performed using Grubbs' test. We used Shapiro-Wilk and Levene's tests included in the statistical calculators to determine if data passed normality and homogeneity of variance assumptions. Most of the data met assumptions, however there were some deviations from normality and equal variance in groups with smaller sample sizes. Graphs were generated in Microsoft Excel and assembled in Microsoft PowerPoint. Environmental and biological variables such as temperature, humidity, food condition, number of flies per vial, age, and sex were not strictly controlled across experiments. Due to limited fly availability, some experiments used the same flies sequentially within the same experiment. Flies were not reused across different experimental parts. Bs (*wpara^{bss1}*) and wt (Canton-S) *D. melanogaster* used in part I were obtained from Dr. Elaine Reynolds of Lafayette College. Bs (*parabss1*) and wt (Canton-S) flies used in parts II, III, and IV were obtained from Dr. Atulya Iyengar of the University of Alabama. The *parabss1* mutation is an established gain-of-function mutation in the voltage-gated sodium channel gene *para* (26). Wt flies were included as controls for bs flies.

Fly medium preparation

A 1:1 ratio of Formula 4-24® Instant *Drosophila* Medium Blue (Carolina Biological; cat. no. 173120) and distilled water was used. About 10 g of medium was distributed into each culture vial and 1-3 grains of active dry yeast (Fleischmann's)

were sprinkled onto the surface of the medium in each vial to promote fermentation. This was consistently maintained across all experiments.

Soursop preparation and treatment

To prepare soursop extract for part I, 25 g of dried soursop leaves (1400s Spices; ASIN B0B1VPX919) were ground with a blender until pulverized to a powder. The powder was mixed with 350 mL distilled water in a beaker and left to soak for 72 hours. Using a sieve, particulates were removed from the mixture. Next, the filtrate was incubated in a glove box for about one week to evaporate the water. This resulted in a powdered soursop extract. This extract was then dissolved in distilled water to create a 1.33 mg/mL concentration of soursop extract powder in distilled water. 1.5 mL of this solution was distributed into each vial of fly medium. The solution was mixed into the medium and vials were left to dry for two days before introducing flies.

BHB preparation and treatment

To prepare the BHB medium for parts III and IV, a 2 mM solution of BHB was created by dissolving 10.4 mg of sodium BHB (BulkSupplements; product no. SBHB-P) in 50 mL of distilled water. This solution was combined with equal parts instant medium formula, about 10 g total in each vial, and 2–3 grains of yeast were sprinkled on top of the medium in both control and experimental vials.

Fly seizure induction and measurement of seizure duration

To induce seizure-like behavior, flies were transferred to empty vials and spun on a bench-top vortex (Fisher Scientific; model no. 945404; cat. no. 02215365) at maximum speed for 10 seconds. To determine recovery time following vortex stimulation, the experimental vials were inspected visually and by video recording to count the number of flies per vial that were on their backs, paralyzed, or shaking (seizure-like behavior) versus the number standing (recovered), at 30 second intervals for 15 minutes. This definition of seizure activity in flies is consistent with Dare et al. (10).

Experimental design and data collection

In part I, recovery time from seizure activity was compared between flies who had fed on soursop extract with those that had not (**Figure 1**). Flies were observed for recovery time after each trial. First, flies without soursop were tested. They were left to feed in a vial without soursop for 48 hours before they were transferred to an empty vial for seizure induction. Seizure-like behavior was induced and recovery time data were collected. Immediately after, the same flies were moved into a vial containing soursop and left to feed for 48 hours before they were transferred to an empty vial for seizure induction. Seizure-like behavior was induced and recovery time data were collected. The same flies were tested first in the untreated condition and then in the soursop-treated condition.

In part II, flies were divided into three experimental groups to compare recovery time after one or two consecutive vortex exposures. Group one was vortexed once, group two was vortexed twice with a three-day interval between each vortex, and group three did not survive to a third round of seizure induction. Recovery time was recorded for each fly after each

seizure induction, excluding flies that died. For analysis, flies were grouped according to the number of vortexes they had experienced.

In part III, recovery times were compared between BHB-treated and untreated bs flies. Seizure-like behavior was induced in BHB-treated and untreated bs flies and recovery time data were collected. Unlike parts I and II, different flies were used between the two treatment groups.

In part IV, recovery times were compared between synchronized and desynchronized flies, with and without BHB treatment (**Figure 5**). For untreated groups (bs sync -, wt sync -, bs desync -, and wt desync -), fly medium was prepared normally. For treated groups (bs sync +, wt sync +, bs desync +, and wt desync +), a 2 mM solution of BHB was created and distributed into vials. The same fly vials were used across conditions, with the four seizure trials separated by seven days. Each vial underwent four vortex exposures total in the following sequence: sync -, desync -, sync +, and DD with BHB (desync +). Flies were entrained using a light-based circadian synchronization protocol (15). Flies were placed into a sealed cardboard box inside of a cabinet to control exposure to outside light. A timed 5000K full-spectrum white light (Barrina; model no. MC05; ASIN B0BKPF8D8G) was attached to the top of the inside of the cardboard box. Seizure-like behavior was induced and measured sequentially across light conditions and treatment. First, flies were placed into vials without BHB and entrained to a 12:12 light:dark (LD) cycle for 7 days (circadian synchronization), after which seizures were induced via vortex in an empty vial and recovery time was recorded. Flies were then entrained under constant darkness (DD) for 7 days (circadian desynchronization), and recovery times were again measured. After that, flies were transferred to BHB-containing vials and entrained under LD, followed by seizure measurement. Finally, flies were entrained under DD on BHB food, and recovery times were measured once more. Seizures were induced during the light phase of the LD cycle for synchronized flies, at approximately 15:00, and at the same clock time for desynchronized flies.

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REFERENCES

1. Stafstrom, Carl E. and Lionel Carmant. "Seizures and Epilepsy: An Overview for Neuroscientists." *Cold Spring Harb Perspect Med*, vol. 5, no. 6, Jun 2015, <https://doi.org/10.1101/cshperspect.a022426>.
2. Mutanana, Ngonidzashe et al. "General Side Effects and Challenges Associated with Anti-Epilepsy Medication: A Review of Related Literature." *Afr J Prim Health Care Fam Med*, vol. 12, no. 1, Jun 2020, pp. e1–e5, <https://doi.org/10.4102/phcfm.v12i1.2162>.
3. Thijs, Roland D. et al. "Epilepsy in Adults." *Lancet*, vol.

- 393, no. 10172, Feb 2019, pp. 689–701, [https://doi.org/10.1016/S0140-6736\(18\)32596-0](https://doi.org/10.1016/S0140-6736(18)32596-0).
4. Stroke, National Institute of Neurological Disorders and. "Epilepsy and Seizures." <https://www.ninds.nih.gov/health-information/disorders/epilepsy-and-seizures>.
5. Perucca, Emilio. "An Introduction to Antiepileptic Drugs." *Epilepsia*, vol. 46 Suppl 4, Jun 2005, pp. 31–37, <https://doi.org/10.1111/j.1528-1167.2005.463007.x>.
6. Terman, Samuel W. et al. "Changes in the Use of Brand Name and Generic Medications and Total Prescription Cost among Medicare Beneficiaries with Epilepsy." *Neurology*, vol. 99, no. 8, Aug 2022, pp. e751–e761, <https://doi.org/10.1212/WNL.0000000000200779>.
7. Kuebler, Daniel and Mark Tanouye. "Anticonvulsant Valproate Reduces Seizure-Susceptibility in Mutant *Drosophila*." *Brain Res*, vol. 958, no. 1, Dec 2002, pp. 36–42, [https://doi.org/10.1016/S0006-8993\(02\)03431-5](https://doi.org/10.1016/S0006-8993(02)03431-5).
8. Reynolds, Elaine R. et al. "Treatment with the Antiepileptic Drugs Phenytoin and Gabapentin Ameliorates Seizure and Paralysis of *Drosophila* Bang-Sensitive Mutants." *J Neurobiol*, vol. 58, no. 4, Mar 2004, pp. 503–513, <https://doi.org/10.1002/neu.10297>.
9. Pavlidis, P. et al. "The *Drosophila* Easily Shocked Gene: A Mutation in a Phospholipid Synthetic Pathway Causes Seizure, Neuronal Failure, and Paralysis." *Cell*, vol. 79, no. 1, Oct 1994, pp. 23–33, [https://doi.org/10.1016/0092-8674\(94\)90397-2](https://doi.org/10.1016/0092-8674(94)90397-2).
10. Dare, Samuel S. et al. "*Drosophila* Para (Bss) Flies as a Screening Model for Traditional Medicine: Anticonvulsant Effects of *Annona Senegalensis*." *Front Neurol*, vol. 11, Jan 2021, p. 606919, <https://doi.org/10.3389/fneur.2020.606919>.
11. Gavamukulya, Yahaya et al. "Phytochemical Screening, Anti-Oxidant Activity and in Vitro Anticancer Potential of Ethanolic and Water Leaves Extracts of *Annona Muricata* (Graviola)." *Asian Pac J Trop Med*, vol. 7s1, Sep 2014, pp. S355–363, [https://doi.org/10.1016/S1995-7645\(14\)60258-3](https://doi.org/10.1016/S1995-7645(14)60258-3).
12. D'Andrea Meira, Isabella et al. "Ketogenic Diet and Epilepsy: What We Know So Far." *Front Neurosci*, vol. 13, Jan 2019, p. 5, <https://doi.org/10.3389/fnins.2019.00005>.
13. Pulford, D. S. "Ketogenic Diets for Epileptics." *Cal West Med*, vol. 27, no. 1, Jul 1927, pp. 50–56, <https://pmc.ncbi.nlm.nih.gov/articles/PMC1655541/>.
14. Li, Jinglu et al. "The Ketogenic Diet Metabolite Beta-Hydroxybutyrate (β -Hb) Reduces Incidence of Seizure-Like Activity (Sla) in a K(Atp)- and Gaba(B)-Dependent Manner in a Whole-Animal *Drosophila Melanogaster* Model." *Epilepsy Res*, vol. 133, Jul 2017, pp. 6–9, <https://doi.org/10.1016/j.epilepsyres.2017.04.003>.
15. Fogle, Keri J. et al. "Ketogenic and Anaplerotic Dietary Modifications Ameliorate Seizure Activity in *Drosophila* Models of Mitochondrial Encephalomyopathy and Glycolytic Enzymopathy." *Mol Genet Metab*, vol. 126, no. 4, Apr 2019, pp. 439–447, <https://doi.org/10.1016/j.ymgme.2019.01.008>.
16. Jin, Bo et al. "Epilepsy and Its Interaction with Sleep and Circadian Rhythm." *Front Neurol*, vol. 11, May 2020, p. 327, <https://doi.org/10.3389/fneur.2020.00327>.
17. Bazhanova, Elena D. "Desynchronization: Types, Main Mechanisms, Role in the Pathogenesis of Epilepsy and Other Diseases: A Literature Review." *Life (Basel)*, vol. 12, no. 8, Aug 2022, <https://doi.org/10.3390/life12081218>.
18. Aschoff, J. et al. "Desynchronization of Human Circadian Rhythms." *Jpn J Physiol*, vol. 17, no. 4, Aug 1967, pp. 450–457, <https://doi.org/10.2170/jphysiol.17.450>.
19. Lucey, Brendan P. et al. "A New Model to Study Sleep Deprivation-Induced Seizure." *Sleep*, vol. 38, no. 5, May 2015, pp. 777–785, <https://doi.org/10.5665/sleep.4674>.
20. Liu, Wei K. et al. "Sleep and Epilepsy." *Semin Pediatr Neurol*, vol. 48, Dec 2023, p. 101087, <https://doi.org/10.1016/j.spen.2023.101087>.
21. Ozkaya, Ozge and Ezio Rosato. "The Circadian Clock of the Fly: A Neurogenetics Journey through Time." *Adv Genet*, vol. 77, Dec 2012, pp. 79–123, <https://doi.org/10.1016/B978-0-12-387687-4.00004-0>.
22. Schlichting, Matthias. "Entrainment of the *Drosophila* Clock by the Visual System." *Neurosci Insights*, vol. 15, Feb 2020, p. 2633105520903708, <https://doi.org/10.1177/2633105520903708>.
23. Tataroglu, Ozgur and Patrick Emery. "Studying Circadian Rhythms in *Drosophila Melanogaster*." *Methods*, vol. 68, no. 1, Jun 2014, pp. 140–150, <https://doi.org/10.1016/j.ymeth.2014.01.001>.
24. "Graphpad Prism." GraphPad Software <https://www.graphpad.com/quickcalcs/ttest1/>. Accessed November 26 2024.
25. "One Way Anova." Statistics Kingdom <https://www.statkingdom.com/180Anova1way.html>. Accessed November 26 2024.
26. Parker, L. et al. "*Drosophila* as a Model for Epilepsy: Bss Is a Gain-of-Function Mutation in the Para Sodium Channel Gene That Leads to Seizures." *Genetics*, vol. 187, no. 2, Feb 2011, pp. 523–534.

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