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Biphasic Neurostimulatory Effects of Caffeine on Dopamine-Mediated Locomotor Circuits in Drosophila melanogaster

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Abstract

This study aimed to examine how caffeine affects the movement of Drosophila melanogaster in a dose-dependent manner. It also aimed to evaluate the use of Drosophila as an ethical and inexpensive model for studying the neurobehavioral effects of stimulants, which are relevant to human health.

Experimental groups were given freshly prepared caffeine solutions from instant coffee (a stock solution of about 3.5 mg/mL). These solutions were diluted to concentrations of 50% (1.75 mg/mL), 75% (2.63 mg/mL), and 100% (3.50 mg/mL). A control group was not treated. Locomotor activity was measured using the negative geotaxis assay over six hours. The average climbing height (in centimeters) and percent of active flies were recorded for each group.

The 75% group demonstrated peak locomotor performance (mean climbing height: 10.1 ± 0.7 cm; 9/10 flies active), significantly exceeding the control (6.2 ± 0.5 cm; 5/10 active) and confirming caffeine's stimulatory effect at moderate doses. The 100% concentration produced a decline in activity (7.5 ± 0.8 cm; 6/10 active) relative to the 75% group, indicating physiological stress and impaired neuromuscular coordination at excessive doses, consistent with a concentration-dependent biphasic response.

This study provides direct quantitative evidence of a biphasic locomotor response across a graded, ecologically relevant caffeine concentration series in wild-type Drosophila under standardised laboratory conditions, establishing that neural stimulation occurs only within an optimal physiological range. The results underscore the utility of Drosophila melanogaster as a reproducible, and accessible model for neuropharmacological investigation and highlight the public health relevance of dosage regulation among young caffeine consumers.

Keywords: Caffeine; Drosophila melanogaster; locomotor activity; negative geotaxis; biphasic response; adenosine receptor antagonism; dopamine; neurostimulant; dose-dependent.

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Introduction

Caffeine (1,3,7-trimethylxanthine) is the most commonly used psychoactive agent in the world and acts primarily by blocking adenosine receptors and thereby increasing the activity of dopamine neurons and stimulating the excitability of neurons. Recent research has illustrated how caffeine has an effect on both locomotor and neurobehavioral activity on a dose-dependent basis such that moderate doses of caffeine have been shown to enhance motor function, alertness and neuroprotection, whereas excessive caffeine supplementation can cause oxidative damage, impair behavioral function and induce neuromuscular dysfunction. Studies conducted on the fruit fly *Drosophila melanogaster* further suggest that caffeine acts as an effective neurobehavioral model for studying the impact of caffeine on locomotor and neurobehavioral activity as a result of conserved systems of neurotransmitters and reliable methods for measuring locomotor activity such as using negative geotaxis. In addition, these studies indicate that stimulation of locomotor activity with caffeine is primarily mediated through dopamine pathways and there is evidence that moderate or low doses of caffeine provide an antioxidant response, whereas extremely high doses of caffeine produce impaired motor coordination and stabilization. However, there are limitations to the above studies because of the use of either pharmacologically or genetically modified strains or because of a lack of quantitative measures of caffeine's effects for all doses and at a rate of caffeine that would be considered physiologically relevant. Further, there is a need for more studies assessing locomotion in wild-type fruit flies under standardized and replicated laboratory conditions to better reflect the consumption patterns typically found in the real world. The study will evaluate and establish the presence of a biphasic dose-response relationship for geotactic behaviour in wild-type *Drosophila melanogaster* over multiple doses of caffeine. This will also allow for the development of a straightforward and reproducible experimental model that will help establish a reliable basis for use in neuropharmacological studies.

Literature Review

Caffeine(1,3,7-trimethylxanthine) primarily acts in the central nervous system by blocking the receptors for two different nucleotides that have been shown to contribute to fatigue (adenosine A1 and A2A), and therefore are thought to enhance one's ability to respond to a stimulus (alertness). There is good supporting evidence for this explanation [1]; however, caffeine may also modulate other neural systems that involve the neurotransmitters dopamine and GABA. The use of Drosophila melanogaster by Nall et al. [2] has shown that caffeine promotes wakefulness via enhancement of presynaptic dopamine release via paired anterior medial PAM neurons. As a result, the speed at which caffeine promotes arousal can be decreased by silencing PAM neurons; thus, when caffeine was given to adenosine receptor knock-out Drosophila, no arousal effect was observed. Coelho et el.[3] are able to determine the metabolic pathway of caffeine in Drosophila, with the major pathways being cytochrome P450 enzymes (specifically CYP6d5, CYP6a8, and CYP12d1), while caffeine was metabolised primarily into paraxanthine in humans. This provides compelling evidence for distinct pathways of stimulant metabolism between Drosophila and humans.

Caffeine produces a biphasic dose-dependent reaction at a behavioural level in Drosophila. Asbah and colleagues [4] found that low doses of caffeine (0.016 mM) improve locomotor performance, enhance expression of some antioxidant genes (sod1 and cat), and increase the lifespan of neurodegeneration-susceptible flies, indicating a hormetic neuroprotective effect. However, Keebaugh and colleagues [5] provided an important caveat: sleep-suppressing and activity-altering effects of caffeine are greatly influenced by dietary habits; reduction in food intake by aversion to bitter-tasting foods puts the animal into a starvation-like condition that mimics the effect of pharmacological arousal. This finding complicates the interpretation of caffeine as a direct stimulant in Drosophila. Additionally, Francikowski et al. [6] explored the effect of caffeine during larval development; they found that moderates doses (0.5 mg/mL) caused the most considerable changes in expression of adenosine receptors (DmAdoR) mRNA and reduced larva-to-adult viability; furthermore, adult flies that emerged from caffeine-exposed larvae had normal long-term regulation of adenosine receptors, suggesting a metamorphic resetting of adenosine signalling.

The recent research by Saldes et al [7] into the translational relevance of Drosophila caffeine studies reinforces that when caffeine is consumed at night, this leads to increased impulsivity in terms of motor activity (i.e., moves quickly) beyond that which would usually be experienced with other stimulants such as coffee; thus, caffeine's effect on behaviours in individuals consumes caffeine at different times may have significant importance to Drosophila melanogaster (a common fruit fly), due to its entire developmental cycle being affected by its consumption of caffeine through its neurochemistry (neurotransmitters) and also its neurobehavioural characteristics. In addition, the similarities between the neuroanatomy/pathways of invertebrate insects and those of vertebrate mammals, primarily via the dopaminergic system, highlight how caffeine produces the same effects among invertebrates and vertebrates, thus allowing for better understanding of how caffeine affects behaviours in humans.

Table 1: Comparative Review of Existing Studies on Caffeine Effects in Drosophila melanogaster

	Methodology / Model	Modality / Focus Used	Key Contribution	Limitation
	Asbah et al. (2021) — Pharmacological effect of caffeine on Drosophila melanogaster	In vivo; behavioral + biochemical assays (T-maze, climbing, oxidative stress markers)	Demonstrated that low-dose caffeine (0.016 mM) improves locomotor performance, cognitive function, and antioxidant gene expression (sod1, cat)	Limited to a narrow dose range; did not examine chronic or high-dose effects systematically

			in Drosophila; extended lifespan in neurodegeneration models	
	Nall et al. (2016) — Caffeine promotes wakefulness via dopamine signaling in Drosophila	Genetic manipulation; sleep/wakefulness assays; neurochemical analysis of dopaminergic circuits	Identified PAM dopaminergic neurons as the key mediators of caffeine-induced wakefulness, independent of classical adenosine receptor (dAdoR) signaling; challenged the adenosine-only model	Study focused primarily on sleep; locomotor effects and dose-response relationships not fully characterized
	Reichert et al. (2022) — Adenosine, caffeine, and sleep-wake regulation	Systematic review; adenosine receptor pharmacology; human and animal data	Provided comprehensive review of caffeine's mechanism as a non-selective adenosine A1/A2A antagonist; detailed its role in neurotransmitter modulation (dopamine, GABA) and sleep-wake cycle regulation	Review-based; limited direct experimental data; translational gaps between human and Drosophila systems

	Coelho et al. (2015) — Cytochrome P450-dependent metabolism of caffeine in Drosophila melanogaster	Biochemical; radiolabeled caffeine; genetic knockdown; P450 enzyme characterization	Identified CYP6d5, CYP6a8, and CYP12d1 as key P450 enzymes in caffeine detoxification; showed Drosophila primarily produces theobromine (unlike paraxanthine in humans); silencing CYP6d5 abolished theobromine formation	Metabolic focus only; behavioral or neurological consequences of altered caffeine metabolism were not assessed
	Francikowski et al. (2016) — Caffeine effects on AdoR mRNA expression in Drosophila melanogaster	Molecular; RT-PCR; larval survival and development assays	Showed caffeine causes dose-dependent changes in adenosine receptor (DmAdoR) mRNA expression during larval stages; moderate concentrations (0.5 mg/mL) had the strongest effect; adult flies showed a developmental 'reset' of receptor expression after metamorphosis	Study limited to larval stage; long-term adult behavioral or locomotor outcomes were not examined

	Keebaugh et al. (2017) — Dietary context and caffeine-induced sleep suppression in Drosophila	Behavioral; sleep activity monitoring; dietary manipulation (sucrose variation)	Demonstrated that caffeine's sleep-suppressing effects are highly dependent on nutritional context (sucrose concentration); showed bitter taste aversion and reduced food intake (not just pharmacology) contribute significantly to sleep loss	Complex nutritional interactions make it difficult to isolate pure pharmacological effects; findings may not generalize to all dietary conditions
	Saldes, Sabandal & Han (2025) — Nighttime caffeine intake increases motor impulsivity	Behavioral; motor impulsivity assays; nighttime caffeine administration in Drosophila and mammalian models	Demonstrated that nighttime caffeine consumption specifically increases motor impulsivity, providing evidence that timing of caffeine intake influences behavioral outcomes beyond simple stimulant effects	Focused on impulsivity; broader locomotor and cognitive dose-response effects require further characterization

Research Methodology

3.1 Experimental Organisms

The adult fruit fly *Drosophila melanogaster* (3 - 7 days old) was chosen as the experimental organism since it is well characterised genetically, reproduces rapidly, and has been shown to be sensitive to dietary stimulation [8]. Flies were trapped from campus using banana-baited traps, then kept on standard yeast-sugar media at approximately 25˚C ± 2 °C. In order to reduce differences in physiology, flies were age-synchronised and not fed at least two hours before treatment, in order to have similar feeding behaviour.

3.2 Preparation of Caffeine Treatments

Caffeine was provided as a crude aqueous solution of instant coffee (i.e. coffee granules). Instant coffee contains approximately 35 mg caffeine per gram, therefore a 50 mL stock solution made from 5 g instant coffee has an estimated caffeine concentration of

~3.5 mg/mL. The experimental concentrations were derived from diluting the stock solution, with the dilution ratios outlined in Table 1. All treatment vials were created and labeled exactly the same. The food media was created by mixing the standard yeast-sugar base with the appropriate solution of caffeine (~10 mL per vial), while the control media only contained a 5% sugar solution.

Table 1: Caffeine treatment groups and estimated concentrations

Group	Caffeine (%)	Estimated Caffeine (mg/mL)	Description
A	0% (Control)	0	No caffeine
B	50%	1.75	Low concentration
C	75%	2.63	Moderate concentration
D	100%	3.50	High concentration

3.3 Experimental Design and Exposure Protocol

We set up three replicate vials of ten adult flies per treatment. After transferring the flies carefully into each vial, the flies were permitted a 10–15 minute period to acclimate at room temperature and normal light conditions (25 ± 2 °C and standard light levels). After the flies were acclimated and before the caffeine exposure began, we collected baseline data regarding how many flies were at the bottom of the vial before the flies were exposed to caffeine for eight hours. The first number of flies were at the bottom of the vial before the caffeine exposure started; this was one of the first times we will have collected information about their activity in an unexposed situation and each case therefore gave us three independent pieces of information about the activities of the flies during a specific time frame.

3.4 Negative Geotaxis Locomotor Activity Assay

We assessed locomotor activity by conducting a negative geotaxis assay. After gently tapping the vials to settle the flies at the bottom, we measured the average height climbed in 30 seconds for the flies that were able to fly. We also recorded the number of flies that crossed the eight-centimeter mark as an indication of their locomotor activity. We conducted baseline (0h) assessments and then continued to assess activity every hour for the next six hours. All measurements were videorecorded for subsequent blind re-evaluation. Other behaviours, such as hyperactivity, wing flapping, lethargy, freezing, and circling, were qualitatively observed as behavioural abnormalities. Each treatment was replicated three times to reduce the effects of experimentation bias.

3.5 Data Processing and Statistical Analysis

Mean climbing height (cm) and mean active fly count by group were averaged across each of the three replicates. Statistical variability is expressed as standard deviation (SD). The concentration activity relationship was drawn to represent the dose-response curve.

3.6 Ethical Considerations

All experimental procedures were done with little to no distress to the flies. After completing the experiment, flies were killed by brief exposure to low temperature (using a freezing method) to reduce their suffering. There were no vertebrate animals used in this study. Data Availability: The raw locomotor activity data can be obtained from the corresponding author upon reasonable request.

Results and Discussion

4.1 Effect of Caffeine on Locomotor Activity

Negative geotaxis was used to evaluate the effects of caffeine on the locomotor activity of *Drosophila melanogaster* (Table 2; Table 3). The locomotor activity of control flies (0% caffeine) was measured by their mean climbing height, which was equal to 6.2 ± 0.5 cm (Table 3) with only 50% (5 out of 10) of the flies climbing above the limit of 8 cm in this experiment. Locomotor activity of *D. melanogaster* significantly increased when treated with 50% caffeine, as illustrated by the mean climbing height of 8.4 ± 0.6 cm (7 out of 10). The mean climbing height of *D. melanogaster* treated with 75% caffeine was significantly higher than control and 50% caffeine-treated flies (10.1 ± 0.7 cm; 9 out of 10). The mean climbing height at the highest concentration of caffeine (100%) decreased significantly (7.5 ± 0.8 cm) compared to both the 50% and 75% caffeine concentrations and the 100% caffeine concentration (6 out of 10). These data demonstrate a non-linear, biphasic dose-response relationship between caffeine and the locomotor activity of *D. melanogaster*, with moderate caffeine concentrations improving performance and excessive caffeine

concentrations impairing performance.

Table 2: Effect of caffeine concentration on mean climbing height

Concentration (%)	Mean Climbing Height (cm)	SD
0% (Control)	6.2	0.5
50%	8.4	0.6
75%	10.1	0.7
100%	7.5	0.8

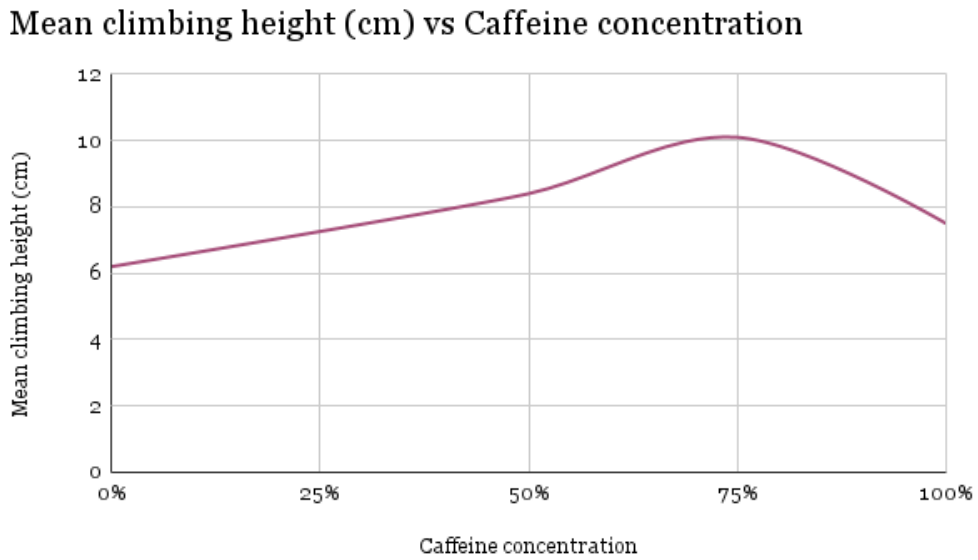
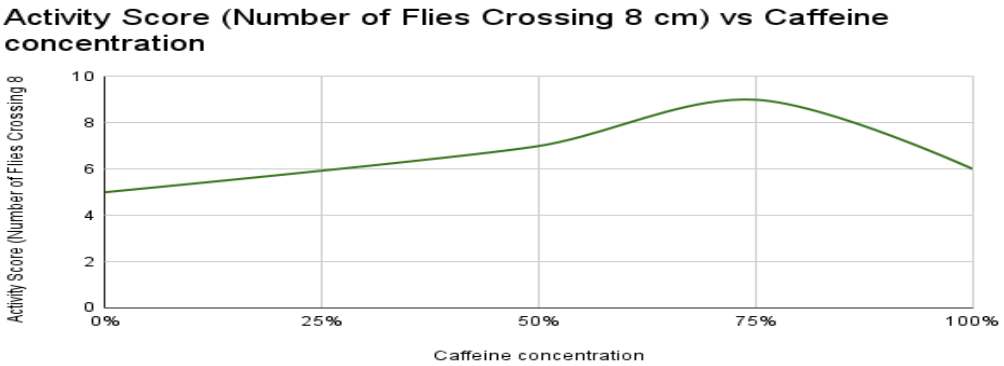


Table 3: Number of active flies crossing the 8 cm mark per treatment group

Group	Active Flies (out of 10)
Control (0%)	5
50%	7
75%	9
100%	6



4.2 Mechanistic Interpretation of Biphasic Response

Caffeine produces a biphasic (two-phase) response due to a combination of two main influences. The first is that caffeine will provide a stimulation to dopamine (or dopaminergic) signalling through actions that inhibit (or block) adenosine (or adenosinergic) signalling. This stimulation results in the depolarization of the excitatory neurons that help to perform different tasks such as motor co-ordination and physical movement capability [3]. The peak effect of caffeine at moderate doses occurs when approximately 75% of the total potential physiological effects are caused by the mobilization of the neuronal tissue.

On the other hand, moderate amounts of caffeine produce the opposite effects when consumed at or close to 100% concentrations. When consumed at or around 100%, the excitatory nature of caffeine will cause excessive stimulation of the neuronal circuits (over-stimulation) that will negatively affect your ability to physically move. You will experience loss of motor co-ordination, increased energy expenditure and/or fatigue due to muscle fatigue. Consuming excessive amounts of caffeine will increase reactive oxygen species (ROS) production (oxidative stress) and disrupt key cellular homeostasis processes (cellular dysfunction), resulting in impaired neuromuscular function and decreased physical performance [9]. Thus, the biphasic (two-phase) response of caffeine highlights the balance between the stimulatory (excitatory) and inhibitory (depressant) biological effects of caffeine at different levels of consumption.

4.3 Comparison with Previous Studies and Novelty

The current findings are consistent with past literature showing increased locomotor behaviour with low doses of caffeine, and decreased performance with higher doses; however, most of those studies did not comprehensively quantify graded dose-response relationships [2]. In contrast, the current study provides a systematic concentration series to demonstrate an optimal concentration for stimulation of locomotor activity, thereby providing a more accurate dose-response relationship.

While other studies have reported an increase in locomotor ability followed by a decrease as caffeine exposure builds, few of these studies provide a specific range of maximum performance to define the transition [8]. The current findings expand upon this work by quantitatively determining that maximum locomotor ability occurs at an intermediate dose (75%), followed by a measurable decline at higher doses.

Recent studies also provide evidence that too much caffeine can produce impulsive motor behaviour and disrupt neural regulation, supporting the decreased locomotor performance seen in the current study at the maximum dose of caffeine [1]. The majority of the research previously done used either genetically modified organisms or extreme experimental paradigms; this study uses wild-type *Drosophila* and physiologically relevant levels of caffeine, therefore increasing the applicability of this research to real-world circumstances.

This research study provides new and innovative systematic experimental designs and has an excellent quantitative analysis of data using ecologically valid doses of caffeine in demonstrating a clear biphasic locomotor response to caffeine. The limitations of prior studies are also addressed in demonstrating more convincing evidence that a stimulatory range for optimal movement exists and that an excessive intake of caffeine will have detrimental effects on such motor function.

Conclusion

The present study has demonstrated that caffeine has a concentration-dependent biphasic effect on locomotion (and therefore behavior) in *Drosophila melanogaster*, and therefore that our primary objective to characterize the dose–response relationship using a simple and reproducible behavioral assay has also now been met. The highest amount of locomotor activity (10.1 ± 0 . 7 cm; 9/10 active flies) was achieved at a moderate concentration of caffeine (75%, 2.63 mg/mL), which represents an increase of 63% compared with controls (6.2 ± 0.5 cm; 5/10 active). A significant decrease in locomotor activity (7.5 ± 0.8 cm; 6/10 active) resulted from use of a greater caffeine concentration (100%, 3.50 mg/mL). Therefore, caffeine contributes to stimulating locomotor activity within a physiological range, but at higher levels results in decreased performance.

Our results also provide direct quantitative evidence of peak stimulatory concentrations of caffeine under controlled experimental conditions, in contrast to other studies that have examined the effects of caffeine and provided a generalized summary of stimulating or inhibiting effects, without specifically identifying an optimal dose– response range. The development of these precise quantitative measures will greatly improve our understanding of how and to what degree the neurobehavioral effects of caffeine occur in a dose-dependent manner, and should provide a more definitive basis for interpreting the responses of *Drosophila melanogaster* to other stimulants.

This study provides insight into the possibility of a systematic analysis of *Drosophila melanogaster* (wild-type fruit fly) responses to graded physiologically relevant doses of caffeine, and so will help facilitate the accurate evaluation and delimitation of caffeine's

biphasic response. The present study also improves upon previous research, which has employed either high or low dose levels of caffeine and/or genetically modified fruit flies, so that there are now more options to replicate the present findings in a natural or more accurate environmental context.

However, there are limitations present in the current study. First, the use of a crude coffee extract to study caffeine's biphasic response may cause variability within the data; second is that there was no long-term evaluation of caffeine's effects; and third, no measurements were taken of the molecular mechanisms that may contribute to caffeine's biphasic response (such as dopamine receptor activity or oxidative stress pathways).

Addressing these limitations in future studies can be achieved through 1) the use of purified caffeine; 2) the evaluation of long-term exposure to caffeine; and 3) the utilization of molecular and biochemical methods to enhance knowledge of caffeine's biphasic responses on biologically and biochemically related levels. Also, in the future there is a possibility that the results of this research will be translated into a wider context through further examination of caffeine's effect on behaviour and physiology over time and with a greater degree of reliability. Overall, the study has increased our understanding of caffeine's biphasic effects on locomotor behaviour due to the availability of quantifiable, experimental, and reproducible evidence, and it demonstrates the need to control the dosage of caffeine to both optimise the positive neuro-stimulation effects of caffeine and reduce the risk of negative effects that may occur with doses that are outside of an optimum range.

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