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Research Article

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QUANTIFYING BEHAVIORAL ENTROPY AS A MECHANISTIC INDICATOR OF NEURAL STATE UNDER SLEEP DEPRIVATION IN DROSOPHILA MELANOGASTER

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Abstract

In the United States, coffee is the most widely consumed beverage, with approximately 68% of Americans drinking it daily. Caffeine is widely used as a neural stimulant; however, its consumption has been associated with negative health effects including cardiovascular issues, insomnia, anxiety, and reduced bone density in women. These concerns motivate the search for alternative dietary compounds capable of sustaining cognitive alertness while minimizing system instability. From a statistical physics standpoint, such compounds may act as external agitators capable of shifting the stability of biological systems away from equilibrium.

This study investigates locomotor dynamics in sleep-deprived Drosophila melanogaster as a model system to evaluate the effects of caffeine and plant-derived polyphenols on neurobehavioral system stability. Drosophila were subjected to chronic sleep deprivation and randomly assigned to one of nine dietary treatment groups containing varying concentrations of caffeine, polyphenols, or a combination of both. Furthermore, two-dimensional motion trajectories were recorded and analyzed as stochastic processes that evolve in phase space.

Entropy, Mean Squared Displacement (MSD), Diffusion Coefficient (D), Anomalous Diffusion (α), and Drift were calculated to quantify individual and system-wide Drosophila behavior. Then, statistical analyses were performed using Kruskal-Wallis tests with a P value of $p<0.05$.

Results indicate that polyphenol-treated Drosophila exhibited the reduced behavioral entropy (H ≈ 0.62-0.68) as well as lower diffusion coefficients (D ≈ 0.48-0.54), indicating more stable system dynamics and decreased behavioral variability. In contrast, caffeine treatments increased both entropy and diffusion (H ≈ 0.72-0.81; D ≈ 0.60-0.[illegible]1), with higher values for higher dosages of caffeine, reflecting increased system movement stochasticity. Control Drosophila displayed intermediate values (H ≈ 0.69; D ≈ 0.55) and provided a baseline for metric comparison.

Overall, these findings show that polyphenols stabilize behavioral dynamics during sleep deprivation while caffeine amplifies system stochasticity. In a wider context, this work connects traditional statistical locomotion physics to neural states, creating a framework for describing macroscopic behavioral dynamics in sophisticated biological systems.

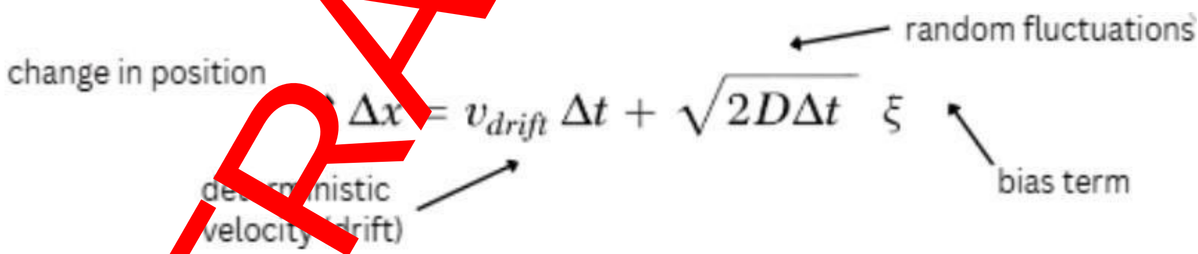
Keywords: Behavioral entropy; Neural state dynamics; Sleep deprivation; Drosophila melanogaster; Neurobehavioral analysis; Mechanistic biomarkers

Introduction

Currently, sleep deprivation is a major issue in society, with approximately 36.8% of Americans reporting getting less than 7 hours of sleep on average. The negative effects of sleep deprivation are often self-medicated by individuals via the consumption of caffeine [1-3]. Coffee, for example, is the most widely consumed beverage in the United States, with 68% of Americans consuming at least one cup of coffee daily. Similarly, there has been a 7% increase in coffee consumption since 2020, highlighting a potential societal trend [1]. Most people report consuming coffee, and other neuro-stimulants, to alleviate fatigue and promote alertness in demanding cognitive environments. Despite its widespread usage, studies have shown that coffee consumption leads to negative health outcomes including cardiovascular issues, anxiety, insomnia, liver cancer, and more [3]. For the vulnerable populations relying on coffee, withdrawal can often have negative effects including headache (79%), fatigue (42%), and irritability (36%) [4]. This creates a unique problem: Consumers rely on coffee to mitigate the effects of sleep deprivation but end up opening doors to potential health issues and dependency.

Beyond biological implications, sleep deprivation can be interpreted through the lens of system dynamics. According to Critical Brain Theory (CBT), neural systems regularly fluctuate between order and disordered states. Thus, the brain functions as a nonlinear system operating near criticality, a system that balances these states to optimize information processing [5]. When neural systems fall into a subcritical state, information sharing between neurons becomes heavily constrained; meanwhile, when neural systems are in a supercritical state, excessive neuron firing makes the system unstable [6]. On a similar note, sleep deprivation tends to disrupt the critical brain, pushing neural systems away from equilibrium and criticality.

Moreover, observable locomotor behavior creates a non-invasive window into underlying neural dynamics. This is because movement trajectories emerge from the combination of sensory information processing, motor planning, and neuro-modulatory regulation. Instead of interpreting motion only as a behavioral output, this study analyzes movement trajectories as stochastic processes that evolve through phase space; in short, allowing biological systems to be examined through a statistical physics lens. In this framework, Entropy, mean squared displacement, Diffusion coefficients, Anomalous diffusion, and Drift serve as the metrics for analysis derived from the Langevin Equation.



$$\Delta x = v_{drift}\,\Delta t + \sqrt{2D\Delta t}\;\xi$$

A Langevin equation is a differential equation that describes the evolution of a system when subjected to deterministic (stagnant) and fluctuating forces. In this study, locomotor motion is modelled as a stochastic process combining directed drift and random fluctuations, consistent with Langevin dynamics. In this context, it serves to model biological diffusion in mm 2/second and random motion, as theorized by Paul Langevin.

Table 1: Showcases different metrics and their equations, meaning, and interpretation. Created by Clara Martins De Bellis Silva in Canva.

Metric	Equation	What it represents	Interpretation of values
Entropy	$H_{norm}=H/\log(n)$	Behavioral unpredictability	Higher entropy relates to more random motion
MSD	$MSD(\tau)=\langle\lvert x(t+\tau)-x(t)\rvert^2\rangle$	Spatial exploration	Higher MSD relates to more system exploration/super diffuse regime
D	$D = MSD(\tau)/4\tau$	Diffusion strength	Higher D values relate to faster movements
α	$\alpha = d\log(MSD)/d\log(\tau)$	System stability	Smaller α related to more stability
Drift	$\langle x(t)\rangle=V_{drift}t$	Directional bias toward food	More negative relates to more bias toward food

All in all, these metrics serve as the macroscopic descriptors of system-level organization due to neural interactions.

On a different note, dietary compounds have the potential to significantly influence these properties. Caffeine primarily functions as a blocker of adenosine receptors, directly increasing neural excitation and alertness. While this process may enhance alertness, the rapid increase in neural activity may lead to constrained neural firing patterns, altering system stability. In other words, caffeine has the potential to force the majority of neurons to activate, rapidly sharing information, and pushing the brain to supercritical states, at the expense of memory retention and healing. In contrast, polyphenols are plant-derived phytochemicals with antioxidant and neuroprotective properties. Polyphenols are found in foods such as green tea (Camellia sinensis) and cocoa (Theobroma cacao) products such as chocolate. Moreover, polyphenols influence cell signalling pathways, modulate transcription factors, and enhance the expression of neurological factors that support neuron growth and synaptic plasticity [7]. Similarly, these compounds have been seen to promote cerebrovascular function, reducing neuroinflammation, and potentially supporting neural flexibility under stressors. Even though polyphenols have been widely studied for their benefits, effects on system-level dynamics remain unstudied.

Particularly, few published studies that examined whether dietary compounds have the ability to alter system stability during sleep deprivation.

Therefore, the present study addresses this research gap by examining the locomotor behavior of Drosophila melanogaster under chronic sleep deprivation and a variety of dietary treatments. Through treating locomotion as an active stochastic process, this study combines statistical physics with biological neuroscience to investigate quantitative metrics of neural state.

Research objectives

The main objective of this study is to quantify how different treatments affect neural stability under sleep deprivation through a physics-based framework. Treatments contained caffeine, polyphenols, and combined compounds of both were applied to the model system. In this study, sleep deprivation is treated as a sustained external perturbator applied to the model system. Neural stability is evaluated through the use of measurable physics observables applied to locomotor trajectories, ex. Entropy, MSD, Diffusion Coefficients, etc.

Furthermore, this research characterizes how sleep deprivation affects large-scale neural dynamics through the aforementioned metrics. Also, it explores the specific role of polyphenols in system dynamics under sleep deprivation. Overall, it interprets locomotor trajectories as stochastic processes that evolve over time, linking microscopic neural activity to macroscopic behavioral outcomes.

Methodology

Subjects and experimental design

Adult Drosophila melanogaster was used as the model organism due to their conserved sleep regulation mechanisms and suitability for studying neural dynamics under controlled perturbations. Drosophila were

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randomly assigned to nine experimental groups representing different dietary conditions under chronic sleep deprivation. Each group contained five biological replicates consisting of five flies per Petri dish, resulting in a total sample size of 225 flies.

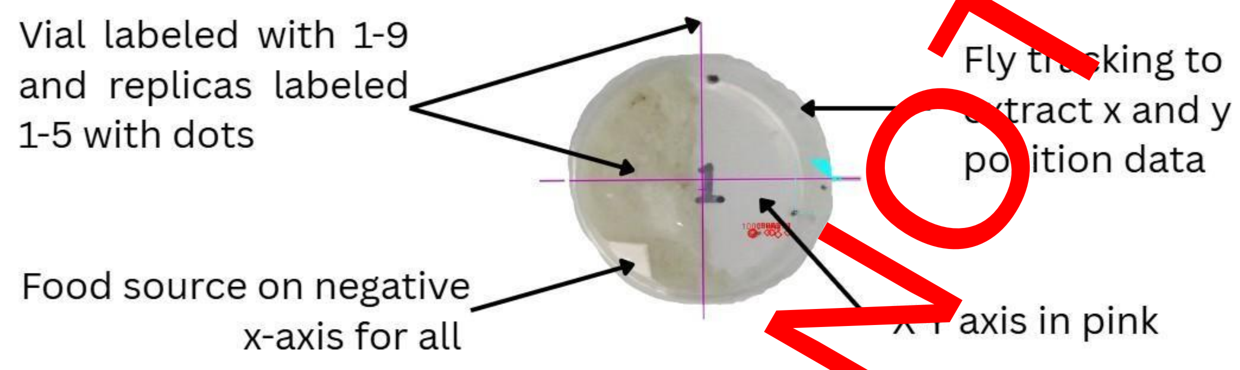

Illustration 1: Petri dish arrangement showing five flies per dish across nine experimental groups.

Dietary treatments: Dietary compounds were incorporated into standard Drosophila food medium. Caffeine was administered using powdered coffee extract, while polyphenols were supplied through matcha green tea powder and cocoa powder. Treatments were prepared at concentrations of 0.5 g, 1.0 g, or 1.5 g per 100 mL of food medium. Combined treatment groups received proportional concentrations of both compounds.

Treatment Group	Concentration (g/100mL)
1. Control	N/A
2. Low Caffeine	0.5 gram
3. Medium Caffeine	1.0 gram
4. High Caffeine	1.5 grams
5. Low Polyphenol	0.5 gram
6. Medium Polyphenol	1.0 gram
7. High Polyphenol	1.5 grams
8. Medium Combined	1.0 gram
9. High Combined	2.0 grams

Illustration 2: Describes the nine treatment groups; all gram values are per 100ml of food medium; 5 Drosophila melanogaster in one petri dish.

Sleep deprivation protocol: All experimental groups were subjected to chronic mechanical sleep deprivation using an orbital shaker operating at [illegible]00 rotations per minute for 16 hours per day.

This mechanical agitation functioned as an external forcing mechanism preventing entry into stable sleep states. Environmental conditions were maintained at 25°C with controlled lighting to minimize extraneous environmental variability.

Behavioral recording & tracking: Locomotor behavior was recorded daily following sleep deprivation. Each Petri dish contained a food source positioned along the negative x-axis to introduce a consistent environmental gradient influencing movement trajectories. Video recordings were conducted for 500 seconds per replicate. Two-dimensional locomotor trajectories were extracted using automated tracking software at a sampling rate of one frame per second.

Statistical analysis: Behavioral metrics exhibited non-normal distributions; therefore, non-parametric statistical methods were applied. Kruskal-Wallis tests were used to compare entropy, diffusion, and drift across treatment groups. Statistical significance was defined as $p<0.05$.

Results

Behavioral entropy

Behavioral entropy values differed across treatment conditions. Median entropy increased progressively across caffeine treatments, rising from 0.6894 in the control group to 0.8092 in the high-caffeine condition. This trend suggests that caffeine exposure increased the randomness of locomotor trajectories, producing greater stochastic variability in movement behavior. In contrast, polyphenol treatments produced a consistent reduction in entropy. Median entropy declined from 0.682[illegible] in the low polyphenol group to 0.6191 in the high polyphenol condition, indicating a progressive stabilization of locomotor behavior. Combined treatments produced intermediate entropy values. The medium combined treatment produced a median entropy of 0.7071, while the high combined condition produced 0.7170, suggesting that caffeine partially counteracted the stabilizing effects of polyphenols.

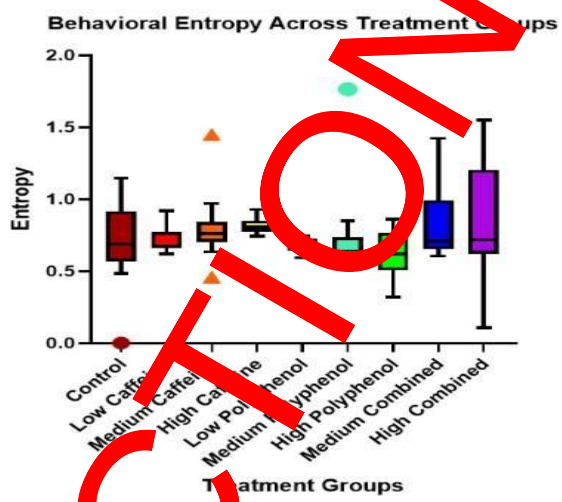

Figure 1: A bar graph with error bars for Entropy across treatment groups; a higher entropy implies more system randomness. Created by Clara Martins De Bellis Silva in GraphPad (2026).

The distribution of entropy values was assessed using a Q-Q plot, which revealed clear deviations from normality. A probability density plot further confirmed non-Gaussian distribution of entropy values across treatment groups. Because the data violated normality assumptions, non-parametric statistical tests were used. A Kruskal-Walli's test revealed statistically significant differences between treatment groups, indicating that dietary interventions significantly altered locomotor entropy.

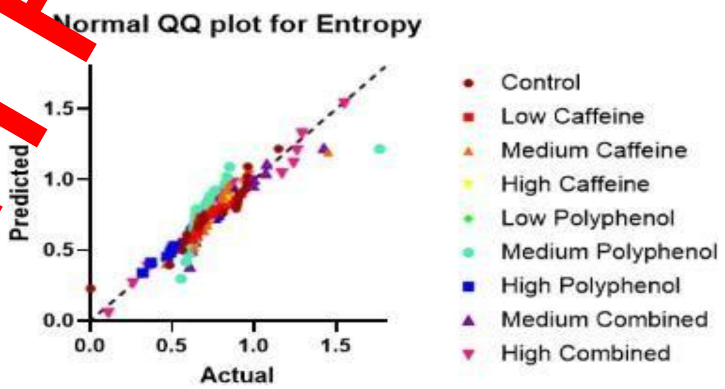

Figure 2: A normal quartile-quartile plot for Entropy implies a non-Gaussian distribution due to values across treatments not matching the predicted values. Created by Clara Martins De Bellis Silva in GraphPad.

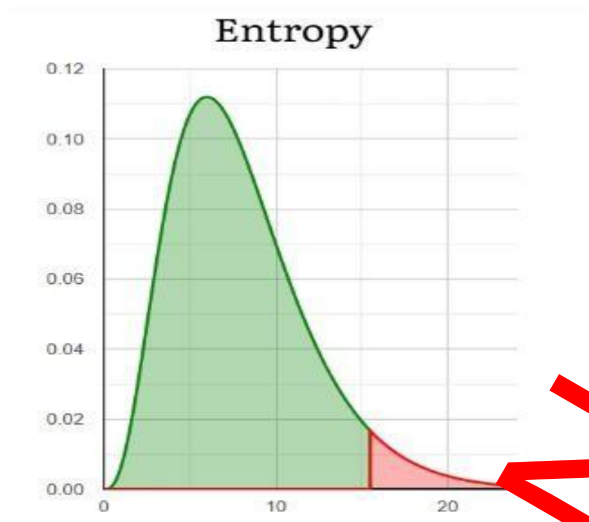

Figure 3: Chi-square distribution and group differences for entropy.

The test statistic (H=25.44, df=8) lies in the upper tail of the chi-square distribution, indicating a significant group effect. The Kruskal-Walli's test showed differences in entropy across groups (p=0.0013), with a moderate-to-large effect size (η^2=0.11). Post hoc comparisons revealed multiple significant pairwise differences, demonstrating meaningful variation in entropy between experimental conditions. Created by Clara Martins De Bellis Silva in GraphPad.

Diffusion Dynamics (MSD & D)

Mean-squared displacement analysis revealed treatment-dependent differences in locomotor diffusion. Diffusion coefficients increased progressively across caffeine treatments, rising from D=0.5545 in controls to D=0.7124 in the high caffeine condition. This increase indicates greater spatial displacement over time and suggesting that caffeine exposure promoted increased behavioral activation and exploratory movement. In contrast, polyphenol treatments reduced diffusion coefficients. The high polyphenol condition exhibited the lowest diffusion value (D=0.4805), indicating constrained locomotor movement and reduced spatial diffusion. Combined treatments again produced intermediate diffusion values, with diffusion coefficients of 0.5760 and 0.6055 in the medium and high combined conditions respectively.

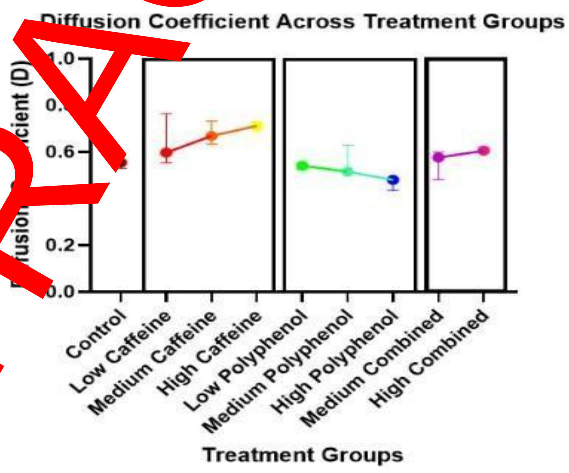

Figure 4: A scatterplot of median with IQR for Diffusion Coefficient across treatment groups; a higher diffusion coefficient implies faster movement across concentrations. Created by Clara Martins De Bellis Silva in GraphPad.

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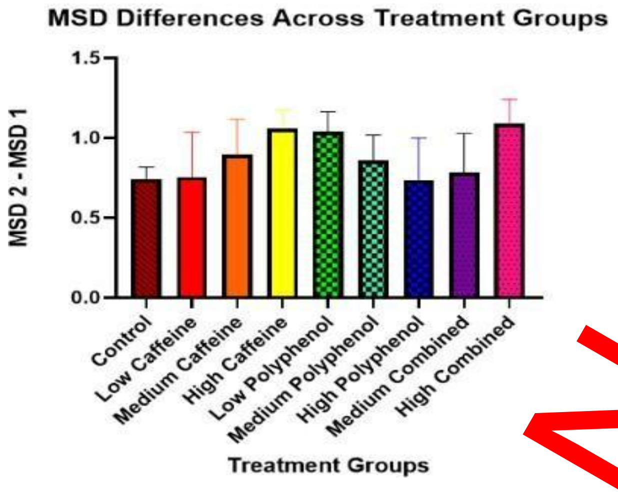

Figure 5: A bar graph of MSD 2 minus MSD 1 across treatment groups with error bars; a higher difference implies increasing spread over time across concentrations. Created by Clara Martins De Bellis Silva in GraphPad.

When entropy and diffusion were mapped in behavioral phase space, treatment groups separated into distinct dynamical regimes. Caffeine groups occupied regions of higher entropy and diffusion, indicating increased system instability. Polyphenol groups clustered in regions of lower entropy and diffusion, reflecting more stable locomotor dynamics.

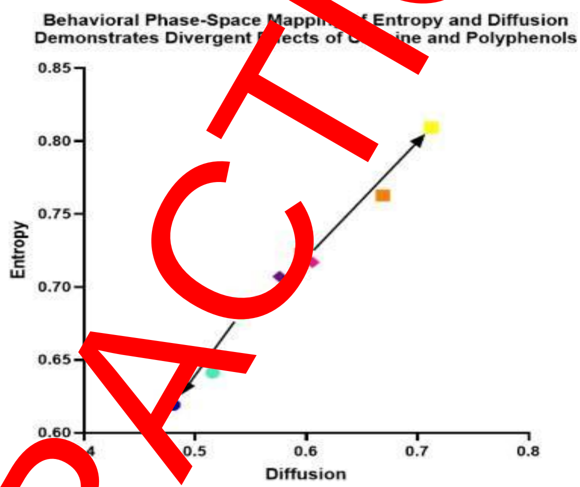

Figure 6: A scatterplot of the behavioral phase-space mapping of Diffusion vs Entropy. Caffeine-treated groups exhibited a positive slope while polyphenol-treated groups exhibited a negative slope. Created by Clara Martins De Bellis Silva in GraphPad.

Anomalous diffusion exponent

Control flies exhibited a median exponent of α=0.59[illegible]4, indicating sub-diffusive behavior consistent with constrained exploratory movement.

Caffeine treatments increased α values, reaching 0.7410 in the high-caffeine group, suggesting a shift toward more persistent locomotor activity. Polyphenol treatments produced lower α values, decreasing to 0.5250 in the high-polyphenol condition, further supporting the interpretation that polyphenols stabilize locomotor dynamics. Combined treatments again produced intermediate exponents (α=0.6185-0.6317).

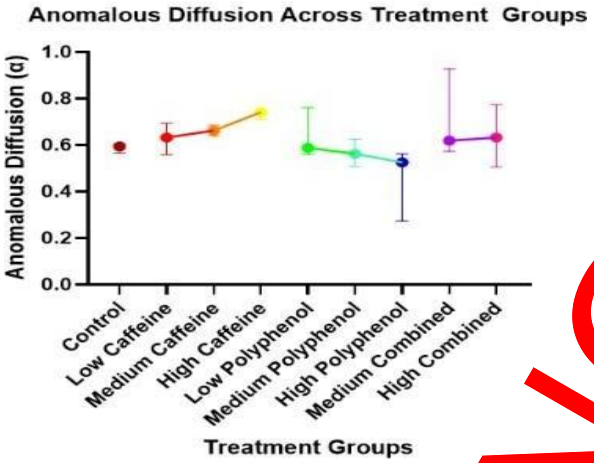

Figure 7: A scatterplot of median with IQR for Anomalous Diffusion across treatment groups; a higher anomalous diffusion implies a less stable system across concentrations. Created by Clara Martins De Bellis Silva in GraphPad.

Drift and directional bias

Directional drift values revealed consistent movement toward the food source across all treatment groups. Drift values were negative in all conditions, reflecting directional bias toward the food positioned along the negative x-axis. Caffeine treatments produced increasingly negative drift values, reaching -0.3416 in the high-caffeine condition, indicating stronger directional dependence on the food source. Polyphenol treatments reduced the magnitude of drift, with the high polyphenol condition exhibiting a drift value of -0.2574. This reduction suggests decreased dependence on environmental food cues and more stable exploratory behavior. Combined treatments again produced intermediate drift values (-0.3222 to -0.3294).

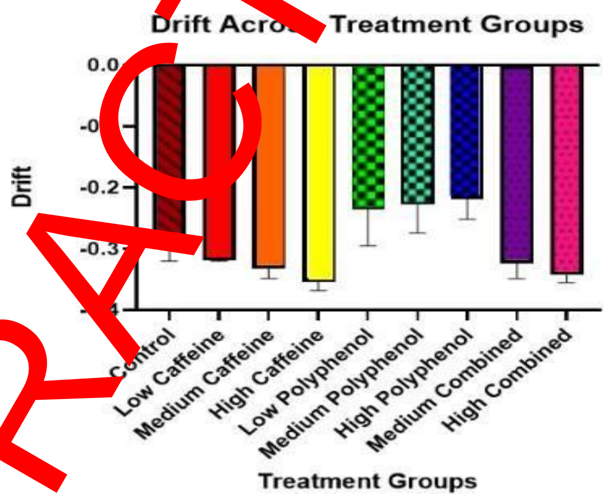

Figure 8: A bar graph with error bars for Drift across treatment groups; a more negative drift implies a higher food bias. Created by Clara Martins De Bellis Silva in GraphPad.

Mortality

Mortality levels increase with increasing levels of caffeine, that is, 12% mortality was observed for control flies, whereas 28%, 36%, and 48% mortality levels were observed for low, medium, and high levels of caffeine, respectively. Treatment with polyphenols resulted in a survival effect, as observed by 16%, 12%, and 12% mortality for low, medium, and high levels of polyphenols, respectively. Caffeine and polyphenols combined resulted in decreased mortality levels compared to when they are used individually; that is, 24% mortality levels were observed for both medium and high levels of caffeine and polyphenols combination. Thus, these results indicate that caffeine causes mortality in flies, and polyphenols help mitigate these adverse effects.

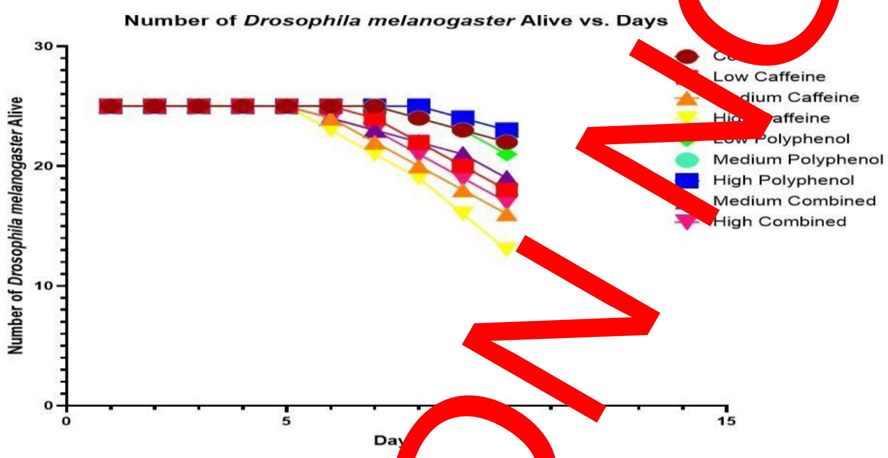

Figure 9: Number of surviving Drosophila melanogaster (n=25 per group) tracked over 10 days across varying conditions.

Survival remained stable through Day 5 for all groups, after which caffeine-treated groups showed a dose-dependent decline, most pronounced in the high-caffeine condition. Polyphenol-treated groups maintained survival comparable to controls, while combined treatments exhibited intermediate survival patterns.

Statistical summary

All behavioral metrics exhibited non-Gaussian distributions; therefore non-parametric analyses were applied. Kruskal-Wallis tests revealed significant differences across treatment groups for entropy, diffusion coefficient, and drift (P<0.0001). Sample size n=168 flies across nine experimental conditions.

Summary of System-Level Behavioral Dynamics (SLBD). Across all behavioral metrics, clear treatment-dependent trends emerged. Baseline behavior in sleep-deprived control flies remained relatively constrained, showing limited exploratory movement. Caffeine treatments increased locomotor activation, producing higher entropy, diffusion, and directional bias toward food resources. These patterns indicate increased system instability and greater dependence on environmental cues. In contrast, polyphenol treatments stabilized locomotor behavior by reducing variability and constraining movement dynamics. Combined treatments produced intermediate behavioral outcomes, suggesting that coffee-derived caffeine partially counteracts the stabilizing effects of polyphenols.

Physics-based locomotion analysis

Locomotor trajectories were analyzed using a stochastic dynamics framework based on the Paul Langevin equation. The Langevin equation describes the evolution of a system under the influence of both deterministic and stochastic forces. In biological systems, this formulation provides a useful model for movement dynamics in which deterministic drift represents directed motion while stochastic fluctuations represent random exploratory behavior.

In this study, the position of each fly was modelled as a function of three interacting components:

- Deterministic velocity (drift)
- Random fluctuations
- A bias term representing attraction toward the food source

These components combine to produce the observed change in position over time. Behavioral diffusion therefore emerges as the macroscopic consequence of microscopic fluctuations in neural and motor activity.

Because behavioral entropy and diffusion metrics remain underused in neuroscience and sleep research, this framework provides a quantitative method for evaluating system-level neural stability. Trajectory data were obtained from 500 seconds of locomotor recording per replicate over a ten-day period, resulting in high-resolution spatial datasets. XY position coordinates for individual flies were extracted using Tracker 6.3.3 software. From these trajectories, entropy, MSD_1, MSD_2, diffusion coefficient (D), anomalous diffusion exponent (α), and drift were calculated for each treatment group. A total of 225 flies were randomly assigned to one of nine experimental treatments, representing varying concentrations of caffeine, polyphenols, and combined compounds.

Discussion

Overall, chronic sleep deprivation generated unique characteristics in the locomotion of different treatment groups. These results illustrate dissociated effects with regard to entropy and diffusion. As a matter of fact, sleep-deprived control flies displayed moderately high entropy values ($H \approx 0.69$) together with moderate diffusion coefficients ($D \approx 0.55$), suggesting baseline locomotor variability under sleep deprivation.

Moreover, caffeine treatments altered the dynamics of locomotor movement by increasing diffusion processes and elevating entropy values relative to controls. Median entropy increased from $H \approx 0.69$ in the control group to approximately $H \approx 0.81$ in the high-caffeine condition, while diffusion coefficients increased from $D \approx 0.55$ to $D \approx 0.71$. These patterns indicate heightened locomotor variability and increased exploratory movement consistent with caffeine-induced neural stimulation. Although caffeine is commonly administered as a solution to fatigue, the results of this study suggest that caffeine may amplify stochastic behavioral dynamics rather than restoring stable locomotor regulation under sleep deprivation.

In contrast, polyphenol-treated flies exhibited reduced entropy values, ranging from $H \approx 0.68$ in the low polyphenol condition to $H \approx 0.62$ in the high polyphenol condition, together with lower diffusion coefficients ($D \approx 0.48$-0.52). These results indicate more constrained locomotor trajectories and reduced stochastic variability. Such behaviours are consistent with increased stabilization of locomotor dynamics under physiological stress. Combined caffeine–polyphenol treatments resulted in entropy and diffusion values that were intermediate between the individual treatments. Median entropy ranged from $H \approx 0.71$-0.72, while diffusion coefficients ranged from $D \approx 0.58$-0.61. These findings suggest that caffeine partially offsets the stabilizing effects of polyphenols on locomotor dynamics.

All together, these findings indicate that entropy and diffusion capture complementary dimensions of behavioral organization and that dietary compounds modulate locomotor system dynamics during sleep deprivation.

Mechanistic interpretation of treatments

Through the combination of past research and this study, it has been uncovered that coffee tends to have detrimental effects, particularly when consumed for prolonged periods of time and at higher concentrations [3,8,9]. On the other hand, polyphenols tend to exhibit more neuroprotective effects [10-12].

With this in mind, it begs the question of why this stark difference exists. The answer therein lies in the mechanisms of caffeine in coffee vs. matcha and cocoa products. Coffee delivers caffeine quickly through the digestive system, with a peak of energy at 30-60 minutes post-consumption [13]. This mechanism explains why coffee tends to cause issues within an organism; the body does not have time to adjust to the changes before being infiltrated with caffeine. Matcha, however, gradually delivers the naturally present caffeine from the green tea leaf over the course of 4 to 6 hours, with no dramatic peaks in energy.

This gradual mechanism allows for caffeine to gently infiltrate the body, preventing jitters and hyperactive, chaotic mental states [14-18].

Moreover, it is important to note that the reason matcha and other polyphenols exhibit these properties is due to an amino acid called L-theanine [19-23]. L-theanine is a naturally occurring amino acid, found mainly in the leaves of certain plants such as Camellia sinensis (teas) and some mushrooms, mainly as its biologically active L-enantiomer [24-29]. Structural analog of glutamate, L-theanine activates glutamate receptors and also affects inhibitory and neuro-modulatory neurotransmission, such as GABA, dopamine, and serotonin [30-32]. These mechanisms appear to be responsible for the regulation of cortical excitability and attentional states. Currently available evidence suggests oral L-theanine supplementation may improve attention and cognitive performance in individuals with normal cognitive abilities.

Conclusions

This study demonstrates that behavioral entropy and diffusion analysis provide a physics-based means to quantify the stability of neural systems under sleep deprivation.

Sleep-deprived control flies exhibited moderate diffusion together with moderately high entropy, reflecting baseline locomotor variability under conditions of physiological stress. Caffeine treatments increased both entropy and diffusion relative to control conditions, indicating heightened locomotor variability and increased exploratory movement. While caffeine is commonly used to counteract fatigue, these results suggest that caffeine amplifies stochastic behavioral dynamics rather than restoring stable locomotor regulation during sleep deprivation.

In contrast, polyphenol treatments reduced both entropy and diffusion values, producing more constrained locomotor trajectories and reduced behavioral variability. These patterns suggest that polyphenols stabilize locomotor dynamics under sleep deprivation and promote more regulated behavioral responses. Flies exposed to combined caffeine–polyphenol treatments exhibited intermediate entropy and diffusion values, indicating that caffeine partially offsets the stabilizing effects of polyphenols. Rather than producing additive benefits, the combined treatment appears to generate a balance between stimulation and stabilization.

Overall, these findings suggest that polyphenol-rich compounds such as matcha and cocoa may promote greater stability in behavioral system dynamics compared to caffeine alone under conditions of sleep deprivation. More broadly, these results demonstrate that system-level behavioral metrics such as entropy and diffusion provide a quantitative framework for evaluating neural flexibility and for investigating how dietary compounds influence complex biological systems.

Limitations

While polyphenols have been seen to serve as an efficient general substitute to coffee, it is important to note that this may not be true for all populations. This is particularly the case for populations that require an immediate peak in energy, rather than a gradual increase. A meta-analysis study caffeine consumption was typically related to decreased levels of hyperactivity in those with Attention Deficit Hyperactivity Disorder (ADHD). This specific meta-analysis focused on caffeine from traditional forms such as coffee, energy drinks, and carbonated beverages; further research would be required to determine if polyphenols hold the same benefits for people with ADHD.

Some additional limitations for this project include the lack of neural imaging for a concrete view of the effects of different treatments on neural systems. Nonetheless, when utilizing Drosophila melanogaster, it is inherently complicated to obtain accurate results from neural imaging, and two-photon microscopy would be needed, which the researcher did not have at their disposal. Similarly, Drosophila melanogaster was used as a model organism, which limits the direct translatability of results to human neural systems. While flies share conserved sleep and neuro-modulatory pathways with mammals, differences in brain complexity and metabolism restrict direct extrapolation to human cognition or dietary effects.

Also, mechanical sleep deprivation may introduce stress-related confounds independent of sleep loss itself. Although all experimental groups were subjected to identical deprivation conditions, mechanical stimulation could influence locomotor behavior or neural state beyond the effects of sleep deprivation alone. Similarly, only short-term effects were examined due to

Drosophila melanogaster's limited lifespan of 15 days to 30 days. Behavioral tracking was conducted over a fixed 500-second window, and the study did not assess long-term adaptation, recovery, or cumulative effects of prolonged dietary exposure.

Implications

These results showcase that behavioral entropy and diffusion can efficiently serve as physics-based metrics for evaluating neural systems under sleep deprivation. By treating locomotor behavior as an emergent property of an underlying dynamical system, these measures function as quantitative observables linking macroscopic behavior to microscopic neural organization. Unlike other traditional studies, this framework uncovers whether increased movement is related to adaptive flexibility or unregulated instability by distinguishing ordered variability from stochastic disorder within system dynamics. Overall, the results indicate that polyphenols tend to promote stability and behavioral adaptation, while the caffeine present in coffee constrains system dynamics. In dynamical terms, polyphenols appear to preserve exploratory variability, whereas caffeine shifts the system toward more constrained motion regimes. This suggests that neural stimulants like caffeine may increase alertness without improving neural system stability.

Similarly, this work supports the use of polyphenols as a dietary supplement to minimize the effects of chronic sleep deprivation rather than coffee. Furthermore, it highlights the essentialness of evaluating system-wide behavior rather than isolated behavior because complex biological systems cannot be fully characterized through single-variable measurements alone.

More broadly, the study creates a low-to-no-cost framework for screening other dietary interventions via non-equilibrium behavioral metrics and demonstrates how statistical physics approaches can provide scalable tools for studying biological regulation under chronic perturbation.

Safety and Ethical Considerations

This research was conducted following standard safety guidelines and ethics for carrying out research involving invertebrates. Since Drosophila melanogaster are not vertebrate animals, it was not necessary to seek approval for carrying out animal research; all due care was taken to handle them and ensure minimal stress was caused to them.

Chemical safety:

- Caffeine (coffee extract) and polyphenols (matcha and cocoa powder) were added at low levels of dietary concentrations, which are well below occupational exposure levels.
- Gloves and safety glasses were properly used during the preparation and handling of all chemical-containing media.
- All chemical handling was conducted in well-ventilated areas and carried out using dedicated balances.
- Unused media and chemical waste were disposed of in accordance with institutional and local chemical waste disposal policies.

Behavioral safety:

- The mechanical sleep deprivation protocols were implemented in a way that minimized injuries while effectively restricting an individual's sleep.
- No manual handling was involved in the locomotor recording, thus minimizing any stress-induced artifacts.
- The environmental factors (temperature, lighting, and humidity) were well-regulated for the well-being of flies.

Ethical considerations:

- Experimental procedures did not involve the use of vertebrates, human subjects, or harmful biological agents.
- All experiments were done according to the principles of the "3Rs" (Replacement, Reduction, Refinement), and only a minimum of flies were used that were necessary to obtain statistically relevant results.

Overall, this study ensured that chemical and behavioral interventions were applied safely, ethically, and consistently across all experimental groups.

Data Availability

The raw data from this study are available upon request. Please email Clara Martins De Bellis

Silva at claramdbs@gmail.com to access the datasets.

References

1. National Coffee Association. More Americans drink coffee each day than any other beverage, bottled water back in second place. 2025. [Google Scholar]
2. Rehm CD, Ratliff JC, Riedt CS, Drewnowski A. Coffee consumption among adults in the United States by demographic variables and purchase location: Analyses of NHANES 2011-2016 data. Nutrients. 2020;12(8):2463. [Crossref][Google Scholar][PubMed]
3. Bordeaux B. Benefits and risks of caffeine and caffeinated beverages. 2025.
4. Caffeine addiction statistics: How common is dependence? ScienceInsights. 2025.
5. Heiney K, Huse Ramstad O, Fiskum V, Christiansen N, Sandvig A, et al. Criticality, connectivity, and neural disorder: A multifaceted approach to neural computation. Front Comput Neurosci. 2021;15:611183. [Crossref][Google Scholar][PubMed]
6. Zimmern V. Why brain criticality is clinically relevant: A scoping review. Front Neural Circuits. 2020;14:565335. [Crossref][Google Scholar][PubMed]
7. Kennedy DO. Polyphenols and the human brain: Plant "secondary metabolite" ecologic roles and endogenous signaling functions drive benefits. Adv Nutr. 2014;5(5):515-533. [Crossref][Google Scholar][PubMed]
8. Rauf MQ, Sharma L, Elsayet E, Elhassan O, Fahim R, et al. Caffeine consumption patterns, health impacts, and media influence: A narrative review. Cureus. 2025;17(6). [Crossref][Google Scholar][PubMed]
9. Čižmárová B, Kraus Jr V, Birková A. Caffeinated beverages-Unveiling their impact on human health. Beverages. 2025;11(1):18. [Crossref][Google Scholar]
10. Arias-Sánchez RA, Torner L, Fenton Navarro B. Polyphenols and neurodegenerative diseases: Potential effects and mechanisms of neuroprotection. Molecules. 2023;28(14):5415. [Crossref][Google Scholar][PubMed]
11. Imran A, Maqbool J, Rauf A, Anwar H, Malik SA, et al. Neuroprotective effects of polyphenols. Frontiers in Natural Product Chemistry. 2021;8:146-172. [Crossref][Google Scholar]
12. Godos J, Caraci F, Caruso G, Micek A, Frias-Toral E, et al. Molecular mechanisms underlying the neuroprotective effects of polyphenols: Implications for cognitive function. EXCLI J. 2025;24:1262. [Crossref][Google Scholar][PubMed]
13. Fiani B, Zhu L, Musch BL, Briceno S, Andel R, et al. The neurophysiology of caffeine as a central nervous system stimulant and the resultant effects on cognitive function. Cureus. 2021;13(5). [Crossref][Google Scholar][PubMed]
14. Frank MG. Sleep and plasticity in the visual cortex: More than meets the eye. Curr Opin Neurobiol. 2017;44:8-12. [Crossref][Google Scholar][PubMed]
15. de Lima EP, Laurindo LF, Catharin VC, Direito R, Tanaka M, et al. Polyphenols, alkaloids, and terpenoids against neurodegeneration: Evaluating the neuroprotective effects of phytocompounds through a comprehensive review of the current evidence. Metabolites. 2025;15(2):124. [Crossref][Google Scholar][PubMed]
16. Sokary S, Al-Asmakh M, Zakaria Z, Bawadi H. The therapeutic potential of matcha tea: A critical review on human and animal studies. Curr Res Food Sci. 2023;6:100396. [Crossref][Google Scholar][PubMed]
17. Donlea JM, Seugnet L, Shaw PJ. Genetics of sleep in drosophila melanogaster. InDrosophila Melanogaster: Life Cycle, Genetics and Development. 2012:141-155. [Google Scholar]
18. Mancini E, Beglinger C, Drewe J, Zanchi D, Lang UE, et al. Green tea effects on cognition, mood and human brain function: A systematic review. Phytomedicine. 2017;34:26-37. [Crossref][Google Scholar][PubMed]
19. James JE. Caffeine and cognitive performance: Persistent methodological

challenges in caffeine research. Pharmacol Biochem Behav. 2014;124:117-122. [Crossref][Google Scholar][PubMed]

20. Society for Science. (n.d.). Rules and guidelines ISEF.
21. ISEF International Rules and Guidelines (2025-2026).
22. SSEF Rules & Rules Supplement (2025-2026).
23. OSHA Hazard Communication Standard (29 CFR 1910.1200).
24. Tononi G, Cirelli C. Sleep and the price of plasticity: From synaptic and cellular homeostasis to memory consolidation and integration. Neuron. 2014;81(1):12-34. [Crossref][Google Scholar][PubMed]
25. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of sleep and waking in Drosophila melanogaster. Science. 2000;287(5459):1834-1837. [Crossref][Google Scholar][PubMed]
26. Peng CK, Havlin S, Stanley HE, Goldberger AL. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos. 1995;5(1):82-87. [Crossref][Google Scholar][PubMed]
27. Costa M, Goldberger AL, Peng CK. Multiscale entropy analysis of biological signals. Phys Rev E Stat Nonlin Soft Matter Phys. 2005;71(2):021906. [Crossref][Google Scholar][PubMed]
28. Parupalli A, Varkey J, Torres-Rocha V, Kethar J. From cup to cure: The impact of matcha tea on Alzheimer's disease. J Student Res. 2024:13(4).
29. Helfrich-Förster C, Bertolini E, Menegazzi P. Flies as models for circadian clock adaptation to environmental challenges. Eur J Neurosci. 2020;51(1):166-181. [Crossref][Google Scholar][PubMed]
30. Dubowy C, Sehgal A. Circadian rhythms and sleep in Drosophila melanogaster. Genetics. 2017;205(4):1373-1397. [Crossref][Google Scholar][PubMed]
31. Huang Q, Braffett BH, Simmens SJ, Young HA, Ogden CL. Dietary polyphenol intake in US adults and 10-year trends: 2007-2016. J Acad Nutr Diet. 2020;120(11):1821-1833. [Crossref][Google Scholar][PubMed]
32. Centers for disease control and prevention. FastStats: Sleep in adults. 2024. [Google Scholar]

