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Mosaic of *Drosophila* Behavior Due to Bisphenol-A Exposure: From Hyperactivity to Aversion

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Abstract. Bisphenol-A (BPA) is classified as an endocrine-disrupting chemical that is widely found in everyday food. However, various studies have reported inconsistent results regarding the effects of this exposure on behavior. This study aimed to evaluate how dose, genetic background, and duration of exposure interact to shape behavioral responses in *Drosophila melanogaster* exposed to BPA. Three strains (Wild-type, *white-eye*, *and black-body*) were cultured on a medium containing 0, 0.25, and 0.5 mg/mL BPA for two generations. Before exposure (G0), after the first generation of exposure (G1), and after the second generation of exposure (G2), three behaviors were measured: larval crawling speed, number of larval contractions, and larval exploration distance. Repeated-measures ANOVA revealed a non-monotonic pattern in behavioral responses. Each behavior exhibited distinct sensitivities to treatment factors, and complex interactions were observed between genetic factors, dose, and time (generation). Furthermore, the effects of BPA are not general but rather specific to the type of behavior observed. Generational effects are evident in some behavioral data. In addition, fly strain plays a role in determining the effect of exposure on behavioral responses.

Keywords: Activity; BPA contamination; *Drosophila melanogaster*; locomotor; neurobehavior

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INTRODUCTION

Bisphenol-A (BPA) is an endocrine-disrupting chemical widely used in producing polycarbonate plastics and epoxy resins (Liao et al., 2025). These materials are found in electronic equipment, construction materials, and food containers (Al-Tameemi et al., 2024). Its extensive use has led to environmental contamination in the air, soil, water, and food (Ulyarti et al., 2021). BPA can leach from packaging into food and beverages (Vilarinho et al., 2019), bind to estrogen-related receptor gamma (ERRγ), and alter DNA (Pathak & Kim, 2024; Cariati et al., 2020), and disrupts the neurotransmitter system (Costa & Cairrao, 2024; Shi et al., 2025). Consequently, behavioral alterations are often used as indicators of BPA-induced neurotoxicity.

Although considered capable of influencing behavior, various studies on BPA have reported inconsistent results. Some studies have reported hyper-locomotion in zebrafish, rats, and fruit flies following BPA exposure (Kochetkov et al., 2024; Ishido et al., 2004; Musachio et al., 2021), whereas others have found reduced activity or exploratory behavior (Wang et al., 2020; Begum et al., 2021). In several cases, no significant behavioral effects were observed (Rubin & Seebacher, 2024; Wang et al., 2023). These discrepancies may arise from methodological variations, single-generation designs, or the lack of genetic diversity in test organisms, which can obscure the complex dose-response and genotype-environment interactions.

In response to the limitations of several previous studies, the use of fruit flies (*Drosophila*

melanogaster) can facilitate the design of BPA exposure studies spanning multiple generations and diverse genetic backgrounds. *D. melanogaster* provides an efficient model for multi-generational and multi-strain toxicological studies due to its short life cycle, ease of culture, and genetic similarity to humans, with approximately 60% of genes and 75% of human disease-related genes having homologs in flies (Atoki et al., 2025) and comparable to other organisms, such as mice and rats (Pertiwi et al., 2025). There are also validated behavioral assays also enable assessment of contaminant effects on neural and motor systems (Neckameyer & Bhatt, 2016; Welch & Mulligan, 2022).

Previous research has investigated BPA's effects on *Drosophila* behavior (Begum et al., 2021; Nguyen et al., 2021). However, studies that simultaneously measure three behavioral parameters at different doses and strains and collect data across generations are still challenging to find. Most cross-generational studies expose only one parental generation, limiting conclusions about long-term or cumulative impacts (Kim et al., 2021; Wolstenholme et al., 2019). The present study addresses these gaps by testing three genetically distinct strains (wild type*, white-eye*, and *black-body*) exposed to three BPA concentrations (0, 0.25, and 0.5 mg/mL) across two successive generations. Behavioral responses were assessed before exposure (G0), after one generation (G1), and after two generations (G2).

This multifactorial design enables analysis of how exposure duration, dose, and genotype interact to shape behavioral outcomes, including potential reversals or accumulations across generations. Moreover, this study examines rarely reported larval behavioral parameters such as crawling speed, contraction frequency, and exploration distance to reveal whether BPA induces a mosaic of phenotype-specific responses. By integrating these behavioral measures, we aim to elucidate how individual genetic variability contributes to differential susceptibility under cumulative toxicant exposure.

This study aimed to evaluate how dose, genetic background, and duration of exposure interact to shape behavioral responses in *Drosophila melanogaster* exposed to BPA

METHODS

Fruit Fly Stock and Maintenance

All experiments were conducted at the Genetics Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang (Malang, East Java, Indonesia; 7°96′ S, 11°62′E). Three *D. melanogaster* strains were used: wild-type (*N*), *white-eye* (*w*), and *black-body* (*b*), obtained from the laboratory stock culture. Flies were maintained under standard laboratory conditions (room temperature, 12 h light:12 h dark cycle, and normal humidity) on a semi-solid medium of mashed banana, brown sugar, and cassava tape (7:2:1), following Fauzi et al. (2020). The cooked medium was poured into 200 mL glass bottles and cooled to room temperature before use.

Experimental Design and BPA Exposure

A mixed factorial design was employed with two between-subject factors, namely BPA concentration (0, 0.25, and 0.5 mg/mL) and strain (*N, w, b*), and one within-subject factor, generation (before exposure/G0, first generation exposure/G1, and second generation exposure/G2). Each combination was replicated four times, resulting in 36 observation units (3 concentrations × 3 strains × 4 replications). Behavioral observations were conducted at all three generations. BPA exposure was administered through food media throughout all developmental stages, from adult parents to their offspring, encompassing the egg, larval, pupal, and adult phases.

Behavioral Assays

Crawling Assay (Speed and Number of Contractions)

The crawling assay for measuring larval speed and contraction frequency was adapted from Post & Paululat (2018). An empty Petri dish was placed on millimeter paper under a stereo microscope equipped with a camera (Figure 1a). The surface of the dish was lightly coated with water using a brush, and a third-instar larva was placed at the center. Video recording was carried out for at least ten seconds while the larva moved continuously without falling, stopping, or rotating. Recordings shorter than ten seconds were repeated. Video analysis was then used to count the number of larval contractions within a ten-second period, interpreted as twitching movements during locomotion. The total distance traveled in ten seconds was measured and converted into larval crawling speed (mm/s).

Exploratory Assay

The exploratory assay measured the distance traveled by freely moving larvae within one minute, adapted from Post & Paululat (2018). A

third-instar larva was placed at the center of the observation arena (Figure 1b). The observation was initiated with a timer, and larval movement was recorded for one minute. The distance from the starting point was then determined in centimeters using a thread as a manual measuring tool to trace the larval path. Video recordings were used to verify the accuracy of the measurement. A summary of the behavioral parameters is presented in Table 1.

Statistical Analysis

The three behavioral data were summarised in comma-separated value files and then evaluated for normality of distribution (Q-Q plot), homogeneity of variance (Levene's test), and sphericity (Mauchly's test). When all assumptions were met, a mixed-design repeated-measures ANOVA was run with BPA concentration and strain as between-subject factors and generation as the within-subject factor. Greenhouse–Geisser correction was applied to adjust for any sphericity violations. Effect sizes are reported as partial η^2. When a significant effect was found for the main effect or interaction ($p < 0.05$), the analysis proceeded to Holm-adjusted post-hoc pairwise comparisons. All analyses were conducted using JASP 0.19.10 and RStudio 2023.12.1+402.

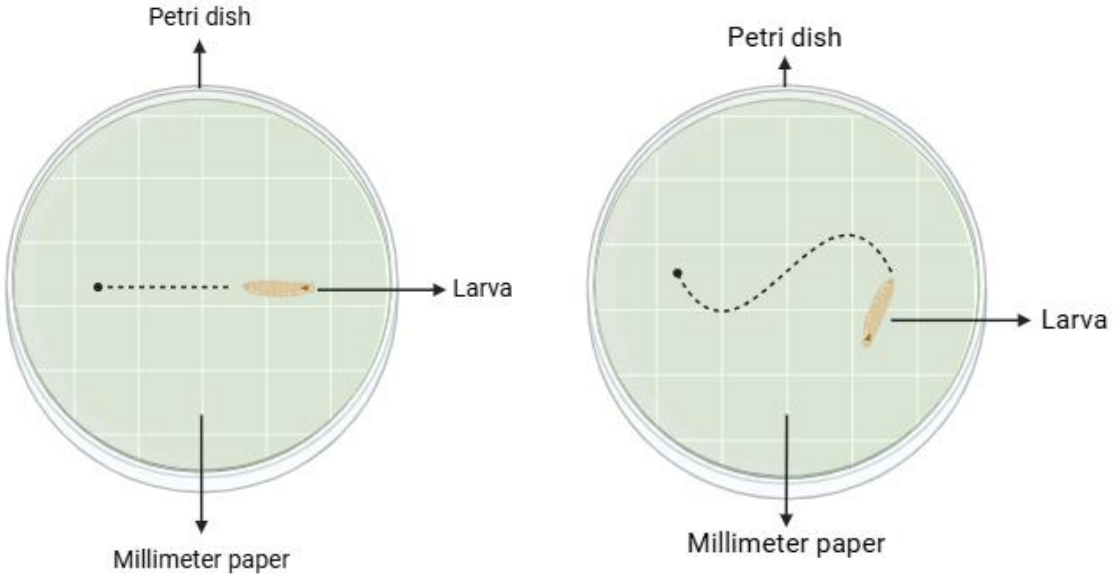


Figure 1. Components for larva behavior assay. A) Larva crawling assay. B) Larva exploratory assay

Table 1. Comparison of *Drosophila* larva behavior data collection

Observed Variable	Measurement Unit	Behavioral Assay	Apparatus and Measurement	Individuals per Replicate
Crawling speed	mm/s	Crawling Assay	Petri dish on mm grid paper under stereo microscope; movement recorded on video; distance measured for 10 seconds	1
Contraction	count	Crawling Assay	Same settings as crawling speed; the number of whole-body twitches over 10 seconds.	1
Exploratory distance	cm	Exploratory Assay	Larvae were placed in a flat arena under soft lighting; the distance from the center was recorded after 1 minute of observation.	1

RESULTS AND DISCUSSION

Overview of the effects of BPA exposure on behavior

This study collected behavioral data of three *Drosophila* strains in three generations. Table 2 presents a summary of the within-subject effects of the three behavioral data from the results of repeated measures ANOVA and their interaction effects. In contrast, Table 3 presents a summary of the between-subject effects.

Based on the results of repeated-measures ANOVA, of the three behavioral parameters measured, the generation factor is the factor that consistently has the most influence. Based on Table 2, four of the six behavioral parameters are significantly affected by the generation factor with moderate to large effect sizes (η^2_p, ranging from 0.145 to 0.407). In addition, the generation factor is also involved in several significant interactions, such as its interaction with BPA concentration in the exploratory distance and its interaction with the strain factor in both crawling speed and exploratory distance.

In contrast to the generation factor, when acting as the main effect, BPA concentration is the factor that least often gives a significant influence. Referring to Table 3, this factor has a significant effect only on the exploratory distance parameter ($F = 8.611$, $p = 0.001$, $\eta^2_p = 0.389$). In other behavioral data, such as crawling speed and contraction count, differences in BPA concentration as the main effect could not significantly impact the observation data. BPA concentration is rarely involved in producing a significant interaction in the interaction effect. It is noted that BPA only interacts significantly twice with generation (Gen x BPA) in the within-subject effect (Table 2) and once with strain (BPA x Strain) in the between-subject impact (Table 3).

The results of repeated-measures ANOVA also revealed that exploratory distance was the behavioral variable with the most consistent response, both within- and between-subject factors. A significant main effect of generation was found ($F(2, 54) = 18.559$, $p < 0.001$, $\eta^2_p = 0.407$), along with significant effects of BPA concentration ($F(2,27) = 8.611$, $p = 0.001$, $\eta^2_p = 0.389$) and strain ($F(2,27) = 9.294$, $p < 0.001$, $\eta^2_p = 0.408$). Significant two-way interactions were also observed between generation and BPA ($F(4,54) = 5.928$, $p < 0.01$, $\eta^2_p = 0.305$), and between generation and strain ($F(2,27) = 4.133$, $p = 0.005$, $\eta^2_p = 0.234$). However, despite having a medium effect size, the three-way interaction (generation x BPA x strain) was not significant ($F(8,54) = 0.845$, $p = 0.568$, $\eta^2_p = 0.111$).

On the other hand, the number of twitches in larvae was the least responsive factor. A significant main effect was found only in the generation factor ($F = 5.176$, $p = 0.009$, $\eta^2_p = 0.161$). In contrast, the single and interactive effects of the other two factors were not statistically significant and had relatively small effect sizes ($\eta^2_p < 0.1$). Furthermore, although the interaction of the three factors had a larger effect size ($\eta^2_p = 0.122$), its influence was also insignificant.

Table 2. Summary of the influence of within-subject effects on fruit fly behavior

Variable	Generation		Gen × BPA		Gen × Strain		Gen × BPA × Strain	
	F	η^2_p	F	η^2_p	F	η^2_p	F	η^2_p
Crawling speed	4.580*	0.145	1.078	0.074	3.469*	0.204	1.538	0.186
Contraction count	5.176**	0.161	0.138	0.01	0.829	0.058	0.94	0.122
Exploratory distance	18.559**	0.407	5.928**	0.305	4.133**	0.234	0.845	0.111

[1] Greenhouse–Geisser correction was applied due to a violation of the sphericity assumption as indicated by Mauchly's test ($p < 0.05$).

*Significant at $\alpha = 0.05$; **Significant at $\alpha = 0.01$

Table 3. Summary of the influence of between-subject effects on fruit fly behavior

Variable	BPA		Strain		BPA × Strain	
	F	η^2_p	F	η^2_p	F	η^2_p
Crawling speed	0.201	0.015	1.928	0.125	2.937*	0.303
Contraction count	0.951	0.066	0.715	0.05	0.681	0.092
Exploratory distance	8.611**	0.389	9.294**	0.408	0.487	0.067

*Significant at $\alpha = 0.05$; **Significant at $\alpha = 0.01$

Crawling speed

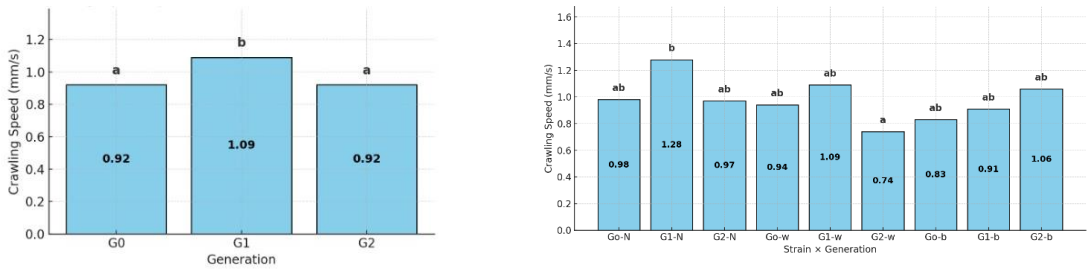
Repeated-measures ANOVA (Tables 2 and 3) showed that larval crawling speed was significantly affected by generation, the interaction between generation and strain, and the interaction between BPA concentration and strain. However, Holm-adjusted post hoc tests revealed significant group differences only for the generation factor (Figure 3a) and the interaction between generation and strain (Figure 3b). No significant pairwise differences were found for the BPA by strain interaction. As shown in Figure 3, larvae in G1 exhibited the highest crawling speed (1.09 mm/s), significantly faster than those in G0 and G2 (each 0.92 mm/s). Within the strain by generation interaction, the N strain in G1 reached the highest value (1.28 mm per second), significantly different from the w strain in G0 (0.74 mm per second). These findings indicate that generation and genetic variation influence larval locomotion under BPA exposure.

Crawling speed reflects neuromotor health and has long been recognized as a sensitive indicator of neural and muscular function in *Drosophila* larvae (Sinadinos et al., 2012). A slower speed may indicate nervous or muscular decline, while a higher speed suggests increased synaptic activity (Nguyen et al., 2021). Neurotoxic agents, including BPA, can trigger hyperactivity (Bedrossiantz et al., 2021), possibly through altered dopamine signaling (Spulber et al., 2014).

The results showed that generation had a significant effect on larval crawling speed. Larvae exposed to BPA for one generation (G1) moved faster than unexposed larvae (G0) and those exposed for two generations (G2). This increase in locomotor activity is consistent with previous studies on *Danio rerio* (Kochetkov et al., 2024) and *D. melanogaster* (Nguyen et al., 2021), although opposite results have also been reported (Wang et al., 2020). Other studies on fly larvae suggested that faster crawling in offspring may represent improved adaptability after prolonged parental exposure to contaminants (Wang & Shen, 2025).

The nonlinear generational pattern (an increase in G1 followed by a decrease in G2) suggests complex intergenerational dynamics. The first generation may have gained a developmental boost from early BPA exposure, such as enhanced synaptic connectivity in the neuromuscular system, resulting in more active larvae (Nguyen et al., 2021). This finding supports the notion that BPA exposure during early development can transiently increase locomotor activity by altering synaptogenesis. The reduced speed in G2 may reflect compensatory or adverse effects following initial adaptation, consistent with the concept that early stimulation can lead to later physiological costs.

In addition to generational effects, genetic variation also shaped larval behavior. Post hoc analysis revealed a significant interaction between generation and strain, with G1 larvae of the N strain moving faster than G2 larvae of the *w* strain. The *N* strain, as a wild type, generally exhibits stable locomotor performance, while the *w* strain carries a null mutation at the *w* locus that produces pleiotropic effects on eye color, neural function, and behavior (Rickle et al., 2025). Previous findings indicate that the *w* strain has lower baseline activity and reduced recovery after stress (Brody, 2008). Therefore, the *N* strain in G1 likely benefited more from environmental stimulation due to its intact adaptive mechanisms, whereas the w strain was less responsive because of neural limitations. These results reinforce the conclusion of Nguyen et al. (2021), that genetic variation modulates sensitivity and adaptation capacity related to neurodevelopment and behavior in flies.



(a) (b)

Figure 3. Comparison of the crawling speed of larvae in (a) three different generations and (b) combinations between strains and generations. Note: different letters above each bar indicate statistically significant differences between groups based on Holm-adjusted post-hoc pairwise comparisons ($p < 0.05$).

Contraction count

Only the generation factor significantly affected the data on larvae's body contractions (twitches) during crawling behavior. The results of further tests for this factor are presented in Figure 4. Based on additional tests, the number of contractions in larvae of G0 (10.67 twitches) was not significantly different from G2 (11.19 twitches). However, both were significantly lower than the number of contractions of larvae in G1 (12.92 twitches). The results of this analysis indicate an increase in motor responses in larvae that had been exposed to BPA for one generation before decreasing again after being exposed for two generations.

Similar to crawling speed, contraction count reflects the neuromuscular condition of larvae. In neurotoxicology, larval contractions involve the coordination of motor neurons, muscles, and neuromuscular synapses. Each contraction propels the larva forward; thus, a higher contraction frequency indicates increased motor stimulation, whereas a lower frequency reflects reduced sensorimotor coordination or impaired neural function. Consistent with other locomotor indicators, peristaltic contractions are widely used to assess the effects of toxicants or disease phenotypes (Sinadinos et al., 2012).

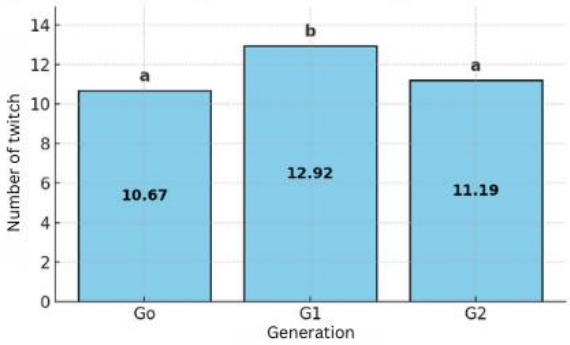


Figure 4. Comparison of the average number of larval twitches while crawling. Note: different letters above each bar indicate statistically significant differences between groups based on Holm- adjusted post-hoc pairwise comparisons ($p < 0.05$).

As observed in crawling speed, generation significantly affected the number of larval contractions. Larvae exposed to BPA for one generation showed a higher average contraction frequency than those in the previous or subsequent generations. Similar findings were reported in *Drosophila* larvae exposed to BPA (Nguyen et al., 2021). Increased contraction frequency elevates crawling rate, indicating hyperactivity possibly induced by altered synaptogenesis that overstimulates the motor circuit. BPA exposure has been associated with repetitive movements and locomotor disturbances in flies (Kaur et al., 2015), and with restlessness or hyperactivity in mammals (Komada et al., 2014).

The decrease in contraction frequency observed in G2 may be related to BPA's endocrine-disrupting nature and its non-monotonic dose-response relationship. Low doses can induce hyperactivity through reduced dopamine levels and oxidative stress, whereas high or prolonged exposure may suppress activity due to neuronal damage or metabolic disruption. Low-dose BPA has been reported to produce stronger effects than higher doses (Pinney et al., 2017). This finding also aligns with other studies involving exposure to other endocrine disruptors, which have also reported a non-monotonic dose-response. Similar U-shaped responses have been observed for other endocrine disruptors (Capela et al., 2018), possibly driven by receptor desensitization, negative feedback (Lagarde et al., 2015), or differences in toxicokinetics and toxicodynamics (More et al., 2021).

BPA is also known to exert transgenerational effects, where exposure in one generation influences subsequent generations. Maternal BPA exposure can alter reproductive, hormonal, genetic, and behavioral traits across species (Bhandari et al., 2015; López-Rodríguez et al., 2021). Similar findings have been reported in *Drosophila* studies (Kim et al., 2021; Musachio et al., 2021). Thus, multigenerational exposure may lead to the accumulation of BPA effects, explaining the U-shaped trend observed in both dose and generational responses in the present study.

Exploratory distance

Larval exploration distance is the behavioral variable most influenced by this study's main factor or interaction. Three main effects and two interaction effects significantly influenced the larval exploration distance. A comparison of each group and the post hoc test results is presented in Figure 5. In the generation factor, larvae in G0 had a significantly greater exploration distance (5.86 mm) than G1 (4.25 mm) and G2 (4.60 mm). In the combination of generation × BPA, the G0 group at all doses showed the highest value and did not differ significantly from each other. Then, there was a significant decrease in almost G1 and G2, especially in the 0.25 and 0.5 mg/mL groups.

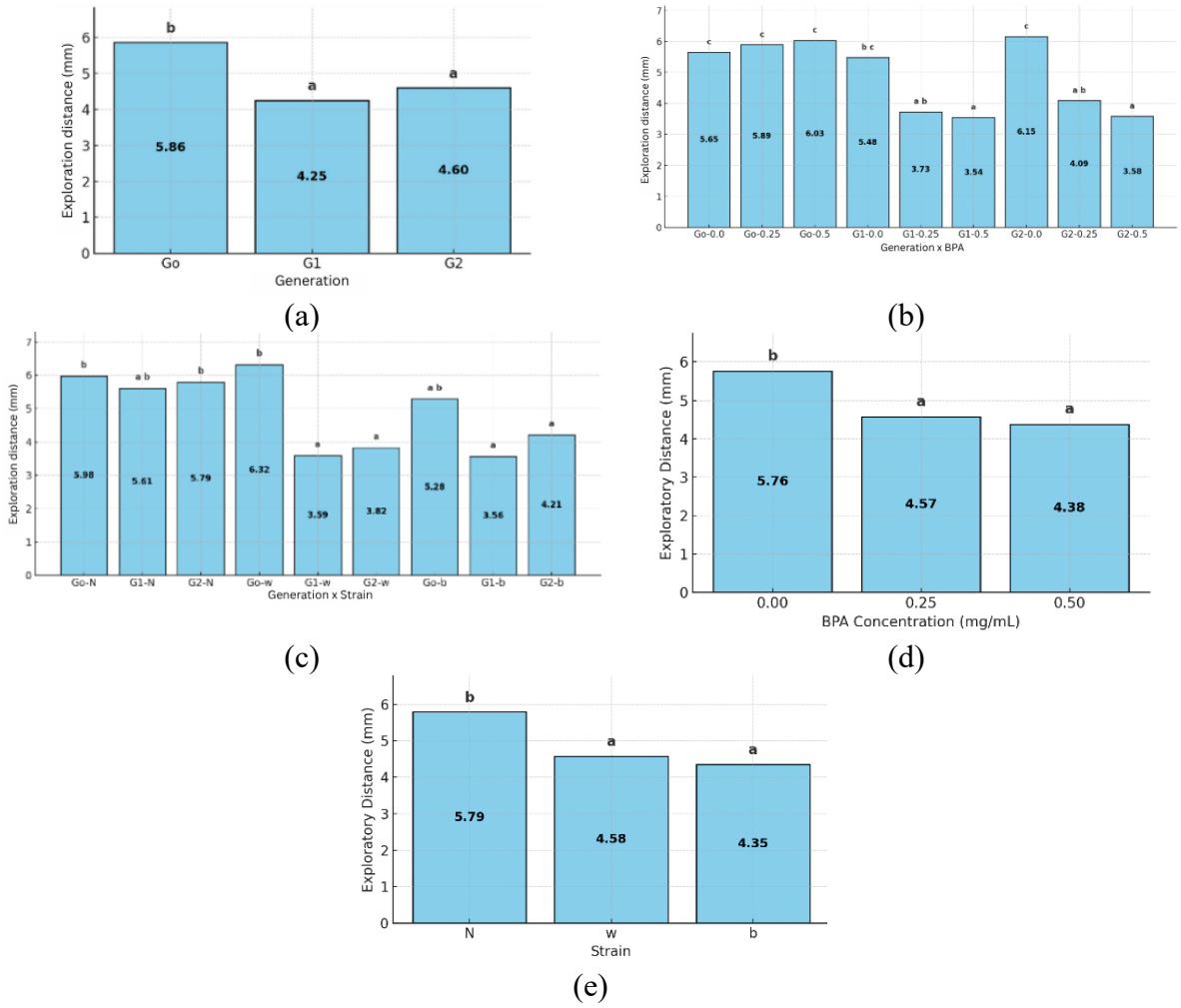


Figure 5. Comparison of larval exploration distances in (a) three different generations, (b) combinations between generations and BPA, (c) combinations between generations and strains, (d) three different concentrations, and (e) three different strains. Note: different letters above each bar indicate statistically significant differences between groups based on Holm-adjusted post-hoc pairwise comparisons ($p < 0.05$).

In the interaction between generation and strain, the longest distance was recorded in the w strain at G0 (6.32 mm), which differed significantly from almost all w and b strains in G1 and G2. For BPA concentration, unexposed flies traveled farther (5.76 mm) than those exposed to 0.25 mg/mL (4.57 mm) or 0.50 mg/mL (4.38 mm). Among strains, the N strain had the greatest exploration distance (5.79 mm), significantly higher than w (4.58 mm) and b (4.35 mm). Thus, the decrease in exploration distance was most evident in post-exposure generations and in mutant strains.

Exploration distance provides an important measure of neurotoxicity, reflecting the function of motor and sensory systems. BPA, as an endocrine disruptor, can also interfere with neuronal proliferation, synapse formation, and synaptic plasticity, which contribute to neurodevelopmental abnormalities (Hyun et al., 2022; Welch & Mulligan, 2022). Larvae with neural impairment typically show altered exploration patterns (Kaur et al., 2015). Consistent with these findings, larvae not exposed to BPA traveled farther than those exposed, suggesting that reduced exploration may result from impaired neuromuscular coordination or decreased motivation to move away from the starting point.

Among all behavioral variables, exploration distance was the only one significantly affected by all main factors (generation, BPA concentration, and strain) and two interactions (generation with BPA and generation with strain). This indicates that exploration distance is highly sensitive to genetic and physiological alterations. Larvae from the pre-exposure generation (G0) exhibited the

longest travel distance, while significant decreases in G1 and G2 indicate cumulative or long-term impacts of BPA. This pattern aligns with previous reports that BPA disrupts neuroblast development (Nguyen et al., 2021). The decline in exploration among exposed generations may also reflect maternal or transgenerational effects, where parental BPA exposure compromises the physiology of their offspring (Musachio et al., 2021). Accumulated BPA effects across generations may amplify neurotoxic symptoms and restrict exploration ability.

Strain differences further emphasize genetic influence on locomotion and toxin sensitivity. The *N* strain consistently exhibited greater travel distances than the w and b strains, suggesting that genetic background determines both baseline motor ability and resistance to BPA. As the wild type, N possesses a more optimal locomotor capacity, while mutations in the *w* and *b* strains cause pleiotropic effects that can alter neurotransmitter levels (Rickle et al., 2025). Consequently, each genotype responds differently to neurotoxic exposure.

The *w* strain at G0 also showed high exploration ability, comparable to N, but both *w* and *b* strains displayed marked reductions from G0 to G2. This suggests that both weaker genetic backgrounds and BPA exposure contribute to diminished movement capacity. In the interaction between generation and BPA, unexposed or control groups (G0 across all BPA levels and G2 at 0 mg/mL) maintained the highest exploration distances. Flies exposed to BPA exhibited immediate and persistent declines, with the steepest decreases observed in *w* and *b* strains, while *N* remained relatively stable. These findings suggest that genetic factors modulate susceptibility to BPA, where *N* may possess better protective or cellular repair mechanisms, whereas *w* and *b* are more vulnerable to neurotoxic stress. A summary of post hoc comparisons illustrating these behavioral patterns is presented in Table 4.

Overall, although all three strains (*N*, *w*, and *b*) were exposed to BPA at similar concentration levels (0, 0.25, and 0.5 mg/mL) and measured using the same six behavioral variables across three generations (Pre-exposure generation to two post-exposure generations), the observed results yielded diverse trends. The diverse data patterns can be attributed to several toxicological and neurobiological principles in Table 5.

Research Implications

This study demonstrates that toxicant effects cannot be accurately evaluated using a single generation, genetic background, or behavioral parameter. BPA exposure induced non-monotonic, strain-dependent, multigenerational responses that differently affected various behavioral measures. These results underscore two key points. *First*, reliance on F1 data or a single behavioral metric may lead to underestimation or overestimation of toxicant risks, as effects can vary or even reverse across parameters. *Second*, the pronounced strain effects highlight the importance of incorporating diverse genetic backgrounds in toxicity testing, including variants associated with neural, motor, detoxification, and morphological traits.

Limitation

Although this study revealed several unique and significant findings, certain limitations should be acknowledged. *First*, only three fly strains were used, with two limited to single mutants for eye and body color. *Second*, the study covered only three generations, whereas real-world populations may experience BPA exposure across many generations. *Third*, the BPA exposure levels were restricted and did not span the full range from acute to chronic doses. *Finally*, the assessment of BPA effects was limited to six behavioral parameters and did not include molecular-level analyses.

Table 4. Summary of post hoc results of the six observed behavioral variables

Behavioral Variable	Significant Factors (Post Hoc Compared)	Direction of Difference
Crawling speed	Generation (G1 > G0, G2); Generation × Strain (G1-N > G2-*w*)	G1 fastest; G0 and G2 similar
Contraction count	Generation (G1 > G0, G2)	G1 most contractions
Exploratory distance	Generation (G0 > G1, G2); BPA (0.00 > 0.25, 0.50); Strain (N > *w, b*); Generation × BPA (G0 > G1, G2 at 0.25 & 0.5); Generation × Strain (G0-*w* > G1-*b*, G2-*w*)	G0 farthest; BPA reduces distance; N strain is highest

Table 5. Several principles of toxicology and neurobiology underlie the diverse data patterns obtained in this study

Principle	Consequence	Manifestation in Behavior
Phenotype-specific molecular targets	Each behavior is controlled by specific neural circuits, such as dopaminergic neurons, which are involved in crawling (Riemensperger et al., 2013). As an endocrine disruptor, BPA can interfere with multiple endocrine glands (Berto-Júnior et al., 2018; Gorini et al., 2020) although the extent of disruption may vary across different gland types.	Crawling speed and contraction count showed a similar pattern, increasing in G1 and decreasing in G2, as both are regulated by the segmental control generator (Gjorgjieva et al., 2013; Kohsaka et al., 2014). In contrast, exploration distance, influenced by multiple stimuli such as visual input (Robie, 2010), declined steadily across generations.
Dose and time-dependent hormones	Exposure to endocrine disruptors under certain conditions can increase synaptogenesis (hyper-function) or have the opposite effect (hypo-function) (Kawato, 2004; Liao et al., 2008; Parent et al., 2016).	Most behavioral data form an inverted U shape where the data peaks in G1 and then declines in G2 (e.g., speed, contraction)
Differences in the genetic conditions of each strain	Genetic background diversity influences sensitivity and adaptive capacity in fly neurodevelopment and behavior (Nguyen et al., 2021). The N strain possesses an optimal wild-type genome, while mutations in the *w* strain cause pleiotropic effects on neural function and behavior (Rickle et al., 2025). The *b* strain carries mutations that alter dopamine levels.	The *b* strain maintained its performance in exploration distance

Future Research: 1. extending BPA exposure to three or more generations to better represent populations, 2. comparing flies exposed for several generations with those where BPA exposure, 3. expanding BPA doses to include more acute and chronic exposure levels, 4. employing diverse fly strains, including conventional mutants and CRISPR-engineered lines lacking detoxification genes or exhibiting neuromuscular deficiencies, 5. integrating multi omics analyses to connect phenotypic observations with underlying molecular pathways, and (6) incorporating additional environmental stressors, such as elevated temperature or light intensity, to better simulate real-world exposure scenarios.

CONCLUSION

Crawling speed, larval contraction, and exploration distance exhibited different data changes, indicating that the interaction of dose, strain, and generation factors could influence these parameters. The first generation often showed the most extreme values (the highest or the lowest), such as the highest crawling speed and number of contractions, and the lowest exploration distance. In contrast, the second generation showed a rebound or change in response direction. Crawling speed and contraction peaked in the first filial generation (≈ 20% above G0), and exploration distance decreased after flies were exposed to BPA. In some parameters, the *b* strain often showed the worst behavioral performance, while the *N* strain performed the best. Although there was no significant effect on every behavioral data point, in general, when showing a significant impact, increasing the concentration of BPA exposure resulted in decreased behavioral performance of the flies.

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AUTHOR CONTRIBUTION STATEMENT

AF: Writing – Original draft preparation, Formal analysis, Data curation. MJR, SK, SK,

NAZ: Investigation, Resources, Project administration. DS: Conceptualization, Writing – Review & Editing, Project administration. HB: Conceptualization, Writing – Review & Editing, Project administration. MRB: Writing – Review & Editing, SZ: Conceptualization, Writing – Review & Editing, Project administration, Supervision.

INFORMED CONSENT STATEMENT

This study was conducted after obtaining approval from the Faculty of Veterinary Medicine, Universitas Brawijaya, Indonesia, No. 7-KEP-FKHUB-2025.

CONFLICT OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest concerning the publication of this manuscript.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors state that no AI tools were involved in the creation, analysis, or writing of this manuscript. Every stage of the research process, from data collection and interpretation to manuscript preparation, was completed solely by the authors without any support from AI technologies.

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