DOES EARLY-LIFE EXPOSURE TO NON-NUTRITIVE SWEETENERS HAVE LONG-TERM EFFECTS ON BEHAVIOR IN MICE?

By

Dhanish Chowdary Venigalla

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University of California, Riverside

APPROVED

Dr. Theodore Garland, Jr.

Department of Evolution, Ecology, and Organismal Biology

Dr. Begoña Echeverria, Howard H Hays, Jr. Chair

University Honors

ABSTRACT

Obesity remains one of the most significant public health challenges in the United States and globally, affecting approximately 42 percent of adults and nearly 20 percent of children (Bryan et al., 2021). Excess consumption of sugar-sweetened foods and beverages is widely recognized as a major contributor to rising obesity rates and associated metabolic disorders (Malik and Hu, 2022). In response, non-nutritive sweeteners have been increasingly adopted as low-calorie alternatives designed to reduce caloric intake while maintaining palatability. Between 1999 and 2012, consumption of non-nutritive sweeteners among children rose from 8.7 percent to 25.1 percent, reflecting their growing prevalence in modern diets (Sylvetsky et al., 2017).

Despite their widespread use, the long-term physiological and behavioral consequences of non-nutritive sweetener exposure, particularly during early development, remain poorly understood. Emerging research suggests that these compounds may influence metabolic regulation, neural reward pathways, and gut microbiota composition, thereby producing lasting changes in behavior and physiology (Suez et al., 2014; Yang, 2010). Additionally, non-nutritive sweeteners may disrupt learned associations between sweetness and caloric intake, potentially altering feeding behavior and energy regulation (Davidson and Swithers, 2004; Swithers and Davidson, 2008).

The High Runner mouse selection experiment (Swallow et al., 1998) provides a powerful model for examining how genetic background interacts with environmental exposures. Mice from the four replicate High Runner lines have been selectively bred for high voluntary wheel-running behavior across more than one hundred generations and exhibit elevated activity levels along with distinct morphological and physiological traits compared to four non-selected

Control lines (Careau et al., 2013; Khan et al., 2024; Schwartz and Garland, 2024; Schwartz et al., 2023). In the present study, juvenile female High Runner and Control mice were exposed for three weeks following weaning to one of four drinking solutions containing water, sucrose, saccharin, or sucralose. After an eight week "washout" period, mice underwent behavioral testing using the open-field assay.

Behavioral outcomes were quantified using total distance traveled, total number of movement bouts, and distance traveled in the center of the arena. In addition, physiological stress indicators including fecal pellet production and urine output were measured. Contrary to expectations, High Runner mice had reduced locomotor activity and reduced center exploration compared to Control mice across all treatments. Early-life exposure to sweeteners, particularly saccharin, was associated with decreased exploratory behavior and increased anxiety-related responses. These findings suggest that early dietary exposure to non-nutritive sweeteners can produce long-term behavioral changes that are influenced by genetic background and environmental context.

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INTRODUCTION

Obesity has become one of the most pressing public health concerns in modern society, with prevalence continuing to rise across both developed and developing nations. In the United States alone, approximately 42 percent of adults and nearly 20 percent of children are classified as obese, placing them at increased risk for cardiovascular disease, type 2 diabetes, and a range of other chronic health conditions (Bryan et al., 2021). These trends highlight the importance of identifying modifiable environmental factors, particularly dietary influences, that contribute to the development of obesity and related metabolic disorders.

Among these factors, the consumption of sugar sweetened foods and beverages has been identified as a major driver of excess caloric intake and metabolic dysregulation (Malik and Hu, 2022). In response to growing awareness of these risks, non-nutritive sweeteners have been introduced as alternatives that provide sweetness without significant caloric contribution. These compounds are now widely used in beverages, processed foods, and products marketed as low-calorie or diet options, and their consumption has increased dramatically over recent decades. In particular, consumption of non-nutritive sweeteners among children in the United States increased from approximately 8.7 percent in 1999 to 25.1 percent in 2012, reflecting a nearly threefold rise in use during a critical developmental period (Sylvetsky et al., 2017). The rapid increase in non-nutritive sweetener consumption, especially during early developmental periods, raises important questions about their long-term physiological and behavioral consequences.

Although non-nutritive sweeteners are often promoted as beneficial substitutes for sugar, their long-term physiological and behavioral effects remain an active area of investigation. These compounds interact with sweet taste receptors not only in the oral cavity but also in the

gastrointestinal tract, where they can influence glucose absorption, insulin signaling, and the release of hormones involved in appetite regulation (Pepino, 2015; Suez et al., 2014). Activation of these receptors may also initiate anticipatory metabolic responses, including early phase insulin release and changes in glucose handling that typically occur in expectation of caloric intake. When sweetness is detected without the accompanying delivery of energy, this may introduce a mismatch between sensory input and metabolic outcome, potentially altering downstream regulatory processes (Yang, 2010; Swithers and Davidson, 2008).

In addition to these anticipatory effects, non-nutritive sweeteners may disrupt the learned association between sweetness and caloric intake, which plays a critical role in maintaining energy balance. Under normal conditions, sweetness serves as a reliable predictor of energy content, allowing organisms to regulate food intake based on expected caloric value. When this predictive relationship is weakened, organisms may experience impairments in energy regulation, leading to compensatory changes in feeding behavior and altered responsiveness to food-related cues (Davidson and Swithers, 2004; Swithers and Davidson, 2008). Furthermore, alterations in gut microbiota composition induced by artificial sweeteners may influence metabolic signaling pathways and contribute to changes in both physiological regulation and behavior (Suez et al., 2014). Together, these mechanisms suggest that non-nutritive sweeteners may affect energy balance and behavioral outcomes through a combination of disrupted sensory signaling, altered metabolic responses, and changes in gut brain communication.

Early life represents a particularly sensitive period for examining the effects of dietary exposures. During development, neural circuits, metabolic pathways, and behavioral patterns are highly plastic and can be shaped by environmental inputs. In rodent models, early-life environmental exposures, including diet, have been shown to influence long-term metabolic

regulation, stress responsiveness, and behavior through lasting changes in neural and physiological systems (Meaney, 2010). Dietary inputs during this developmental window may therefore produce persistent effects that extend into adulthood. However, the extent to which these outcomes vary as a function of genetic background remains an important area of investigation.

The High Runner mouse selection experiment provides a powerful framework for examining interactions between genetic background and environmental exposure. This long-term selection experiment consists of four replicate lines bred for high voluntary wheel-running behavior and four non-selected control lines (Swallow et al., 1998; Careau et al., 2013). Over many generations, selective breeding has resulted in mice that exhibit consistently elevated voluntary activity levels, along with a suite of correlated physiological and behavioral traits. High Runner mice also differ from control mice in neural systems related to motivation and reward, which may influence how they respond to such environmental factors as dietary exposure (Rhodes et al., 2005).

The present study builds upon this model by examining whether early-life exposure to non-nutritive sweeteners leads to lasting behavioral effects in adulthood and whether these effects differ between High Runner and Control mice. Behavioral outcomes were assessed using the open-field assay, which evaluates exploratory behavior, activity patterns, and anxiety related responses in a novel environment rather than serving as a direct measure of locomotor performance. By focusing on behavioral activity, exploratory patterns, and anxiety-related responses, this study aims to provide a more comprehensive understanding of how early dietary environments shape long-term behavioral outcomes.

High Runner Mouse Selection Experiment

The High Runner mouse selection experiment was designed to test whether behavior can evolve as a primary target of selection. This long-term experiment consists of four replicate lines selectively bred for high voluntary wheel running behavior and four non-selected control lines maintained without directional selection (Swallow et al., 1998; Careau et al., 2013). Selection is based on voluntary wheel-running activity measured over a standardized testing period (total revolutions run on days 5 and 6 pf a 6-day exposure to wheels when mice are young adults), with the most active individuals chosen as breeders for subsequent generations.

Over many generations, this selection process has resulted in a consistent and substantial divergence in activity levels. High Runner mice exhibit approximately a 2.5- to 3- fold increase in voluntary wheel running compared to control lines, a difference that has remained approximately stable across generations and replicates since the High Runner lines reached apparent selection limits around generations 17–27 (Careau et al., 2013). Because voluntary wheel running is the trait under direct selection, additional differences observed in these mice are interpreted as correlated responses rather than traits that were independently selected (Khan et al. 2024).

In addition to elevated activity, High Runner mice exhibit a range of correlated physiological and behavioral traits, including reduced adiposity, increased food intake, elevated energy expenditure, and enhanced cardiovascular function. These changes reflect coordinated shifts across multiple biological systems that have emerged alongside selection for increased activity, supporting the idea that selecting for behavior can drive broader organismal changes.

Previous research has also demonstrated that High Runner mice exhibit differences in neural systems involved in motivation and reward processing, particularly within dopaminergic pathways that regulate voluntary activity and reinforcement behaviors (Rhodes et al., 2005). Alterations in these neural systems may contribute to the elevated activity phenotype and may also influence how these animals respond to environmental factors such as dietary exposure, novelty, and stress. As a result, the High Runner model provides a valuable framework for examining how genetic selection on behavior interacts with environmental influences to shape long-term physiological and behavioral outcomes.

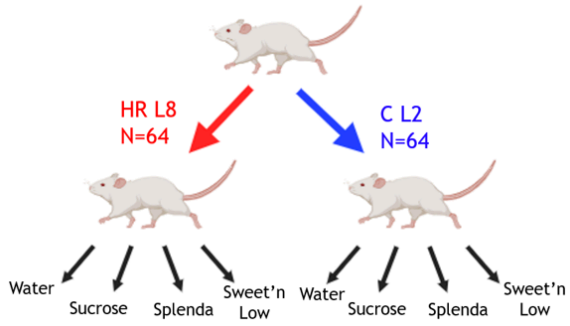
METHODOLOGY

Experimental Design

A total of 96 female mice from generation 104 were used in this study. Only female mice were included due to availability at the time of the experiment. Mice were divided equally between one representative High Runner line 8 and one Control line 2 (see Figure 1), allowing for balanced comparisons between genetic backgrounds while maintaining sufficient sample size within each treatment condition.

Within each line, mice were assigned to one of four treatment conditions consisting of water, sucrose, saccharin (Sweet'n Low), or sucralose (Splenda). Each treatment group included an equal number of individuals from each genetic line, resulting in balanced group sizes across conditions.

Figure 1: illustrates the distribution of mice across genetic lines and treatment conditions, providing a visual representation of sample sizes and group structure.



Sweeteners were administered by mixing them directly into the drinking water at defined concentrations, allowing for voluntary consumption throughout the treatment period. The concentrations were selected to reflect commonly used levels in experimental studies and to ensure consistent exposure across individuals. Sucrose was provided at 105 g/L, saccharin (Sweet'n Low) at 2.0 g/L, and sucralose (Splenda) at 1.6 g/L. Mice had continuous access to these solutions, allowing intake to occur naturally through normal drinking behavior.

Exposure took place from weaning at three weeks of age until six weeks of age, representing a critical developmental window during which neural circuits, metabolic pathways, and behavioral patterns are undergoing significant maturation. In rodent models, early-life dietary exposures during this period have been shown to produce lasting effects on metabolic regulation, neural development, and behavior that persist into adulthood (Swithers and Davidson, 2008; Meaney, 2010). As a result, this time frame is particularly well suited for examining the long-term impact of early-life dietary exposure.

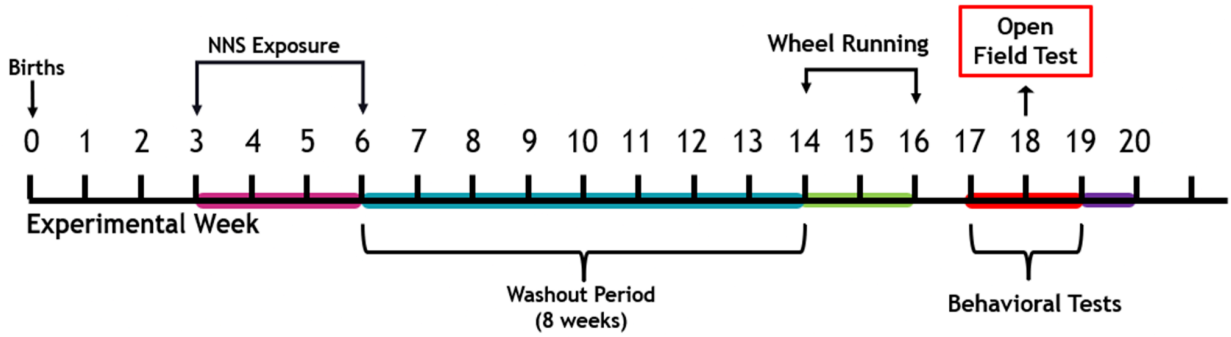
Washout Period

Following the three week treatment period, all dietary treatments were removed and all mice were provided with only tap water for the duration of an eight week washout period. This ensured that any subsequent behavioral or physiological differences were not influenced by ongoing exposure to sweeteners.

The washout period was included to eliminate short term or transient effects of the dietary treatments and to allow physiological systems to return to baseline prior to behavioral testing. Because the focus of this study was on long-term behavioral and physiological changes, this design allowed for isolation of persistent effects resulting from early-life exposure rather than immediate responses to recent consumption.

In developmental terms, this extended period of 8 weeks is roughly equivalent to several years of maturation in humans. This approach strengthens the interpretation that any observed differences in exploratory behavior, anxiety related responses, or stress indicators reflect lasting alterations rather than acute effects of the treatments.

Figure 2: Timeline of the Experimental Design



Mice from one High Runner line and one Control line were assigned to four treatment conditions (water, sucrose, saccharin, or sucralose) from three to six weeks of age. The washout period (blue) indicates the eight week phase during which all treatments were removed and only water was provided. The open-field test (red) marks the behavioral testing period used to assess long-term effects of early-life exposure.

Behavioral Testing: Open-Field Assay

Following the washout period, mice underwent behavioral testing using the open-field assay, a widely used paradigm for assessing exploratory behavior, anxiety related responses, and

general activity patterns in rodents (Prut and Belzung, 2003). Rather than serving as a direct measure of physical performance, this assay primarily reflects the animal's emotional state and response to a novel environment.

The open-field assay is based on the natural tendency of rodents to balance exploratory drive with avoidance of open and potentially threatening spaces. Rodents typically remain close to the walls of an arena in a behavior known as thigmotaxis, which is associated with increased anxiety-like behavior (Prut and Belzung, 2003). As a result, movement patterns within the arena provide insight into both exploratory behavior and anxiety related responses.

Testing was conducted during the photoperiod in a dimly lit room at approximately 6 lux to encourage exploration while minimizing excessive stress. Each mouse was placed individually in the center of the arena and allowed to explore freely for a five minute trial. During testing, physiological indicators of stress were also recorded, including fecal pellet production and urine output, which are commonly used as indirect markers of stress and autonomic activation (Hall, 1934). Because the focus of this assay is on emotional and behavioral responses, these physiological measures provide important complementary information beyond movement patterns alone.

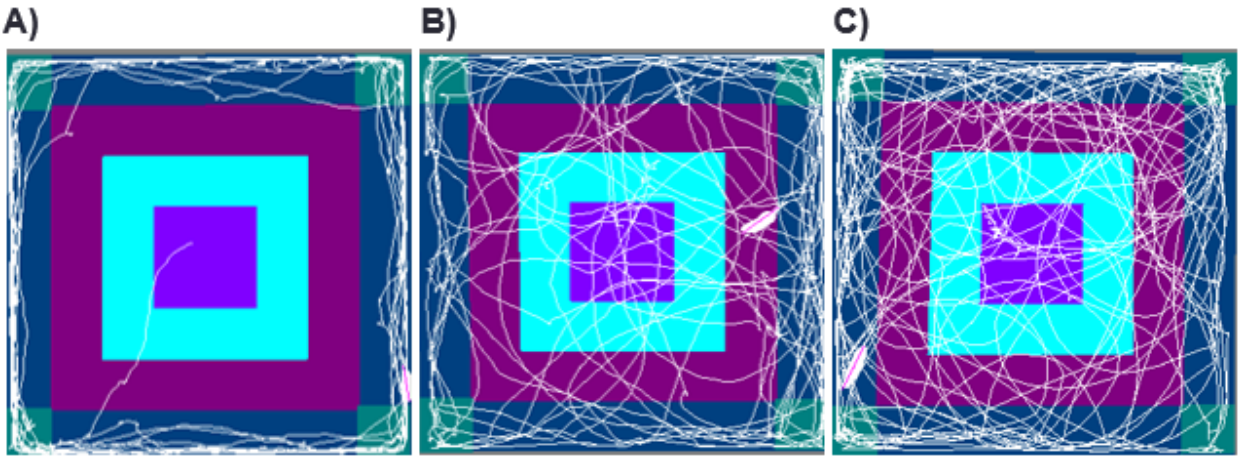
Behavioral Tracking and Analysis

Movement was recorded using an overhead camera system and analyzed using automated tracking software capable of quantifying spatial position and movement trajectories over time. The arena was divided into multiple predefined zones, including outer, inner, middle, center, and limbo regions, as well as corner areas. This zoning allowed for detailed analysis of spatial preferences and movement patterns throughout the trial.

Several behavioral variables were extracted from the tracking data. Total distance traveled was used as a measure of overall activity within the arena, while the number of movement bouts provided information on how frequently the animal initiated movement. In addition to these general activity measures, spatial metrics were used to assess anxiety-related behavior.

Distance traveled in the center of the arena was used as a key indicator of anxiety-like behavior. Time or movement within central regions, as compared to outer regions, is commonly used as an index of anxiety related responses in open-field testing (Prut and Belzung, 2003). Increased center activity is interpreted as reduced anxiety or greater exploratory behavior, whereas reduced center activity reflects increased anxiety-like behavior and a preference for safer, peripheral zones.

Figure 3: Examples of Movement Paths through the Open-Field Behavioral Test, as analyzed with CleverSys Arena Tracking Images: (A) high wall activity, (B) moderate movement throughout, (C) high exploration.



Statistical Analysis

Data were analyzed using SAS (version 9.1), employing a mixed-model analysis of variance (ANOVA). The main effects included:

Genetic line (HR vs. Control)

Treatment (water, sucrose, saccharin, sucralose)

Line × Treatment interaction

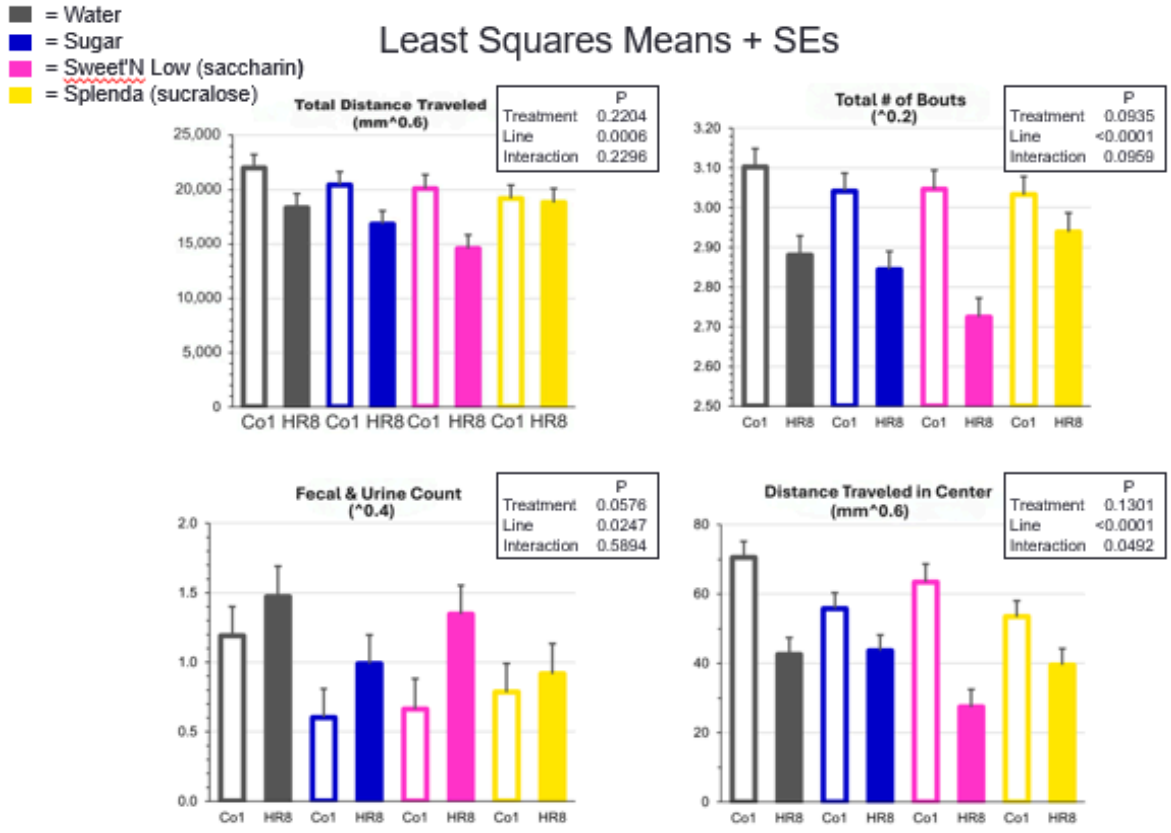
Statistical analyses were conducted using a mixed model analysis of variance framework to evaluate the effects of genetic line, treatment condition, and their interaction on behavioral and physiological outcomes. This approach allowed for the simultaneous assessment of multiple independent variables while accounting for potential variability within experimental groups.

The primary fixed effects included genetic line, defined as High Runner or Control, and treatment condition, defined as water, sucrose, saccharin, or sucralose. The interaction between genetic line and treatment was also included in the model to determine whether the effects of early-life dietary exposure differed depending on genetic background. This interaction term was particularly important for assessing gene by environment relationships.

Dependent variables analyzed included total distance traveled, total number of movement bouts, distance traveled in the center of the arena, fecal pellet count, and urine output. For each variable, least squares means were calculated to estimate group-level differences while controlling for variability within the dataset. Standard errors were also computed to provide an estimate of variability around these means.

Statistical significance was evaluated using a predefined threshold of $p < 0.05$, and p values were reported for both main effects and interaction effects. Interpretation of results considered not only statistically significant differences but also patterns of variation across groups, providing a more comprehensive understanding of how genetic and environmental factors interact to influence behavioral outcomes.

Figure 4: Statistical Analyses: SAS 9.1 Procedure Mixed to assess the effects of line, treatment (water, sucrose, saccharin, and sucralose), and their interactions. Statistical significance judged at $P < 0.05$.



RESULTS

Locomotor Activity

High Runner mice exhibited significantly lower total distance traveled in the open field compared to Control mice across treatment conditions ($p = 0.006$)(Fig. 4). A similar pattern was observed for the number of movement bouts ($p = <0.0001$), indicating differences in how mice moved throughout the arena rather than a complete reduction in behavioral activity. Because

movement bouts reflect transitions between areas of the arena, this finding indicates differences in how frequently mice moved between zones.

Importantly, High Runner mice remained active throughout the behavioral test but displayed a different pattern of movement compared to Control mice. Rather than indicating reduced engagement with the open-field environment, these results suggest an alternative behavioral strategy characterized by fewer transitions between zones and more restricted spatial exploration. High Runner mice were not inactive; instead, their movement appeared to be more localized, potentially reflecting differences in how they navigated the arena.

The open-field assay is a behavioral test conducted in a novel environment, and patterns of movement within the arena are commonly interpreted in the context of anxiety-related behavior. In this framework, reduced movement through central or exposed regions of the arena and fewer transitions across zones are associated with increased anxiety-like behavior, whereas greater movement across zones and increased exploration of central regions are associated with lower anxiety and increased exploratory behavior (Prut and Belzung, 2003).

High Runner mice remained behaviorally engaged throughout the open-field test but displayed patterns of movement that differed from Control mice. Rather than indicating reduced overall behavioral output, the lower total distance traveled and fewer movement bouts suggest differences in spatial exploration and how mice navigated the arena. This behavioral profile is consistent with previous findings from the Garland laboratory demonstrating that High Runner mice can exhibit elevated anxiety-like responses and altered exploratory behavior in novel environments despite their high voluntary wheel-running phenotype (Rhodes et al., 2003; Careau et al., 2013). Consequently, the reduced transitions between zones and more restricted

exploration observed in the present study were not unexpected and likely reflect differences in behavioral responses to novelty rather than generalized reductions in movement.

Additionally, because total distance traveled and movement bouts do not directly measure time spent in specific regions of the arena, these variables should be interpreted with caution. Reduced distance traveled may reflect slower movement, fewer transitions between zones, or increased time spent within a single region rather than reduced behavioral engagement. Similarly, fewer movement bouts may indicate reduced transitions between zones rather than prolonged inactivity. Together, these findings suggest that High Runner mice differ in how they move through and interact with the open-field environment, particularly in response to a novel setting, in a manner consistent with increased anxiety-like behavior.

Effects of Sweetener Exposure

Treatment effects on open-field behavioral measures varied across groups; however, not all differences between treatment conditions were statistically significant. For total distance traveled, there was no consistent main effect of treatment across all groups ($p = 0.2204$)(Fig. 4). Similarly, movement bouts did not show a uniform pattern of significant differences between treatment conditions ($p = 0.0935$), indicating that early-life sweetener exposure did not produce a consistent effect on behavioral measures across all mice.

Although descriptive trends were observed, with some treatment groups appearing to differ in movement patterns, these differences were not statistically significant across all comparisons. As a result, conclusions regarding overall treatment effects on behavior should be interpreted with caution.

Among the treatment groups, variation in behavioral patterns appeared to depend on genetic background. In particular, High Runner and Control mice responded differently to

sweetener exposure, suggesting that genotype plays an important role in shaping behavioral responses to early-life dietary conditions. These differences were most evident when comparing patterns across treatment groups within each genetic line, rather than when examining treatment effects alone.

Variation in behavioral patterns was influenced by the interaction between treatment and genetic background. For saccharin exposure, the interaction between genetic line and treatment approached significance for movement bouts ($p = 0.0959$) and reached statistical significance for distance traveled in the center of the arena ($p = 0.0492$)(Fig. 4). These findings suggest that the behavioral effects of saccharin differ between High Runner and Control mice, particularly in measures related to zone transitions and anxiety-like responses.

DISCUSSION

Behavioral Responses in the Open-Field Assay

The behavioral differences observed in the open-field assay suggest that early-life exposure to non-nutritive sweeteners may influence long-term responses to novel environments. Rather than reflecting simple reductions in behavior, the observed patterns appear to involve changes in spatial exploration, zone transitions, and anxiety-related responses that differed depending on genetic background.

High Runner mice remained behaviorally engaged throughout the open-field test but displayed patterns of movement that differed from Control mice. Rather than indicating reduced overall behavioral output, the lower total distance traveled ($p = 0.006$) and fewer movement bouts suggest differences in spatial exploration and how mice navigated the arena. This behavioral profile is consistent with previous findings from the Garland laboratory demonstrating that High Runner mice can exhibit elevated anxiety-like responses and altered

exploratory behavior in novel environments despite their high voluntary wheel-running phenotype (Rhodes et al., 2003; Careau et al., 2013). Consequently, the reduced transitions between zones and more restricted exploration observed in the present study were not unexpected and likely reflect differences in behavioral responses to novelty rather than generalized reductions in movement.

Center-related behavior provided additional insight into how mice responded to the open-field arena. Rodents naturally avoid exposed central regions in a behavior known as thigmotaxis, preferring to remain near the walls where perceived risk is lower (Prut & Belzung, 2003). Reduced movement through the center therefore suggests greater anxiety-like behavior or altered exploratory strategy rather than reduced behavioral capacity. High Runner mice generally displayed lower center exploration than Control mice, indicating differences in how each line responded to the novel environment.

Effects of Early-Life Sweetener Exposure

Behavioral responses also varied according to early-life dietary exposure, although these effects depended on genetic background rather than treatment alone. A significant interaction between genetic line and treatment condition was observed for distance traveled in the center region of the arena (interaction $p = 0.0492$), while movement bouts showed a similar interaction trend (interaction $p = 0.0959$). These findings indicate that High Runner and Control mice responded differently to specific dietary exposures.

Saccharin exposure produced the clearest divergence between genetic lines. High Runner mice exposed to saccharin showed reduced movement through central regions of the arena, whereas Control mice exposed to saccharin displayed comparatively greater center exploration

relative to several other treatment groups. This suggests that the same non-nutritive sweetener can influence behavioral responses differently depending on underlying genetic background.

One possible explanation involves differences in how specific sweeteners interact with physiological and neural systems. Saccharin has been reported to produce stronger alterations in glucose intolerance and gut microbiota composition compared with some other non-nutritive sweeteners, including sucralose (Suez et al., 2014). In addition, because saccharin provides intense sweetness without caloric reinforcement, it may more strongly disrupt learned associations between sweetness and energy intake, potentially altering dopaminergic reward signaling and behavioral responses to novel environments (Yang, 2010; Davidson & Swithers, 2004). While the precise mechanisms remain unclear, the present findings suggest that responses to non-nutritive sweeteners are not uniform and may depend on interactions between developmental exposure and inherited behavioral predispositions.

Physiological Stress Indicators

The physiological stress measures collected during behavioral testing further demonstrated that behavioral and physiological responses do not always align directly. High Runner mice exhibited elevated fecal boli and urine output relative to Control mice, suggesting increased stress responsiveness during exposure to the open-field arena. However, the water treatment group displayed relatively high levels of these stress indicators overall, indicating that the relationship between dietary exposure and stress physiology is complex.

These findings suggest that early-life dietary exposure may influence multiple interacting systems involved in behavioral regulation, stress responsiveness, and physiological adaptation. Behavioral outcomes observed in the open-field assay likely reflect contributions from neural, endocrine, autonomic, and metabolic systems rather than a single isolated mechanism.

Alterations in gut–brain communication pathways may also contribute to these responses by influencing physiological regulation and behavioral responses to novel environments (Suez et al., 2014).

Gene–Environment Interactions

The interaction between genetic background and dietary treatment highlights the importance of gene–environment interactions in shaping long-term behavioral outcomes. Although both High Runner and Control mice experienced the same experimental exposures, their behavioral responses differed substantially across treatment conditions. These findings emphasize that inherited behavioral predispositions can influence how organisms respond to environmental stimuli during development.

Selective breeding in the High Runner model has altered multiple interconnected systems associated with voluntary exercise behavior, including reward processing, stress responsiveness, and motivational circuitry (Rhodes et al., 2003; Careau et al., 2013). As a result, developmental exposure to non-nutritive sweeteners may differentially affect these mice because the underlying neural and physiological systems regulating behavior already differ between genetic lines.

More broadly, the present findings support the idea that environmental exposures during sensitive developmental periods may not produce uniform effects across individuals. Instead, outcomes may depend heavily on interactions between environmental conditions and preexisting genetic or physiological characteristics.

Broader Implications

The increasing use of non-nutritive sweeteners during childhood and adolescence has raised important questions regarding their long-term effects on physiology and behavior. Although these compounds are commonly promoted as alternatives to sugar, the present findings

suggest that developmental exposure may influence behavioral regulation in ways that depend on genetic background and environmental context.

Importantly, controlled rodent models remain valuable for examining these questions because they allow researchers to isolate specific dietary exposures while minimizing many confounding variables present in human studies. Human dietary data are frequently collected through self-reported questionnaires, which may not fully capture broader dietary patterns or associated lifestyle factors. In contrast, animal models provide precise control over exposure timing, dosage, and environmental conditions, allowing clearer interpretation of developmental effects.

Future research should continue examining how early-life exposure to different non-nutritive sweeteners influences neural systems involved in reward processing, stress regulation, metabolic signaling, and gut–brain communication. Additional studies incorporating both sexes and broader developmental time points will also be important for understanding how these exposures shape long-term behavioral and physiological outcomes across populations.

CONCLUSION

This study demonstrates that early-life exposure to non-nutritive sweeteners can influence long-term behavioral responses in adulthood, particularly in how animals respond to novel environments. Using the High Runner mouse model allowed examination of how genetic background interacts with developmental dietary exposure under tightly controlled experimental conditions. Compared to human studies, controlled rodent models reduce many confounding variables related to lifestyle, diet composition, and self-reported intake, allowing clearer interpretation of causal relationships between early-life exposure and behavioral outcomes.

High Runner mice displayed behavioral patterns in the open-field assay that differed from Control mice, including reduced movement through central regions of the arena and fewer transitions between zones. Rather than reflecting reduced behavioral capacity, these findings are consistent with altered exploratory strategies and increased anxiety-like responses in a novel environment, aligning with previous Garland laboratory findings in High Runner mice. Importantly, behavioral responses to early-life sweetener exposure depended on the interaction between genetic background and treatment condition rather than treatment alone. Saccharin exposure produced the clearest divergence between lines, particularly for center-related behavior and movement patterns, suggesting that inherited behavioral predispositions influence susceptibility to environmental exposures.

The inclusion of physiological stress indicators further demonstrated that behavioral and physiological responses are shaped by multiple interacting systems, including neural, endocrine, autonomic, and metabolic pathways. Potential mechanisms underlying these effects may involve disruption of learned associations between sweetness and caloric intake, altered dopaminergic reward signaling, and changes in gut–brain communication resulting from shifts in gut microbiota composition.

Although caution must be taken when translating animal findings directly to humans, these results contribute to growing evidence that non-nutritive sweeteners are not behaviorally neutral during development. Future studies should further investigate the neural and physiological mechanisms involved, including dopaminergic reward pathways, hypothalamic and stress-related circuits, and gut microbiota interactions, while also examining potential sex differences and broader developmental windows. Overall, this study highlights the importance of

considering both genetic background and developmental environment when evaluating the long-term effects of modern dietary exposures.

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