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Dopamine Homeostasis Links Age-Dependent Metabolic Stress Resistance to Longevity in Drosophila melanogaster

Anathbandhu Chaudhuri

achaudhuri@stillman.edu

Stillman College

Brittany Holloman

HBCU C.A.R.E.S. (Consortium to Advance Research and Economic Strength)

Poulami Sarkar

Indian Institute of Packaging

Natraj Krishnan

Mississippi State University

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Abstract

Dopamine (DA) functions beyond neurotransmission, yet its relationship with age-dependent stress adaptation and physiological aging remains incompletely understood. Here, we investigated whether genetic alterations in DA biosynthesis are associated with coordinated changes in dopamine homeostasis, metabolic stress resistance, and lifespan in *Drosophila melanogaster*. We examined *Catsup*^{26}, which exhibits elevated DA, and the DA-deficient *Pu*^{22} and *ple*^{2} mutants, together with isogenized *w*^{1118} controls. HPLC analysis revealed genotype-, tissue-, and sex-dependent alterations in DA and tetrahydrobiopterin (BH_4), linking disruption of the DA biosynthetic pathway to altered homeostasis. *Catsup*^{26} flies exhibited enhanced starvation resistance and extended lifespan, whereas *Pu*^{22} and *ple*^{2} mutants displayed reduced starvation tolerance and longevity. Starvation resistance declined with advancing age, with significant effects of genotype and age and significant genotype × age interactions in both sexes. Acute starvation also increased head DA and BH_4 and produced tissue- and sex-specific changes in peripheral DA and BH_4 levels. Together, these findings identify DA homeostasis as a physiological link between age-dependent metabolic stress resistance and longevity and support modulation of the DA biosynthetic pathway as a potential mechanism influencing stress adaptation during aging in *Drosophila*.

1. Introduction

Aging and senescence are characterized by progressive, time-dependent declines in cellular, biochemical, and physiological functions, accompanied by reduced functional capacity and resilience to environmental and physiological challenges. Aging is also a major risk factor for numerous chronic and life-threatening disorders, including cardiovascular disease, cancer, Alzheimer's disease, and Parkinson's disease. A prominent feature of aging is the progressive deterioration of stress-response capacity, which can influence health span, survival, and ultimately longevity. Stress resistance and lifespan are therefore closely interconnected, and enhanced resistance to environmental and metabolic stress has frequently been associated with increased longevity in *Drosophila* (Vermeulen and Loeschcke 2007). *Drosophila melanogaster* provides a powerful experimental model for investigating the genetic, physiological, and metabolic mechanisms underlying aging, functional decline, and longevity (Promislow et al. 2022; Wodrich et al. 2023).

Age-dependent changes in neuronal signaling, hormones, metabolism, and cellular stress-response pathways may contribute to the progressive loss of physiological homeostasis. Among the neurochemical systems potentially involved in these processes, dopamine (DA) is particularly important because its functions extend beyond classical neurotransmission to the regulation of behavior, locomotion, motivation, reward, learning, and physiological responses to environmental challenges. Dopaminergic function itself is sensitive to aging, with age-associated alterations in DA signaling contributing to changes in reward processing, cognition, and neurological function (Dreher et al. 2008; Jia et al. 2013). In *Drosophila*, disruption of DA homeostasis has also been associated with age- and sex-dependent alterations in physiological characteristics of senescence (Bednářová et al. 2017).

The dopaminergic system is particularly vulnerable to oxidative and age-associated cellular stress. Exposure of *Drosophila* to the oxidative stress-inducing herbicide paraquat causes selective degeneration of dopaminergic neurons and produces Parkinsonian-like phenotypes, supporting a relationship between oxidative stress and dopaminergic dysfunction (Chaudhuri et al. 2007). More recently, natural variation among *Drosophila* strains revealed a strong relationship between lifespan and age-related degeneration of dopaminergic neurons, with shorter-lived strains exhibiting greater loss of DA neurons and longer-lived strains showing greater preservation of these neurons during aging (Coleman et al. 2024). Together, these observations indicate an important intersection among DA homeostasis, cellular stress responses, and aging. However, whether genetically altered DA biosynthesis is associated with coordinated changes in age-dependent stress tolerance and organismal lifespan remains incompletely understood.

The DA biosynthetic pathway is tightly regulated, and alterations in its enzymatic and regulatory components can substantially disrupt DA homeostasis and associated physiological functions. DA is synthesized from the amino acid tyrosine through L-3,4-dihydroxyphenylalanine (L-DOPA), with tyrosine hydroxylase (TH) catalyzing the first and rate-limiting step in DA biosynthesis (Daubner et al. 2011). TH requires tetrahydrobiopterin (BH_4) as an essential cofactor, while GTP cyclohydrolase I (GTPCH) catalyzes the rate-limiting step in de novo BH_4 biosynthesis (Thöny et al. 2000). In Drosophila, the pale (ple) gene encodes TH (Neckameyer and White 1993), whereas Punch (Pu) encodes GTPCH (Mackay and O'Donnell 1983). DA biosynthesis is further regulated by Catecholamines up (Catsup), which acts as a negative regulator of DA production through post-translational regulation of both TH and GTPCH (Stathakis et al. 1999). Homozygous mutations affecting these essential components of the pathway can be lethal, whereas viable heterozygous mutants provide a useful genetic model for examining the physiological consequences of altered DA biosynthesis (Chaudhuri et al. 2007). Accordingly, genetic alterations in ple, Pu, and Catsup can perturb DA-pathway homeostasis, producing distinct changes in DA and BH_4 levels depending on the affected gene, tissue, and physiological context.

BH_4 is widely distributed among cells and tissues and is synthesized de novo from GTP through a pathway initiated by GTPCH, the major rate-limiting enzyme in BH_4 biosynthesis. Cellular BH_4 availability and regulation vary among tissues and cell types (Thöny et al. 2000; Werner et al. 2011). In the nervous system, BH_4 serves as an essential cofactor for aromatic amino acid hydroxylases and is therefore critical for the biosynthesis of monoamine neurotransmitters, including DA and serotonin. Alterations in BH_4 synthesis or availability have been associated with neurological and neuropsychiatric disorders involving disrupted monoaminergic function (Werner et al. 2011; Fanet et al. 2021). In peripheral tissues, BH_4 also contributes to diverse physiological processes through its role as a cofactor for nitric oxide synthases and has been implicated in vascular function, inflammation, glucose homeostasis, and cellular redox regulation (Werner et al. 2011; Fanet et al. 2021). However, the relationship among peripheral BH_4 homeostasis, metabolic stress responses, and aging remains comparatively poorly characterized.

Dopaminergic signaling also extends beyond its classical roles in neurotransmission and reward, contributing to the regulation of feeding, nutrient sensing, and energy homeostasis (Onimus et al. 2026). Recent evidence indicates that dopaminergic circuits integrate internal metabolic state, nutrient availability, and peripheral signals to coordinate adaptive feeding behavior, energy expenditure, and glucose homeostasis (Onimus et al. 2026). Starvation represents a severe metabolic stress that disrupts energy homeostasis and activates coordinated physiological and neuroendocrine responses required for survival. The capacity to withstand such stresses generally declines with advancing age and is considered an important feature of functional and biological aging (Simm et al. 2008; Vasto et al. 2010). Age-dependent changes are also evident in responses to oxidative stress, with chronological and reproductive aging influencing tissues' ability to withstand oxidative challenges (King et al. 2024). In Drosophila, stress resistance has been closely associated with longevity, although resistance to an individual stressor is not necessarily sufficient to extend lifespan (Rose et al. 1992; Harshman et al. 1999). Collectively, these observations suggest that age-dependent changes in metabolic and stress-response capacity provide an important framework for investigating whether alterations in DA and BH_4 homeostasis are associated with stress tolerance and longevity.

Although DA has been extensively studied in neuronal function and age-related neurological disorders, comparatively little is known about how genetically altered regulation of DA biosynthesis influences the capacity to withstand metabolic stress across the lifespan and organismal longevity. In particular, whether genetically driven increases or decreases in endogenous DA biosynthesis are associated with corresponding changes in age-dependent stress resistance remains incompletely understood.

To address these questions, we used three *Drosophila melanogaster* DA-pathway mutants, *Catsup*^{26}, *ple*^{2}, and *Pu*^{22}, representing distinct regulatory components of the DA biosynthetic pathway. We first quantified basal DA and BH_4 levels in head and peripheral (body) tissues to characterize genotype-dependent alterations in DA/BH_4 homeostasis and then subjected flies at 5, 15, 30, and 45 days of age to starvation to determine whether genetically altered DA biosynthesis was associated with differences in age-dependent metabolic stress resistance. Finally, DA and BH_4 levels were measured in head and body tissues of starved and non-starved *w*^{1118} flies to characterize the biochemical response of the dopaminergic pathway to acute metabolic stress. Finally, we determined lifespan in these fly lines to examine the relationship of DA homeostatic mechanisms with longevity. We hypothesized that genetic alterations that differentially regulate DA biosynthesis would be associated with corresponding differences in age-dependent starvation resistance and longevity.

2.2. HPLC analysis of dopamine and BH_4 in *Drosophila* head and body

Dopamine (DA) and the pteridine cofactor tetrahydrobiopterin (BH_4) were quantified in adult *Drosophila* heads and bodies using high-performance liquid chromatography (HPLC). Two sets of experiments were performed. First, basal DA and BH_4 levels were measured separately in heads and bodies of 48-h-old male and female *w*^{1118} control flies and the DA-pathway mutant genotypes (*Catsup*^{26}, *Pu*^{22}, and *ple*^{2})

under normal culture conditions to determine genotype-associated differences. Second, 5-day-old male and female w^{1118} flies were subjected to 24 h of food deprivation, after which DA and BH_4 levels were measured separately in heads and bodies and compared with those of corresponding non-starved controls to determine whether starvation stress altered DA and BH_4 homeostasis.

HPLC analysis was performed using a CoulArray HPLC system (Model 5600A; ESA, Chelmsford, MA, USA) equipped with a Synergi 4 µm Hydro-RP column (4.6 × 150 mm; Phenomenex, Torrance, CA, USA). The analytical procedure was based on the method of McClung and Hirsh (1999), with modifications described previously by Chaudhuri et al. (2007). For each sample, 100–150 adult heads or 10–15 adult bodies were collected and homogenized in 100–400 µL of 0.1 M perchloric acid. The resulting homogenates were filtered through 0.2 µm filters, and 10 µL of each filtered extract was injected into the HPLC system. Chromatographic separation was performed using a mobile phase containing 75 mM sodium phosphate (pH 3.0), 1.4 mM octanesulfonic acid, 25 µM ethylenediaminetetraacetic acid (EDTA), 100 µL/L triethylamine, and 7% acetonitrile. The mobile phase was delivered isocratically at a flow rate of 1 mL/min.

DA was detected electrochemically using an ESA Model 5011 analytical cell, with channel 1 maintained at − 50 mV and channel 2 at 300 mV. BH_4 was detected using a Linear Model LC305 fluorescence detector at excitation and emission wavelengths of 360 and 465 nm, respectively. DA and BH_4 levels were quantified by comparison with freshly prepared standards (Sigma), and chromatographic data were processed using ESA CoulArray software.

2.3. Starvation stress and body weight measurement

Starvation resistance was assessed in male and female w^{1118}, *Catsup*^{26}, *Pu*^{22}, and *ple*^{2} flies. For each genotype and sex, 10 flies were placed in each of three replicate vials, yielding a total of 30 flies per sex per genotype. Flies were distributed across replicate vials to minimize crowding and maintain comparable experimental density across groups. Flies were transferred to vials containing filter paper saturated with water to prevent desiccation while providing no food. Mortality was recorded at 6–12 h intervals until all flies had died.

To examine age-dependent changes in starvation resistance, assays were performed at 5, 15, 30, and 45 days of age. Males and females were analyzed separately at each age. Individual fly survival times were used as observations for statistical analysis (n = 30 per genotype, sex, and age group), and average survival duration under starvation was calculated for each group and compared among genotypes and across ages to assess the relationship between altered DA-pathway regulation, chronological age, and resistance to food-deprivation stress.

For body-weight analysis, 5-day-old male and female w^{1118} flies were subjected to 24 h of food deprivation and compared with corresponding non-starved controls. Flies were rapidly frozen in liquid nitrogen and immediately vortexed to separate the heads from the bodies. For each sex and experimental condition, 25 heads and 25 corresponding bodies were pooled separately for each

replicate, with three independent replicates, yielding a total of 75 heads and 75 bodies per condition. Each pooled sample was weighed, and the mean individual head and body weights were calculated by dividing the respective pooled weight by the number of heads or bodies in each sample. Total fly weight was calculated as the sum of the mean individual head and body weights.

2.4. Lifespan analysis

Male and female adult flies were separated within 0–12 h post-eclosion and maintained on the standard cornmeal medium described above. For each genotype and sex, 10 flies were placed in each of three replicate vials, yielding a total of 30 flies per sex per genotype. Mortality was recorded once weekly until all flies had died. Survival curves were generated from the mortality data, and average lifespan was calculated for each genotype and sex to assess the association between altered DA-pathway regulation and longevity.

2.5. Statistical analysis

For the genotype-dependent biochemical experiments, basal DA and BH_4 levels in each DA-pathway mutant genotype (*Catsup*^{26}, *Pu*^{22}, and *ple*^{2}) were compared individually with the w^{1118} control using two-tailed unpaired Student's *t*-tests. Additional pairwise comparisons between mutant genotypes were performed using two-tailed unpaired Student's *t*-tests, where appropriate, to evaluate specific genotype-dependent differences.

For the age-dependent starvation-resistance experiments, average survival duration under starvation was calculated for each genotype at 5, 15, 30, and 45 days of age. Males and females were analyzed separately. The effects of genotype and age on average starvation survival were evaluated using a two-way analysis of variance (ANOVA), with genotype and age as independent factors and the genotype × age interaction included as the interaction term. Specific differences among genotypes at individual ages and among age groups within each genotype were further evaluated by one-way ANOVA followed by Tukey's multiple-comparisons test.

For the starvation-induced biochemical experiments, DA and BH_4 levels in starved w^{1118} flies were compared with the corresponding non-starved w^{1118} controls using two-tailed unpaired Student's *t*-tests. Body-weight measurements were similarly compared between starved and corresponding non-starved controls using two-tailed unpaired Student's *t*-tests.

For the lifespan experiments, Kaplan–Meier survival curves were generated using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA), and differences between survival curves were evaluated using the log-rank (Mantel–Cox) test. Mean lifespan was calculated for each genotype and sex, and comparisons between each mutant genotype and the corresponding w^{1118} control were performed using two-tailed unpaired Student's *t*-tests.

Data are presented as mean ± SEM unless otherwise indicated. Differences were considered statistically significant at $P < 0.05$. Additional details of individual statistical comparisons are provided in the

corresponding figure legends.

3. Results

3.1. Dopamine-pathway mutations alter basal DA and BH_4 levels in *Drosophila*

Basal DA and BH_4 levels were quantified separately in the heads and bodies of 48-h-old *Drosophila* to determine how mutations affecting DA biosynthesis alter neuronal-enriched and peripheral biochemical profiles. Distinct genotype-, tissue-, and sex-dependent differences were observed in both DA and BH_4 levels (Fig. 1A–D).

Basal DA levels showed a consistent genotype-dependent pattern in both heads and bodies. *Catsup*^{26} flies exhibited significantly elevated DA levels in both the head and body compared with *w*^{1118} controls in males and females (Fig. 1A, B). In contrast, *Pu*^{22} and *ple*^{2} mutants, which have impaired DA biosynthesis, exhibited significantly reduced head and body DA levels relative to *w*^{1118}. DA levels were generally similar between *Pu*^{22} and *ple*^{2}, indicating comparable reductions in basal DA in these two low-DA genotypes. An exception was observed in the female body, where *ple*^{2} flies exhibited significantly lower ($P < 0.05$) DA levels than *Pu*^{22} flies.

In contrast to DA, basal BH_4 levels did not exhibit a uniform pattern across genotypes, tissues, and sexes (Fig. 1C, D). In males, *Catsup*^{26} flies exhibited significantly elevated head BH_4 levels relative to *w*^{1118}, whereas head BH_4 levels in *Pu*^{22} and *ple*^{2} flies were not significantly different from those in *w*^{1118} controls. In females, head BH_4 levels were significantly elevated in *Catsup*^{26} flies relative to *w*^{1118} controls. Interestingly, *ple*^{2} females also exhibited significantly elevated head BH_4 despite their reduced DA levels, whereas *Pu*^{22} females showed significantly lower head BH_4 levels than *w*^{1118} controls. Thus, alterations in BH_4 did not consistently parallel the genotype-dependent changes observed for DA.

Peripheral BH_4 levels showed a different pattern from those observed in the head. Body BH_4 levels were significantly reduced in both male and female mutant genotypes relative to *w*^{1118} controls, with the exception of *ple*^{2} females, in which no significant reduction was observed. Thus, changes in basal BH_4 levels varied according to genotype, tissue, and sex and did not consistently parallel the corresponding changes in DA.

Sex-dependent differences in basal DA and BH_4 levels varied markedly between the head and body. In the head, significant male–female differences were primarily observed in *Catsup*^{26} flies, with males exhibiting significantly higher levels of both DA and BH_4 than females ($P < 0.05$). In contrast, no significant sex-dependent differences in head DA or BH_4 were detected in *w*^{1118}, *Pu*^{22}, or *ple*^{2} flies. A broader sex-dependent pattern was evident in the body, where significant male–female differences in DA and BH_4 were observed across the examined genotypes, with the exception of body DA in *Pu*^{22} flies, which did not show a significant sex-dependent difference. Thus, the influence of sex on basal DA and

BH_4 levels was dependent on both genotype and tissue, with *Catsup*^{26} exhibiting the most pronounced sex-dependent biochemical differences. Overall, these findings establish distinct basal DA and BH_4 profiles in the heads and bodies of DA-pathway mutants and demonstrate genotype-, tissue-, and sex-dependent alterations in DA and BH_4 homeostasis (Fig. 1A–D).

3.2. Dopamine-pathway mutations differentially affect age-dependent starvation resistance

To determine whether alterations in DA biosynthesis influence age-dependent stress tolerance, male and female flies at 5, 15, 30, and 45 days of age were subjected to starvation stress. Two-way ANOVA performed separately for each sex revealed significant effects of genotype and age on starvation resistance. In males, significant effects were detected for genotype ($F_{3,464}$ = 570.52, P < 0.0001) and age ($F_{3,464}$ = 522.26, P < 0.0001), together with a significant genotype × age interaction ($F_{9,464}$ = 24.21, P < 0.0001). Similarly, females showed significant effects of genotype ($F_{3,464}$ = 762.94, P < 0.0001) and age ($F_{3,464}$ = 436.74, P < 0.0001), as well as a significant genotype × age interaction ($F_{9,464}$ = 3.81, P = 0.00012) (Fig. 2A, B). These interactions indicate that the magnitude of genotype-dependent differences in starvation resistance changed with age in both sexes.

Consistent with the significant overall effect of age, starvation resistance generally declined with advancing age across all genotypes in both sexes (Fig. 2A, B). One-way ANOVA followed by Tukey's multiple-comparisons test showed, however, that the decline was not significant between 30 and 45 days in *w*^{1118} males or females (P > 0.05). A similar plateau between 30 and 45 days was observed in *ple*^{2} males (P > 0.05). In *Pu□*^{22} males, the difference between 15 and 30 days was also not significant (P > 0.05). In contrast, *Catsup*^{26} males and females showed significant decreases in starvation resistance across successive age groups (P < 0.05). Successive age-dependent decreases were also significant in *Pu□*^{22} and *ple*^{2} females (P < 0.05).

The high-DA *Catsup*^{26} mutant exhibited significantly greater starvation resistance than age-matched *w*^{1118} controls at 5, 15, and 30 days in both sexes (P < 0.05) (Fig. 2A, B). At 45 days, however, this difference was no longer significant in males (P > 0.05), whereas *Catsup*^{26} females continued to exhibit significantly greater starvation resistance than age-matched *w*^{1118} females (P < 0.05). Thus, although the enhanced starvation resistance associated with *Catsup*^{26} diminished with advancing age, the genotype-associated advantage was retained to a greater extent in females.

In contrast, the low-DA *Pu□*^{22} and *ple*^{2} mutants exhibited significantly lower starvation resistance than age-matched *w*^{1118} controls, and *Catsup*^{26} flies at 5, 15, 30, and 45 days in both sexes (P < 0.05). The starvation-survival responses of *Pu□*^{22} and *ple*^{2} were generally similar. No significant differences between these two mutants were detected at 5, 15, or 45 days in males, whereas a significant difference was detected at 30 days (P < 0.05). In females, *Pu□*^{22} and *ple*^{2} did not differ significantly from one another at any of the four matched ages (P > 0.05).

The magnitude of the age-associated decline was further illustrated by the average survival durations. In w^{1118} flies, starvation survival decreased from approximately 3.10 to 1.41 days in males and from 4.51 to 2.32 days in females between 5 and 45 days of age. Similarly, survival of $Catsup^{26}$ flies decreased from 4.32 to 1.60 days in males and from 5.88 to 3.68 days in females. Starvation survival was substantially lower in the low-DA mutants: Pu^{22} and ple^{2} males survived approximately 2.28 and 2.31 days, respectively, at 5 days, declining to 0.81 and 0.82 days at 45 days. Corresponding survival durations in females declined from approximately 3.10 days at 5 days in both mutants to 1.40 days in Pu^{22} and 1.11 days in ple^{2} at 45 days.

A clear sex-associated pattern in starvation resistance was evident across the data, with females generally showing longer survival under food deprivation than males. This pattern was particularly apparent in older $Catsup^{26}$ flies, in which 45-day-old females showed greater starvation resistance than age-matched males. Collectively, these findings demonstrate that starvation resistance declines with advancing age and is strongly associated with DA-pathway genotype. The significant genotype × age interactions further demonstrate that the magnitude of genotype-dependent differences changes during aging. The high-DA $Catsup^{26}$ genotype was associated with enhanced starvation resistance, whereas the low-DA Pu^{22} and ple^{2} genotypes were associated with substantially reduced starvation tolerance (Fig. 2A, B).

3.3. Starvation alters DA and BH_4 levels and reduces body weight in *Drosophila*

To determine whether acute metabolic stress alters DA and BH_4 homeostasis, 5-day-old male and female w^{1118} flies were subjected to 24 h of starvation, followed by quantification of DA and BH_4 in the head and body. Starvation produced significant alterations in both biochemical components, with responses that varied according to tissue and sex (Fig. 3A, B).

In males, starvation resulted in a significant increase in DA levels in both the head and body compared with non-starved controls ($P < 0.05$; Fig. 3A). In females, head DA was also significantly elevated following starvation ($P < 0.05$), whereas body DA showed no significant change ($P > 0.05$). Thus, the starvation-induced increase in DA was observed in both tissues in males but was restricted to the head in females.

BH_4 showed a more consistent response to starvation. Following 24 h of food deprivation, BH_4 levels increased significantly in both the head and body of males and females compared with their respective non-starved controls ($P < 0.05$; Fig. 3B). Thus, unlike DA, which showed a tissue- and sex-dependent response, BH_4 exhibited a consistent starvation-induced increase across both tissues and sexes.

Starvation was also accompanied by a significant reduction in fly weight (Fig. 4). Following 24 h of starvation, head, body, and total fly weights were significantly reduced in both males and females compared with their respective non-starved controls ($P < 0.05$). Females exhibited significantly greater head, body, and total fly weights than males under both control and starvation conditions ($P < 0.05$).

Despite these sex-dependent differences in absolute weight, both sexes showed a consistent decline in weight following food deprivation.

Collectively, these findings demonstrate that 24 h of starvation induces significant alterations in DA and BH_4 levels together with a reduction in body mass, revealing distinct biochemical and physiological responses to acute nutrient deprivation (Figs. 3 and 4).

3.4. Altered dopamine biosynthesis is associated with genotype- and sex-dependent changes in lifespan

Lifespan analysis revealed marked differences among w^{1118}, $Catsup^{26}$, Pu^{22}, and ple^{2} flies (Fig. 5). Kaplan–Meier survival curves showed that $Catsup^{26}$ flies, which exhibited elevated DA levels, had significantly prolonged survival compared with w^{1118} controls in both males and females (Fig. 5A, B). In contrast, Pu^{22} and ple^{2} mutants, which exhibited reduced DA levels, displayed significantly shorter survival than w^{1118} controls and the high-DA $Catsup^{26}$ mutant. However, Pu^{22} and ple^{2} exhibited similar lifespan profiles and mean survival, with only minor differences between the two genotypes.

Survival of $Catsup^{26}$ flies extended to approximately 14 weeks in males and 15 weeks in females, at which point survival reached 0%, compared with approximately 11 weeks in male and 12 weeks in female w^{1118} controls. In contrast, survival reached 0% by approximately 8 weeks in both male and female Pu^{22} flies and by approximately 8 weeks in male and 9 weeks in female ple^{2} flies (Fig. 5A, B). Thus, the Kaplan–Meier survival profiles demonstrated an extension of lifespan in $Catsup^{26}$ and shortened lifespan in Pu^{22} and ple^{2} relative to w^{1118} controls.

Consistent with the Kaplan–Meier survival profiles, mean lifespan was substantially greater in $Catsup^{26}$ flies, reaching approximately 76 days in males and 85 days in females, compared with 46 and 49 days, respectively, in w^{1118} controls (Fig. 5C, D). Conversely, mean lifespan was reduced to approximately 35 days in males and 39 days in females for Pu^{22}, and 37 days in males and 40 days in females for ple^{2}.

Sex-dependent differences in lifespan were also evident across most genotypes, with females generally exhibiting longer survival than males. This difference was particularly pronounced in $Catsup^{26}$ flies, whereas male–female differences were comparatively small in Pu^{22} and ple^{2}. Collectively, these findings demonstrate that genetic alterations affecting the dopaminergic biosynthetic pathway are associated with pronounced differences in adult lifespan, with the high-DA $Catsup^{26}$ mutant exhibiting extended longevity and the low-DA Pu^{22} and ple^{2} mutants exhibiting shortened longevity relative to w^{1118} controls.

4. Discussion

Aging and senescence are complex biological processes driven by multiple interacting genetic, physiological, metabolic, and environmental factors. Although DA is widely recognized for its roles in neuronal function, behavior, metabolism, and stress responses, the contribution of DA biosynthesis and endogenous DA levels to organismal aging and age-dependent stress tolerance remains incompletely

understood. In the present study, genetic perturbations of the DA biosynthetic pathway were closely associated with alterations in lifespan and age-dependent stress resistance in *Drosophila melanogaster*, indicating that differences in endogenous DA status are accompanied by distinct effects on physiological responses to metabolic stress and longevity.

Our biochemical findings further revealed that BH_4 levels did not always parallel DA, particularly in head tissues. Nevertheless, the markedly elevated head BH_4 in *Catsup*^{26} was accompanied by increased head DA in both sexes, consistent with the role of BH_4 as an essential TH cofactor and with loss of *Catsup*-mediated negative regulation of TH and GTPCH. Conversely, reduced head BH_4 in *Pu*^{22} was accompanied by lower DA, consistent with impaired GTPCH-dependent BH_4 synthesis. An interesting exception occurred in *ple*^{2} females, in which head BH_4 was relatively elevated despite markedly reduced DA. Because *ple* encodes TH, greater availability of its BH_4 cofactor would not necessarily restore DA synthesis when TH function is compromised. This may reflect altered BH_4 utilization, turnover, or compensatory regulation. Excessive BH_4 may also have biological consequences independent of its cofactor function, as BH_4 can undergo autoxidation and generate reactive oxygen species, and elevated or extracellular BH_4 can induce oxidative damage in catecholaminergic cells (Choi et al. 2000). Interactions among BH_4, DA, and oxidative/quinone-mediated cytotoxicity have also been reported (Choi et al. 2003; Kim et al. 2005). Thus, the observed dissociation between BH_4 and DA emphasizes the importance of BH_4 synthesis, utilization, DA production, and cellular redox status in determining the biochemical phenotype.

Genotype-dependent changes were also evident in peripheral tissues. Body DA was elevated in *Catsup*^{26} but reduced in *Pu*^{22} and *ple*^{2}, while body BH_4 was generally reduced in the mutant genotypes, except in *ple*^{2} females. Previous studies have shown that whole-body DA declines with age in *Drosophila* and that disruption of DA homeostasis is associated with several senescence-related phenotypes (Bednářová et al. 2017). In the present study, the higher peripheral DA observed in the longer-lived *Catsup*^{26} flies and the lower DA in the shorter-lived *Pu*^{22} and *ple*^{2} mutants suggest a possible relationship between peripheral DA status and longevity. However, the specific contribution of peripheral DA to lifespan remains unclear, and these associations do not establish a direct causal relationship.

The physiological significance of reduced peripheral BH_4 remains less clear. Beyond its role in TH-dependent DA synthesis, BH_4 participates in other redox-sensitive enzymatic processes. In mammalian peripheral tissues, alterations in BH_4 availability have been implicated in age-related dysfunction of nitric oxide synthase, oxidative stress, and impaired vascular function (Blackwell et al. 2004; Pierce and LaRocca 2008; Schmidt and Alp 2007). Experimental studies in rat skeletal-muscle arterioles further demonstrated an approximately 60% reduction in BH_4 with aging (Delp et al. 2008). Although these vascular mechanisms cannot be directly extrapolated to *Drosophila*, they demonstrate that age-associated changes in peripheral BH_4 can have physiological consequences beyond catecholamine biosynthesis. Reduced body BH_4 in the present mutants may therefore reflect altered synthesis, utilization, turnover, or oxidative loss. Further studies will be required to determine whether peripheral DA

and BH_4 directly influence aging and stress resistance or are merely biochemical consequences of altered DA metabolism.

A major finding was the strong influence of age and DA biosynthetic status on starvation resistance. Starvation survival declined progressively with age in both sexes, but *Catsup*^{26} generally showed greater resistance, whereas *Pu*^{22} and *ple*^{2} were more susceptible. These observations extend previous findings from oxidative-stress models. *Catsup* mutants with elevated DA pools showed greater paraquat resistance, whereas *Pu*^{22} and *ple*^{2} were more susceptible, and supplementation with DA or L-DOPA ameliorated paraquat-induced survival and locomotor deficits (Chaudhuri et al. 2007). Similarly, *Catsup*^{26} exhibited greater resistance to paraquat and hydrogen peroxide than DA-deficient mutants, with altered antioxidant defenses potentially contributing to these phenotypes (Hanna et al. 2015). Our findings therefore extend the association between DA homeostasis and stress tolerance from chemical/oxidative stress to nutrient-deprivation stress.

Dopaminergic signaling is also linked to metabolic regulation in *Drosophila*. Disruption of the DA transporter (dDAT) in *fumin* mutants, which alters extracellular DA homeostasis, increased metabolic rate, while restoration of dDAT in dopaminergic neurons rescued associated phenotypes (Ueno et al. 2012). Starvation resistance itself is closely associated with metabolic adaptations involving energy storage and metabolic rate (Baldal et al. 2006; Brown et al. 2019). Thus, DA effects on starvation resistance may involve interactions between dopaminergic regulation and systemic energy utilization during nutrient deprivation. Importantly, our significant genotype × age interaction further indicates that these effects vary with age. Although *Catsup*^{26} maintained a starvation-survival advantage at younger and intermediate ages, this advantage was no longer evident in 45-day-old males. Age-dependent interactions between dopaminergic signaling and stress vulnerability have also been demonstrated in *Drosophila*: an age-related decline in paraquat resistance was associated with increased expression of the D1-like dopamine receptor Dop1R2 (DAMB), whereas this aging-associated increase in vulnerability was absent in DAMB-deficient flies (Cassar et al. 2015). These findings suggest that the contribution of DA biosynthetic status to stress resistance progressively changes with age.

Our findings further demonstrate that acute starvation itself alters DA and BH_4 homeostasis. Following 24 h of food deprivation, DA increased in the head and body of males and in the head of females, whereas female body DA remained unchanged; BH_4 increased in both tissues in both sexes. Thus, the DA/BH_4 pathway appears to respond dynamically to nutrient deprivation rather than serving only as a static predictor of stress resistance. DA is linked to food sensing and nutrient-dependent signaling, including dietary restriction-associated pathways in *C. elegans* (Suo et al. 2009; Jiang et al. 2022). However, appropriate homeostatic regulation may be critical, as dopaminergic signaling can modulate oxidative stress vulnerability, while BH_4 can generate reactive oxygen species under certain conditions (Choi et al. 2000; Cassar et al. 2015). The starvation-induced increases in DA and BH_4 may therefore represent an adaptive metabolic stress response, although prolonged or excessive elevations could contribute to oxidative stress. These responses were also sex-dependent: females generally exhibited greater lifespan and starvation resistance, while basal and starvation-induced DA/BH_4 profiles differed

between sexes. Such differences may involve sex-specific energy metabolism, nutrient reserves, reproductive physiology, and dopaminergic regulation rather than DA alone.

Finally, 24 h of food deprivation significantly reduced total, head, and body weights in both sexes, consistent with depletion of energy reserves during acute nutrient stress. These changes occurred concurrently with increased DA and BH_4, particularly in head tissues, further suggesting that modulation of this pathway accompanies physiological adaptation to altered energy availability. Another major finding of this study was the association between genetic alterations in DA biosynthesis and lifespan. *Catsup*^{26} flies, characterized by elevated head DA, exhibited increased lifespan and greater starvation resistance, whereas the DA-deficient *Pu*^{22} and *ple*^{2} mutants showed reduced lifespan and stress resistance. These observations are consistent with recent findings in the silkworm *Bombyx mori*, in which endogenous DA levels were positively associated with adult lifespan; reduced DA shortened lifespan, whereas moderate peripheral DA administration improved health span and locomotor performance, with effects dependent on dose, age, and sex and associated with enhanced antioxidant capacity (He et al. 2026). The contrasting phenotypes of our mutants are consistent with their established roles in DA biosynthesis. *pale* (*ple*) encodes tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis (Neckameyer and White 1993), whereas *Punch* (*Pu*) encodes GTP cyclohydrolase I (GTPCH), required for synthesis of tetrahydrobiopterin (BH_4), an essential TH cofactor. In contrast, *Catecholamines up* (*Catsup*) negatively regulates DA biosynthesis through post-translational regulation of TH and GTPCH (Stathakis et al. 1999). Thus, the biochemical and longevity phenotypes observed here are consistent with the known functions of these components of the DA biosynthetic pathway.

Evidence from other experimental models further supports a role for dopaminergic pathways in longevity. Genetic variation in *Drosophila* dopa decarboxylase (*Ddc*) has been associated with natural variation in lifespan (De Luca et al. 2003). Manipulation of specific dopaminergic neurons through enhanced Multiple Ankyrin repeats Single KH domain (Mask) activity has also been reported to extend lifespan in flies; importantly, this effect required dopaminergic transmission but was not reproduced simply by increasing DA production (Tian 2021). Selection for divergent virgin lifespan in *Drosophila* resulted in differences in DA levels, although the relationship was not uniform across all selected lines, suggesting that DA is one component of a broader physiological network regulating longevity (Vermeulen et al. 2006). Earlier studies also reported increased lifespan following prolonged L-DOPA administration in mice (Cotzias et al. 1974). Together with the recent *Bombyx* findings (He et al. 2026), these observations support a relationship between DA homeostasis and organismal longevity while emphasizing that its effects depend on genetic background and physiological context.

Studies in *Caenorhabditis elegans* provide additional mechanistic evidence linking DA signaling with lifespan. Dopaminergic signaling participates in nutrient sensing and food-dependent regulation of longevity through interactions with octopaminergic signaling (Suo et al. 2009). Moreover, dopamine D1-like (DOP-4) and D2-like (DOP-2) receptors exert opposing effects on lifespan, with D2-like signaling influencing longevity through a dietary restriction-associated AAK-2/AMPK–DAF-16/FOXO pathway

(Jiang et al. 2022). Stimulation of DA secretion by the peptide Acein also prolonged lifespan and ameliorated age-related motor decline in *C. elegans* (Wang et al. 2023). More recently, pharmacological inhibition of vesicular monoamine transporter (VMAT) by reserpine extended lifespan in *Drosophila*, although this was accompanied by reduced locomotor activity and impaired heat-stress resistance (Tiwary et al. 2026). Because VMAT transports multiple monoamines, this effect is not DA-specific, but it illustrates that alterations in monoaminergic signaling can dissociate longevity from stress resilience. Collectively, these studies indicate that DA-associated regulation of lifespan is receptor-, pathway-, and context-dependent rather than being determined simply by absolute DA concentration.

Together, our findings support an integrated relationship in which aging progressively reduces stress tolerance, DA biosynthetic status modifies the capacity to withstand metabolic stress, and acute nutrient deprivation alters DA/BH_4 homeostasis while reducing body mass. Nevertheless, the mutant genotypes affect components of the DA biosynthetic pathway and may therefore produce physiological effects beyond alterations in DA alone, while DA and BH_4 were measured under selected experimental conditions. Based on these observations, we propose a model in which genetic alterations in the DA biosynthetic pathway modify DA and BH_4 homeostasis and are associated with corresponding differences in stress resistance and lifespan (Fig. 6). Importantly, these associations do not establish that DA alone determines stress resistance or lifespan. Overall, our findings identify DA biosynthetic homeostasis as an important component linking physiological adaptation to metabolic stress to aging and longevity in *Drosophila*.

Declarations

Author Contributions

Anathbandhu Chaudhuri: Conceptualization, Methodology, Supervision, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; Brittany Holloman: Investigation, Writing – review & editing; Poulomi Sarkar: Formal analysis, Writing – review & editing; Natraj Krishnan: Conceptualization, Formal analysis, Writing – review & editing. All authors reviewed and approved the final version of the manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Statement

This study used the fruit fly, *Drosophila melanogaster*, an invertebrate model organism. Experiments involving *Drosophila* are not subject to Institutional Animal Care and Use Committee (IACUC) approval under current institutional and U.S. regulatory guidelines. All experiments were performed in accordance with established laboratory practices for the ethical use and care of invertebrate research organisms. Both male and female flies were included in the study, and sex-specific effects were evaluated where appropriate.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) for English language and grammatical editing and to assist in refining the presentation of the schematic illustration (Fig. 6). The authors reviewed and edited all AI-assisted content and take full responsibility for the final content of the manuscript.

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Figures

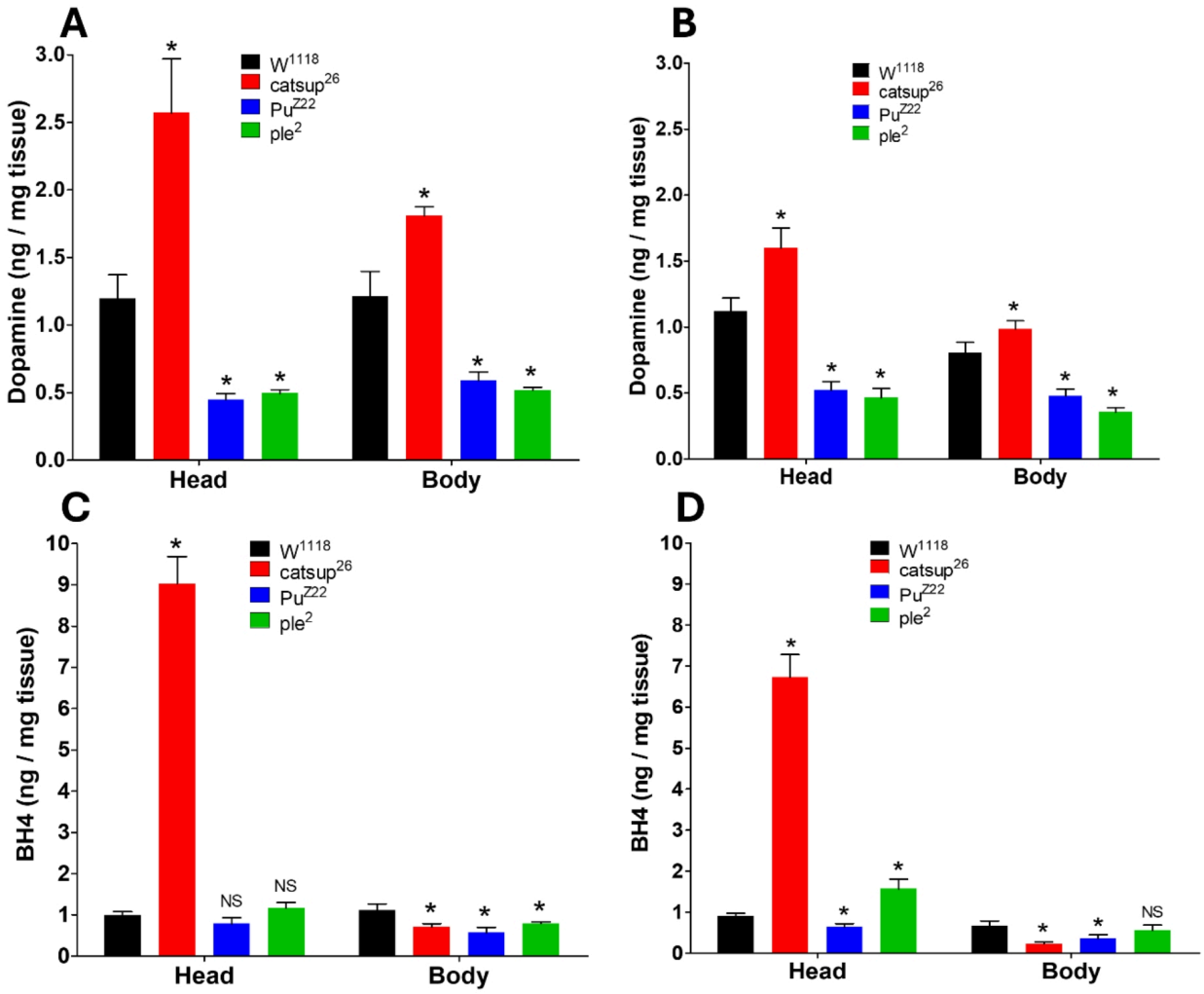


Figure 1

Basal dopamine and tetrahydrobiopterin levels in the head and body of dopamine-pathway mutant *Drosophila*. Dopamine (DA) and tetrahydrobiopterin (BH_4) levels were quantified in the heads and bodies of 48-h-old adult *w*¹¹¹⁸, *Catsup*²⁶, *Pu*□²², and *ple*² flies using high-performance liquid chromatography (HPLC). Heads and bodies were separated following freezing in liquid nitrogen and vortexing. Approximately 100–150 heads and 10–15 separated bodies were collected for HPLC analysis, with three independent biological replicates per genotype. (A) DA levels in male flies; (B) DA levels in female flies; (C) BH_4 levels in male flies; and (D) BH_4 levels in female flies. Data are presented as mean ± SEM from three independent biological replicates. Statistical comparisons indicated in the figure were performed using Student's *t*-test, with each mutant genotype compared with the corresponding *w*¹¹¹⁸ control. $P < 0.05$ was considered statistically significant; * indicates $P < 0.05$ and NS indicates not significant.

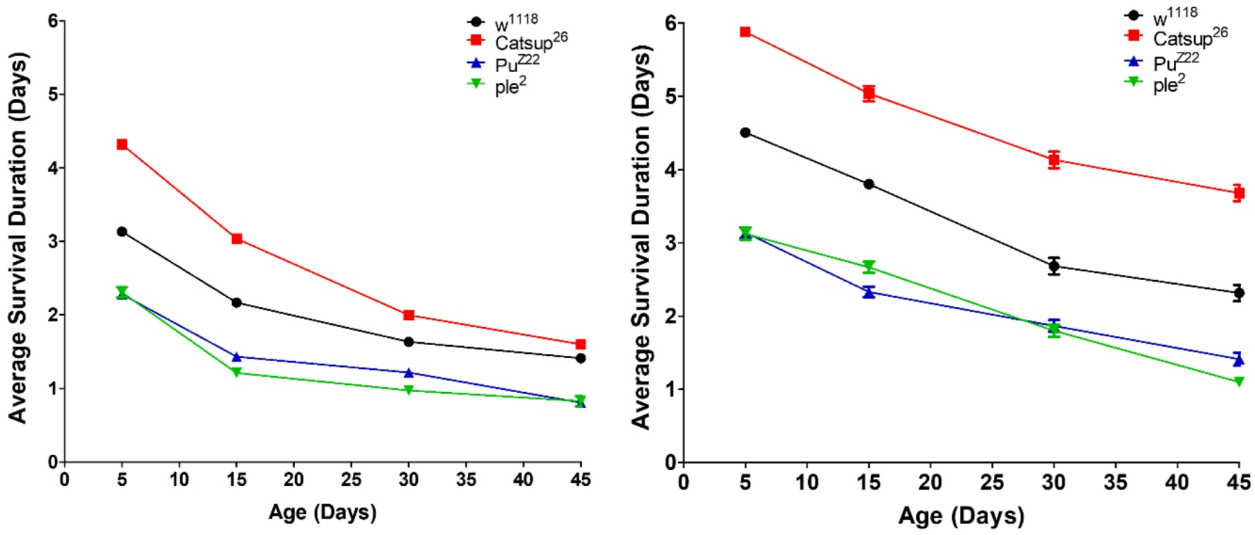


Figure 2

Age-dependent starvation resistance in dopamine-pathway mutant *Drosophila*. Male (A) and female (B) w^{1118}, $Catsup^{26}$, Pu^{Z22}, and ple^{2} flies were subjected to starvation at 5, 15, 30, and 45 days of age. For each sex, genotype, and age group, 30 individual flies were analyzed (10 flies per vial × 3 replicate vials), with individual fly survival times used as observations for statistical analysis (n = 30 per group). Average survival duration under starvation is presented as mean ± SEM. The effects of genotype and age, including the genotype × age interaction, were analyzed separately for males and females using two-way ANOVA. Specific differences among genotypes at individual ages and among age groups within each genotype were evaluated by one-way ANOVA followed by Tukey's multiple-comparisons test. Statistical significance was defined as $P < 0.05$. Significance symbols are omitted from the figure for clarity; detailed statistical comparisons are described in the Results.

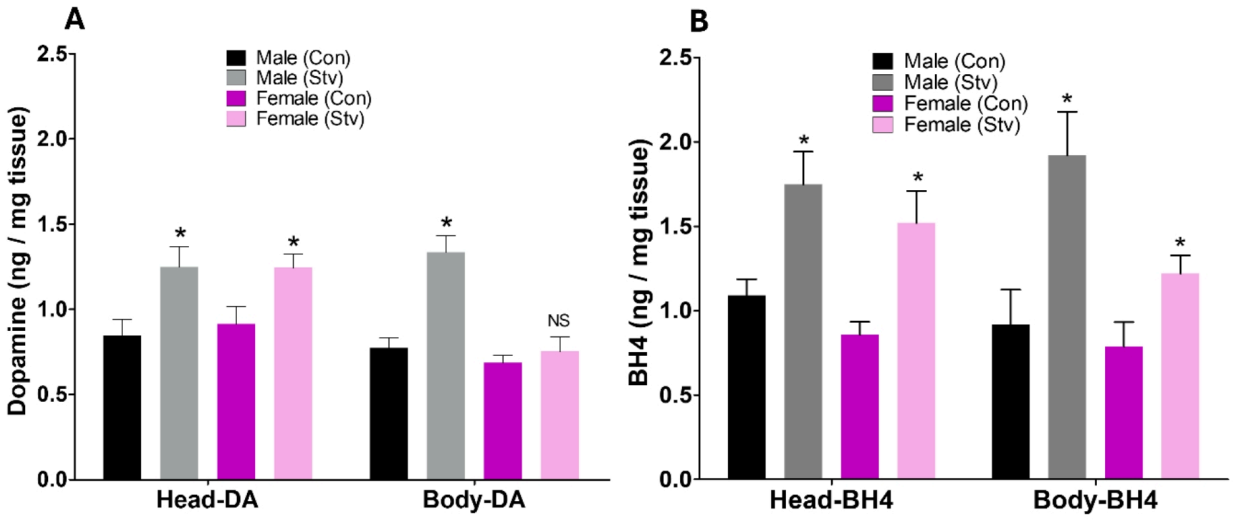


Figure 3

Effects of acute starvation on dopamine and tetrahydrobiopterin levels in *Drosophila*. Five-day-old male and female w^{1118} flies were subjected to 24 h of food deprivation and compared with corresponding non-starved controls. Heads and bodies were separated following freezing in liquid nitrogen and vortexing. Approximately 100–150 heads and 10–15 separated bodies were collected from control and starved flies for HPLC analysis, with three independent biological replicates analyzed for each sex and experimental condition. (A) Dopamine (DA) levels in the heads and bodies of male and female flies; (B) tetrahydrobiopterin (BH_4) levels in the heads and bodies of male and female flies. Data are presented as mean ± SEM from three independent biological replicates. Statistical comparisons between corresponding control and starved groups were performed using a two-tailed unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant; * indicates $P < 0.05$.

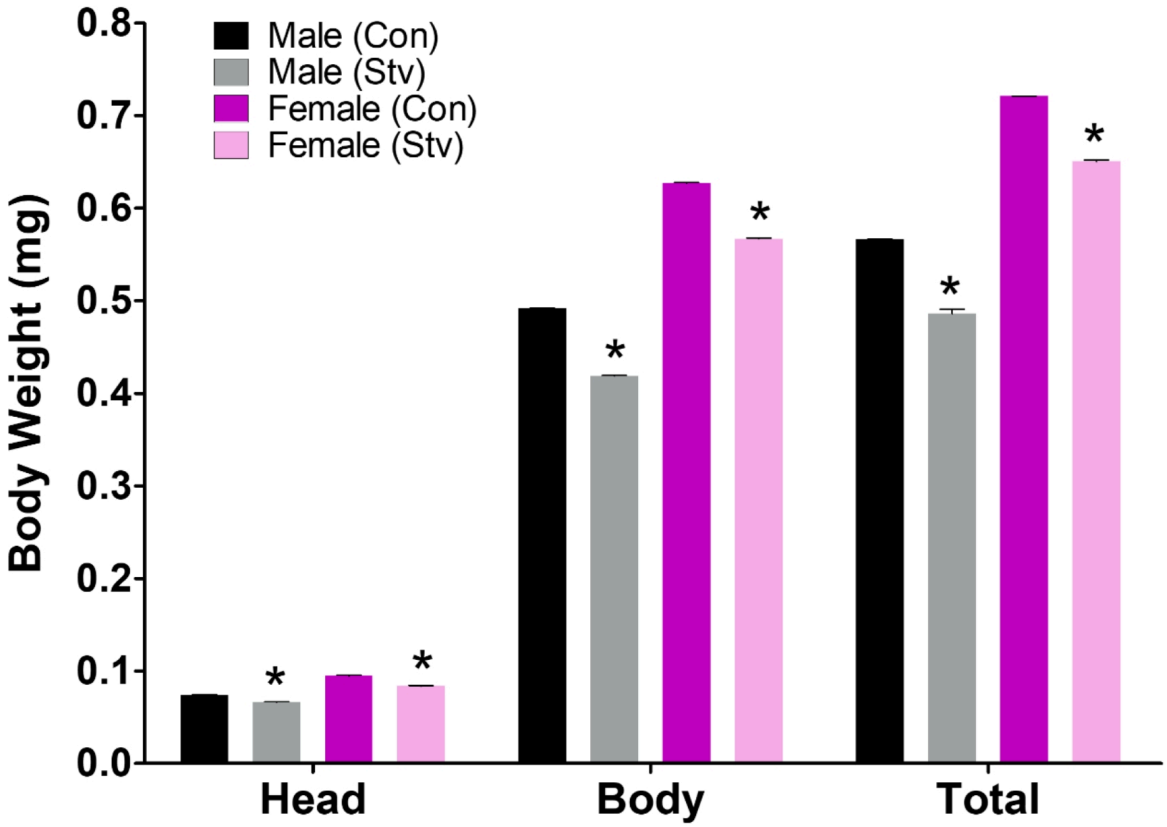


Figure 4

Effects of acute starvation on body weight in *Drosophila*. Five-day-old male and female w^{1118} flies were subjected to 24 h of food deprivation and compared with corresponding non-starved controls. Flies were rapidly frozen in liquid nitrogen and vortexed to separate the heads from the bodies. For each sex and experimental condition, 25 heads and 25 corresponding bodies were pooled separately for each replicate, with three independent biological replicates analyzed. Mean individual head and body weights were calculated by dividing the respective pooled weights by the number of heads or bodies in each sample, and total fly weight was calculated as the sum of the mean individual head and body weights. Data are presented as mean ± SEM from three independent biological replicates. Statistical comparisons between corresponding control and starved groups were performed using a two-tailed unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant; * indicates $P < 0.05$.

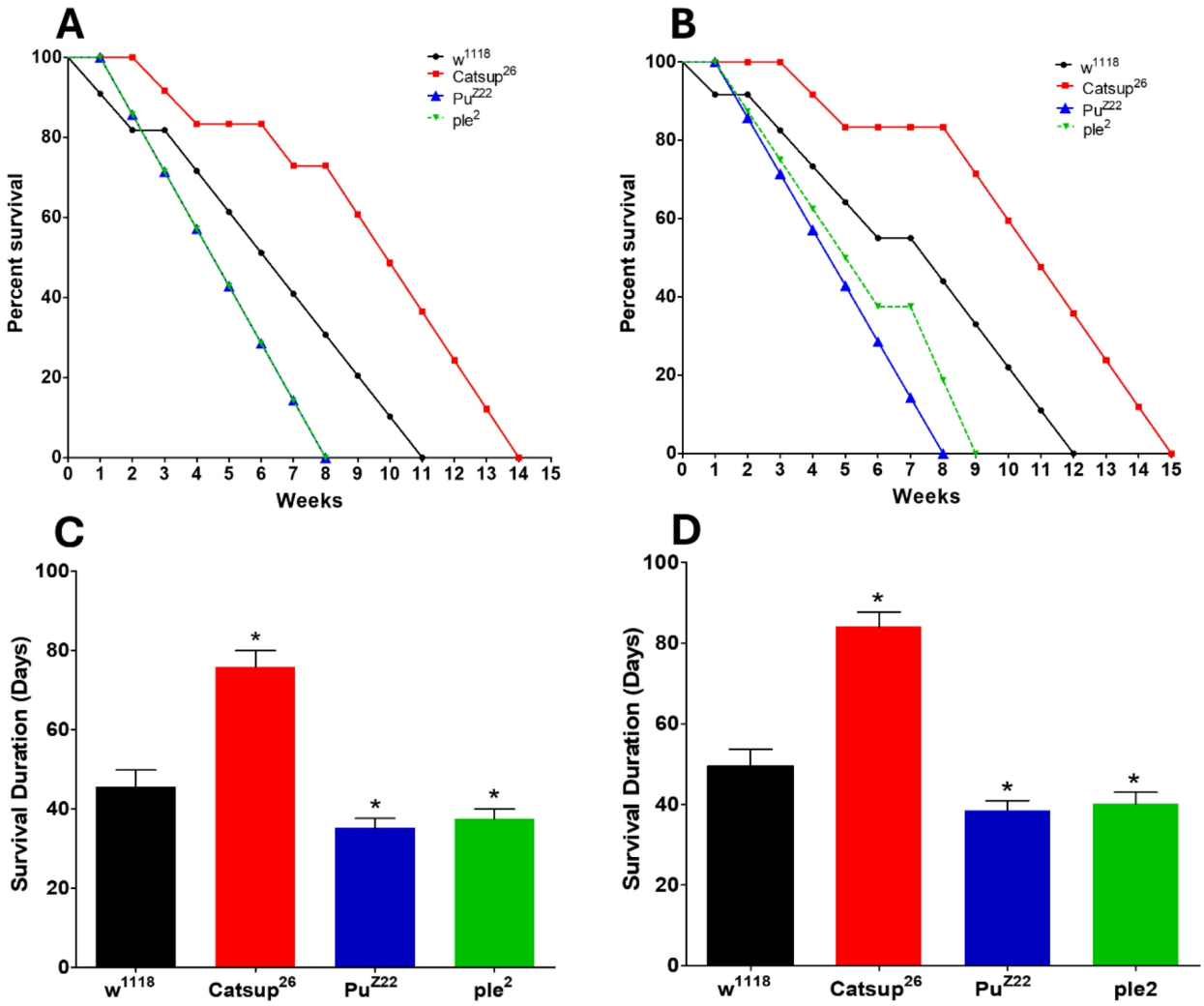


Figure 5

Lifespan and mean survival of dopamine-pathway mutant *Drosophila*. Lifespan was assessed in newly emerged (0–12 h old) male and female *w*¹¹¹⁸, *Catsup*²⁶, *Pu*□²², and *ple*² flies (n = 30 flies per sex and genotype). Survival was monitored weekly until all flies had died. Kaplan–Meier survival curves for males (A) and females (B) were generated using GraphPad Prism, and differences in survival curves were evaluated using the log-rank (Mantel–Cox) test. Mean lifespan of males (C) and females (D) is presented as mean ± SEM. Statistical comparisons of mean lifespan between each mutant genotype and the corresponding *w*¹¹¹⁸ control were performed using a two-tailed unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant; * indicates $P< 0.05$.

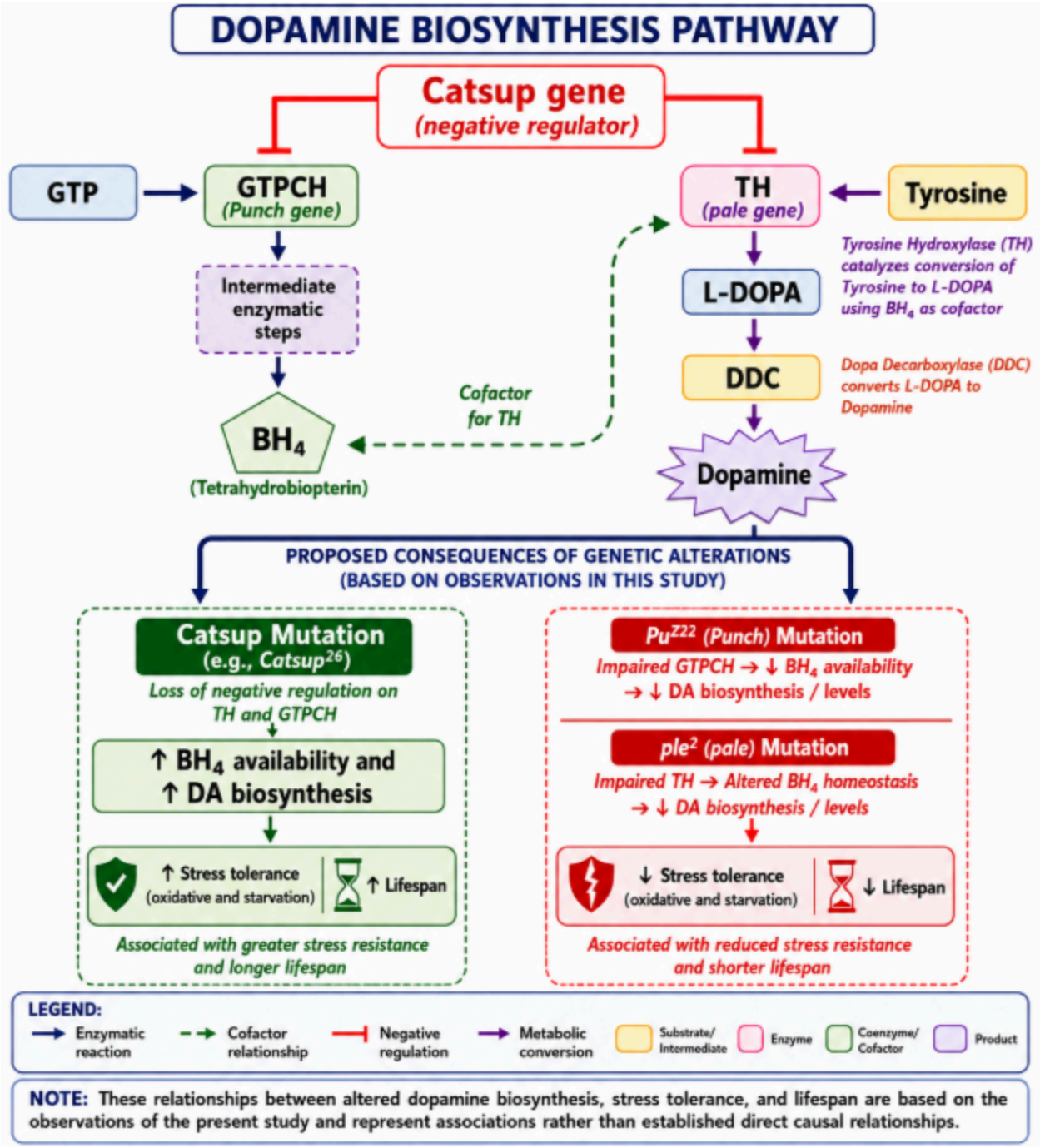


Figure 6

Proposed model linking alterations in dopamine biosynthesis with stress resistance and lifespan in *Drosophila*. The schematic summarizes the dopamine (DA) biosynthetic pathway and the proposed physiological consequences of genetic alterations in *Catsup*, *Punch*(*Pu*), and *pale* (*ple*) based on the findings of the present study. GTP cyclohydrolase I (GTPCH), encoded by *Punch*, catalyzes the first and rate-limiting step in tetrahydrobiopterin (BH_4) biosynthesis, whereas tyrosine hydroxylase (TH), encoded by *pale*, uses BH_4 as a cofactor to convert tyrosine to L-DOPA, which is subsequently converted to DA by dopa decarboxylase (DDC). *Catsup* negatively regulates TH and GTPCH. Consistent with this pathway, *Catsup*^{26} was associated with elevated DA and BH_4, greater stress resistance, and longer lifespan,

whereas *Pu*[22] and *ple*[2] were associated with reduced DA, lower stress resistance, and shorter lifespan. BH_4 alterations in these mutants were tissue- and sex-dependent and did not always parallel changes in DA. The proposed relationships between DA/BH_4 homeostasis, stress resistance, and lifespan are associations based on the present findings and do not imply direct causality. ChatGPT (OpenAI, GPT-5.6 Sol) was used to assist with refinement of the visual presentation of this schematic illustration; the scientific content was reviewed and verified by the authors.