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EFFECTS OF HIGH CONCENTRATION SPERMIDINE SUPPLEMENTATION DURING LARVAL FEEDING IN *DROSOPHILA MELANOGASTER SWISS CHEESE* MUTANTS

Aim. The aim of this study was to show the effects of spermidine supplementation during the larval stage on lifespan, neurodegenerative phenotype, and locomotor behavior in adult *sws¹* mutant flies. ***Methods.*** Experiments were performed using *Drosophila melanogaster* Meigen *swiss cheese (sws¹)* mutants and *Oregon-R* wild-type controls. Flies were maintained at 24 °C on standard medium. Spermidine (5 mM) was added to the food during the larval stage only, while lifespan, locomotor behavioral (negative geotaxis and open-field assays), and histological analysis were conducted in adult flies. Statistical analyses were performed using GraphPad Prism and Microsoft Excel. ***Results.*** Larval-stage spermidine supplementation showed no neuroprotective benefit in the *sws¹* neurodegeneration model, as 5 mM treatment shortened lifespan, failed to restore locomotor or motor activity, and did not reduce histological signs of brain degeneration. ***Conclusions.*** Larval-stage spermidine treatment did not improve survival, behavior, or histological markers of neurodegeneration in adult *sws¹* flies. Further studies are required to evaluate the effects of lower spermidine concentrations.

Keywords: genes, age-related disease, *Drosophila melanogaster*, behavior.

Neurodegenerative diseases are incurable conditions that lead to the progressive loss of nervous system function due to neuronal death. Spermidine levels have been correlated with the development of neurodegenerative diseases. Spermidine belongs to a class of polyamines that interact with negatively charged DNA, RNA, and lipids, enabling it to participate in crucial cellular processes such as DNA stabilization, cell proliferation, growth, and cell death. Dietary supplementation with spermidine has shown distinct anti-aging and lifespan-extending effects across species, including yeast, worms, fruit flies, and mice (Satarker et al., 2024, Michiels et al., 2016).

The fruit fly, *Drosophila melanogaster* Meigen, is a well-established model organism for studying the molecular mechanisms underlying many human neurological disorders due to several key characteristics. Cellular and molecular pathways, including membrane excitability, synaptic plasticity, and neuronal signaling, are highly conserved between flies and humans (Giansanti et al., 2025).

Swiss-Cheese (SWS) is the *Drosophila* homolog of patatin-like phospholipase domain-containing protein 6 (PNPLA6), also known as Neuropathy Target Esterase (NTE). SWS contains a highly conserved domain required for phospholipase activity as well as a domain homologous to the regulatory subunit of protein kinase A, highlighting its important role in neuronal homeostasis (Law et al., 2025).

A mutagenesis screen identified an allele of the *sws* gene (*sws¹*) that introduces a premature stop codon, likely resulting in complete loss of SWS function. Loss of SWS leads to progressive neuronal degeneration and locomotor defects. Phenotypically, *swiss cheese* mutant flies exhibit adult-onset neurodegeneration characterized by spongiform brain lesions detectable around day 5 of adulthood, which worsen with age and ultimately result in premature death (Kretzschmar et al., 1997).

Based on these findings, we sought to determine whether spermidine supplementation during the larval stage could influence lifespan, neurodegeneration, and locomotor behavior in adult *sws¹* mutant flies.

Materials and methods

All experiments were performed with *D. melanogaster swiss cheese* (*sws*[1]) mutant, a line kindly provided by Prof. Doris Kretzschmar (Kretzschmar et al., 1997). As a control, the *Oregon-R* wild-type line served. Flies were kept in vials at 24 ºC, on standard medium (0.7% sugar, yeast, raisins, and semolina, 5% of each). Spermidine was fed during the larval stage at a concentration of 5 mM in the medium, selected based on previously reported effective doses in mouse studies (Satarker et al., 2024, Michiels et al., 2016). Adult flies were kept on standard medium.

Feeding assay. The amount of consumed food was calculated from the optical absorption of the consumed dye described previously (Matiytsiv et al., 2023).

Survival assays were performed using at least 100 males per genotype. Flies were transferred to fresh food every 2–3 days, and survival was recorded. Data were analyzed using GraphPad Prism 8, with significance determined by the log-rank test.

Histological sections and quantification of brain tissue degeneration. Degenerative changes in brain tissue were assessed using histological sections. prepared as previously described (Heisenberg and Böhl, 1979). Flies were anesthetized, positioned with the head properly oriented, and fixed in Carnoy's solution (ethanol : chloroform : acetic acid, 6 : 3 : 1) for 12 h at 4 °C. Samples were dehydrated in graded ethanol, cleared, and embedded in paraffin at 65 °C. Serial sections (7 μm) were cut using a Leica rotary microtome, mounted on SuperFrost slides, deparaffinized in xylene, and permanently coverslipped using DPX mounting medium. Images were captured with an Olympus IX73 microscope equipped with a DP-74 digital camera (Japan) using green fluorescent at 40× magnification. Brains from 20 flies per group were analyzed. Images were processed using ImageJ software (Matiytsiv et al., 2023).

Climbing activity assay. Locomotor performance was evaluated using a negative geotaxis (climbing) assay based on the phototaxis detection system with minor modifications Matiytsiv et al., 2023). The apparatus consisted of five vertically connected vials. Flies were allowed 30 s to climb, after which the number reaching the upper vial was recorded. Without rest, flies were sequentially transferred to the next vial, and the procedure was repeated until the final chamber was reached. The locomotor activity index (L-index) was calculated based on the number of flies reaching each level.

Open-field assay. Locomotor activity was evaluated in a custom-built open-field arena consisting of a 7-cm glass Petri dish divided into 0.5 × 0.5 cm squares and covered with a glass lid. After 2 min of acclimation, flies were video recorded for 10 min. Distance traveled was calculated offline by counting crossed squares (0.5 cm each). At least 25 males per genotype and age group were analyzed (Matiytsiv et al., 2013).

Statistical analysis was conducted using GraphPad Prism 8 and Microsoft Excel 2016. Differences between groups were evaluated using an unpaired two-tailed Student's *t*-test.

Results and discussion

Quantitative spermidine intake and survival analysis. Clear phenotypic differences between genotypes were observed, as *sws¹* mutants exhibited pronounced neurodegenerative features compared with *Oregon-R* wild-type flies. To ensure reliable comparisons between groups, spermidine consumption was quantified during the larval stage, when flies were exposed to spermidine-supplemented food. Wild-type and *sws¹* mutant larvae ingested 5 mM spermidine in amounts comparable to control food, indicating no genotype-dependent differences in intake (Fig. 1 a). After eclosion, all survival and behavioral assessments were performed in adult flies. Survival curves demonstrated that *sws¹* mutants had a significantly shorter lifespan than wild-type flies, confirming the suitability of this model for neurodegeneration studies. Larval-stage supplementation with 5 mM spermidine did not improve survival; instead, it reduced maximal lifespan in *sws¹* adults (Fig. 1 b). Median survival decreased from 15 to 13 days in *sws¹* mutants and from 43 to 39 days in *Oregon-R* controls. Log-rank test showed a significant reduction in survival in spermidine-treated *sws¹* flies.

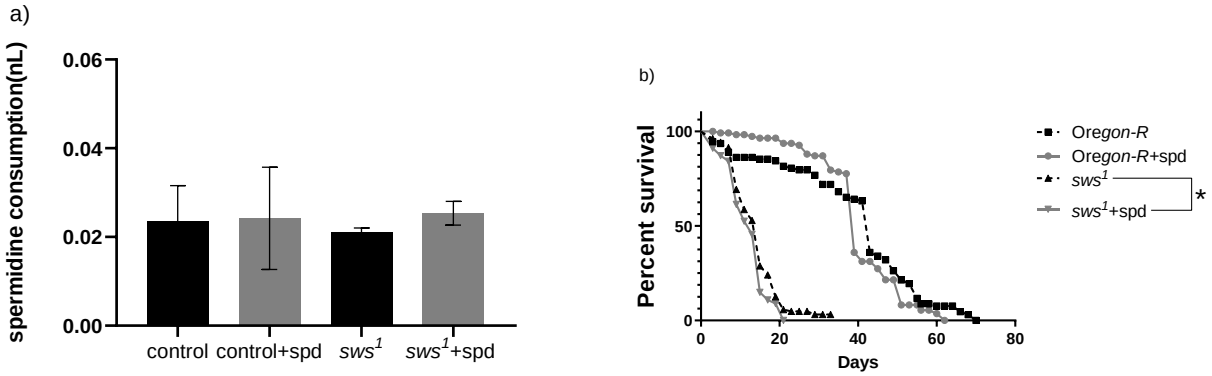

Fig. 1. a) Spermidine intake of the larva. No significant difference. b) Survival curves of *Oregon-R* and *sws¹* flies maintained on standard medium (dashed lines) or after being supplemented with spermidine (5 mM) (n = 100 per group). *Oregon-R* n=102, *Oregon-R*+spd (100), P= 0.1031; *sws*¹(n = 100), *sws¹+spd* (n = 100), P = 0,0231.

As locomotor activity is an important indicator of nervous system function, two additional behavioral assays were performed: climbing ability and the open field test. As locomotor activity is a sensitive indicator of nervous system integrity, two complementary behavioral assays were used to achieve a comprehensive assessment of motor function. Mechanical stimulation was used to elicit climbing capacity, while the open-field test measured spontaneous activity under physiological conditions, free from external disturbance. This combined approach allowed us to differentiate between deficits in baseline activity and impaired responsiveness to stimulation, providing a more reliable characterization of neurodegenerative changes in locomotor behavior. (Fig. 2 a, b). Under standard conditions, *sws¹* mutants demonstrated significantly impaired locomotor activity compared to the *Oregon-R* wild-type flies, indicating pronounced neurodegenerative alterations affecting motor performance. Spermidine supplementation did not significantly modify locomotor behavior in either wild-type or mutant flies compared to their respective controls maintained under standard conditions.

Polyamines in the brain act as modulators of neuron–glial networks and predominantly accumulate in glial cells, where they help maintain cellular homeostasis (Skatchkov et al., 2014). Therefore, polyamine availability may contribute to neuroprotection. Based on this, we examined whether spermidine supplementation affects brain vacuolization in *Drosophila*.

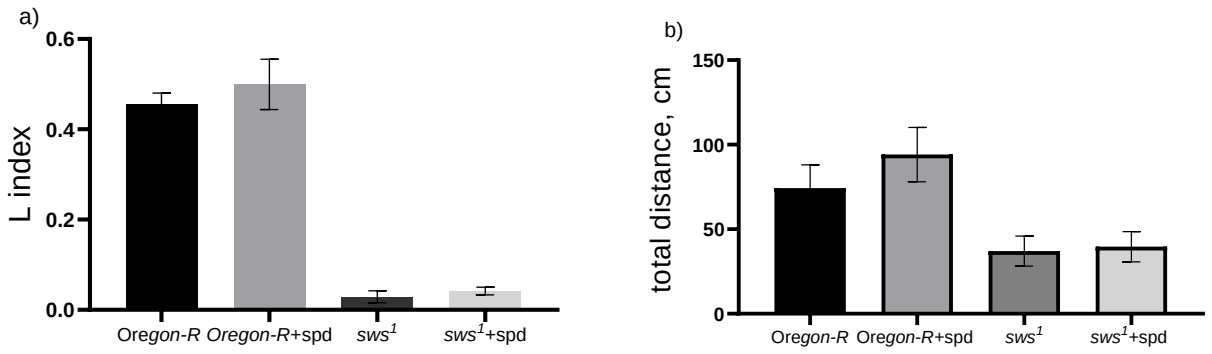

Fig. 2. Locomotor behavior of *Oregon-R* and *sws¹* flies after larval spermidine treatment: a) Locomotor activity index. b) Open-field assay of motor activity.

Histological sections of brains from 13-day-old male *sws¹* flies on control, or 5 mM spermidine-supplemented medium were compared. Phenotypically, *sws¹* mutants exhibited glial hyperwrapping and pronounced vacuolization due to the absence of functional SWS protein in neurons and glia (Fig. 3 a).

Degenerative lesions were widespread throughout the mutant brain. No visible phenotypic differences were observed between control and spermidine-treated *sws¹* flies, which prompted further quantitative analysis. Quantitative analysis of neurodegenerative areas revealed no improvement in either the lamina or the medulla following spermidine treatment, which correlates with results of lifespan and behavior tests (Fig. 3 c, d).

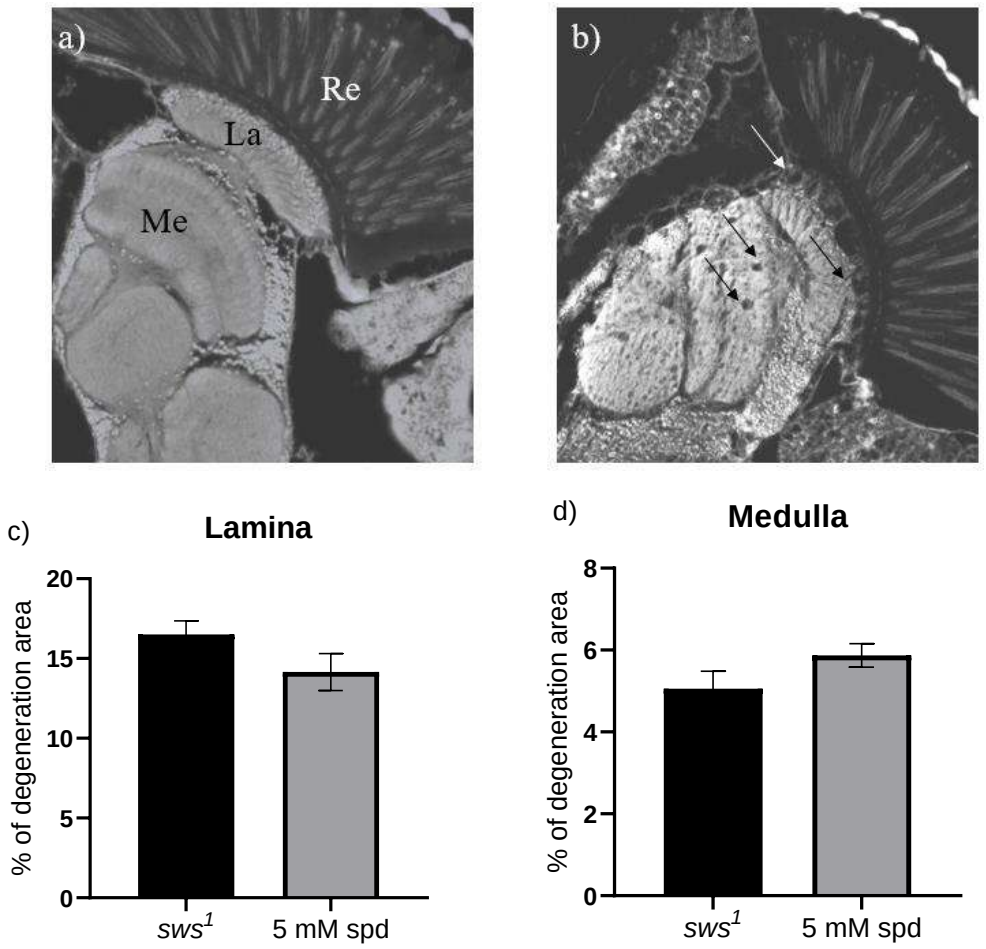

Fig. 3. a) Brain tissue of *Oregon-R*, 13-day-old males; b) *sws¹* mutant brain showing neurodegenerative vacuolization; (c,d) Quantitative analysis of degeneration areas in the lamina and medulla of *sws¹* mutants before and after spermidine treatment. Arrows indicate regions of neurodegeneration. Re – retina; La – lamina; Me – medulla.

Conclusions

Spermidine supplementation during the larval stage did not show neuroprotective effects in the *sws¹* model of neurodegeneration. 5mM spermidine reduced lifespan in both wild-type and mutant flies and failed to improve locomotor performance or brain histopathology. Neither evoked nor spontaneous motor activity showed functional recovery, and quantitative analysis revealed no attenuation of neurodegenerative vacuolization in mutant brains.

These findings indicate that exogenous spermidine at high concentrations may have detrimental rather than protective effects under conditions of genetically determined neurodegeneration. Further studies are required to evaluate lower spermidine concentrations and to investigate molecular and antioxidant stress markers to better define dose-

dependent effects and the mechanisms underlying spermidine action.

ChatGPT was used for grammar and language editing. The authors reviewed and approved the final manuscript and take full responsibility for its content.

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ВПЛИВ ВИСОКОЇ КОНЦЕНТРАЦІЇ СПЕРМІДИНУ НА МУТАНТІВ *SWISS CHEESE DROSOPHILA MELANOGASTER* ЗА ЛИЧИНКОВОГО ЗГОДОВУВАННЯ

Мета. Метою цього дослідження було визначити вплив додавання спермідину на личинковій стадії розвитку на тривалість життя, нейродегенеративний фенотип і рухову поведінку дорослих мутантних мух *sws¹*. ***Методи.*** Експерименти проводили з використанням мутантів *Drosophila melanogaster* Meigen *swiss cheese (sws¹)* та контрольної лінії дикого типу *Oregon-R*. Мух утримували при 24 °C на стандартному живильному середовищі. Спермідин (5 мМ) додавали до корму лише на личинковій стадії, тоді як аналіз тривалості життя, локомоторної активності (тести негативного геотаксису та відкритого поля) і гістологічні дослідження виконували у дорослих особин. Статистичну обробку даних проводили за допомогою програм GraphPad Prism та Microsoft Excel. ***Результати.*** Додавання спермідину на личинковій стадії не продемонструвало нейропротекторного ефекту в моделі нейродегенерації *sws¹*, оскільки обробка 5 мМ спермідином скорочувала тривалість життя, не відновлювала рухову активність і не зменшувала гістологічні ознаки дегенерації мозку. ***Висновки.*** Обробка спермідином на личинковій стадії не покращувала виживаність, поведінкові показники чи гістологічні маркери нейродегенерації у дорослих мух *sws¹*. Подальші дослідження необхідні для оцінки ефектів нижчих концентрацій спермідину.

Ключові слова: гени, вікові захворювання, *Drosophila melanogaster*, поведінка.

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