Gene–environment interplay in *Drosophila melanogaster*: Chronic food deprivation in early life affects adult exploratory and fitness traits

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Early life adversity has known impacts on adult health and behavior, yet little is known about the gene–environment interactions (GEIs) that underlie these consequences. We used the fruit fly *Drosophila melanogaster* to show that chronic early nutritional adversity interacts with rover and sitter allelic variants of *foraging* (*for*) to affect adult exploratory behavior, a phenotype that is critical for foraging, and reproductive fitness. Chronic nutritional adversity during adulthood did not affect rover or sitter adult exploratory behavior; however, early nutritional adversity in the larval period increased sitter but not rover adult exploratory behavior. Increasing *for* gene expression in the mushroom bodies, an important center of integration in the fly brain, changed the amount of exploratory behavior exhibited by sitter adults when they did not experience early nutritional adversity but had no effect in sitters that experienced early nutritional adversity. Manipulation of the larval nutritional environment also affected adult reproductive output of sitters but not rovers, indicating GEIs on fitness itself. The natural *for* variants are an excellent model to examine how GEIs underlie the biological embedding of early experience.

genotype–environment interaction | plasticity

The question of how individual differences arise is fundamental to biology, psychology, and precision medicine (1, 2). From studies on mammals, we know that early adversity places individuals on developmental trajectories for health and behavior that can last a lifetime (3, 4). However, for the most part, this idea has not been investigated in simple model genetic organisms, such as the worm *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*, in which gene manipulation can be readily accomplished. Two biological mechanisms that can underlie individual differences, and that go beyond the obsolete notion of nature or nurture, are gene–environment interactions (GEIs) and epigenetics. Here, we explore GEIs in the context of early adversity. In particular, we address how early adversity interacts with natural variants of the *foraging* (*for*) gene to affect adult behavior and fitness in *D. melanogaster*.

Within human populations, nutritional adversity can lead to substantive effects on cognitive and behavioral development (5, 6). The question of how early adversities perturb normal development is a difficult one, because both the strength and timing of adversity can matter. For example, in humans, detrimental effects are seen after chronic protein or carbohydrate deprivation, yet one or a few acute deprivations have little long-term effect (7). Similarly, in fruit flies, levels of hemolymph carbohydrate affected by 3 h of food deprivation return to normal levels after just 2 h of refeeding (8). Moreover, not all individuals are affected equally by early nutritional adversity (9, 10), and these individual differences arise from GEIs.

In evolutionary biology, GEIs can be important for the maintenance and expression of both phenotypic plasticity and genetic variation (11, 12). Plasticity is defined here as the variation in phenotypes expressed by a single genotype as a function of environmental variation. In the presence of GEIs, the rank order of the fitness of genotypes can change across environments (13, 14). Fluctuations in the nutritional environment will support plasticity because different phenotypes will be favored at different times and places. For example, if early life deprivations are predictive of later life deprivation, which is more likely in a chronic adversity scenario, there should be natural selection for a plastic response that produces an adult phenotype that performs well in harsh conditions (15, 16). The optimal response will depend on the genotype as well as costs and benefits associated with the particular plastic response (17). Manipulating the nutritional environment is thus a logical approach to testing principles of GEI in naturalistic conditions.

In the present study, we hypothesized that early life nutritional adversity would have carryover effects on adult phenotypes and that these effects would be influenced by *for*. In *D. melanogaster*, *for* encodes a cGMP-dependent protein kinase (PKG) known to contribute to behavioral plasticity in larvae and adults (8, 18, 19). Many species have the *for* gene or one of its homologs (20). In *D. melanogaster*, *for* is known to have important effects on a number of traits, including sleep (21) and memory (22). PRKG1, the mammalian homolog of PKG, is implicated in synaptic plasticity and fear conditioning (23, 24). A naturally occurring *for* polymorphism with two variants, for^R (rover) and for^s (sitter), is maintained in wild populations of *D. melanogaster*. Rovers move farther while foraging than sitters and have a higher tendency to leave food patches (25, 26). In nutrient-rich environments, rovers have higher PKG enzyme activity than sitters (19, 27), whereas PKG levels drop in both variants in nutrient-poor environments (19). In these nutrient-poor environments, rover larvae have higher survivorship and faster development than sitter larvae when grown in groups composed of single variants (19). Although not examined in the present paper, complex interactions between *for* genotype and the nutritive and social environments can influence fitness (28, 29).

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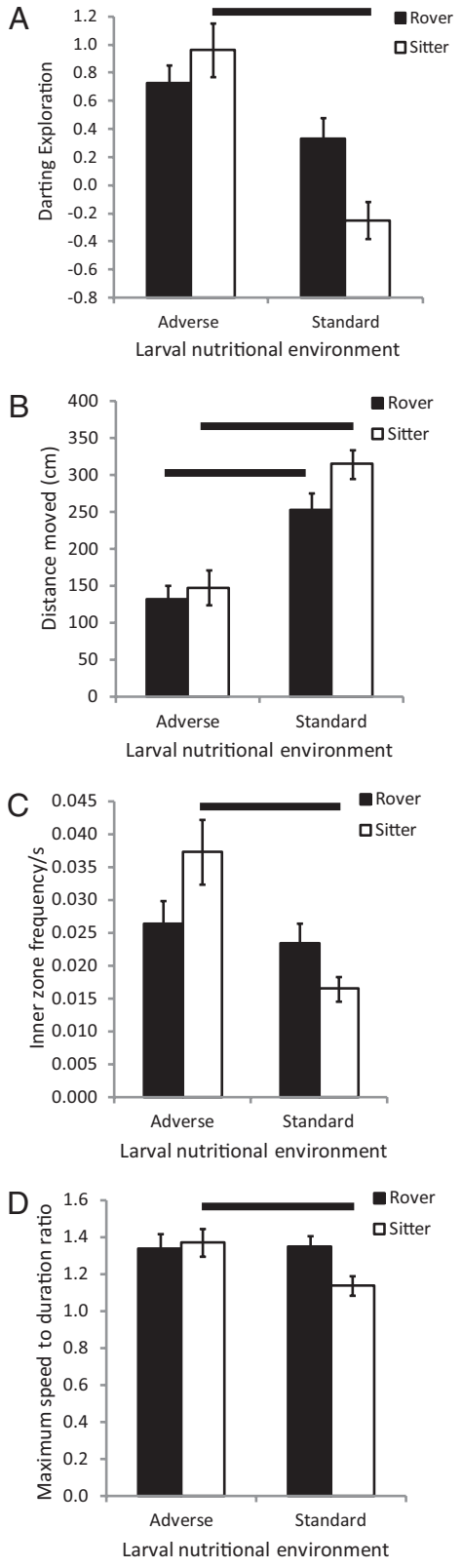

Fig. 1. Behavior of female sitters in the open field was more sensitive to larval nutritional adversity than the behavior of female rovers. (*A*) Darting exploration of sitters was significantly higher when reared in early nutritional adversity than when reared in standard conditions (post hoc ANOVA, $P < 0.001$), but rover behavior was not significantly different between food treatments ($P = 0.11$). Darting exploration is the first component of a principal components analysis of distance, inner zone frequency per second of movement, and mean MSDR during movement periods. (*B*) Both sitters ($P < 0.001$) and rovers ($P = 0.002$) walked shorter distances when reared in early nutritional adversity than when reared in standard conditions. (*C*) Number of crossings into the inner zone of the open field, per second of movement, of sitters was significantly higher when reared in early nutritional adversity than when reared in standard conditions ($P < 0.001$), but rover behavior was not significantly different between food treatments ($P = 0.58$). (*D*) MSDR of sitters was significantly higher when reared in early nutritional adversity than when reared in standard conditions ($P = 0.016$), but rover behavior was not significantly different between food treatments ($P = 0.89$). Lines above bars indicate a significant difference between groups at the terminus of the line. Error bars represent SEM.

Results and Discussion

Exploratory Behavior. The tendency to investigate a novel area, consisting of behaviors or postures that collect information about that environment, is termed exploratory behavior (30). It can affect fitness through the discovery of new resources, such as food, propensity to disperse (31, 32), and competition for territories (33). During exploration, an animal can obtain information, via scanning and locomotion, about the layout of its surroundings, as well as patterns of resources and predation threat. Animals spend a great deal of their time in nature engaged in exploratory behavior. We measured this behavior in the open field, where the fly is put in a novel, open environment and its movement pattern is monitored (*Methods*). These tests have been used for decades to assess exploratory behavior in many species (34, 35), as well as in *D. melanogaster* more recently (36–39). Walking exploration is an ecologically valid measure because flies spend a considerable amount of time walking on fruits and other substrates in search of food, mates, and oviposition sites (40, 41).

We addressed the effects of both larval and adult nutritional adversity on exploratory behavior in an open field (Fig. S1). Adult rovers and sitters were exposed to adverse or standard nutritional conditions throughout their larval life and/or the first 5–7 d of their adult life in a full-factorial design. Three behaviors were measured: distance moved, inner zone exploration, and maximum speed-to-duration ratio (MSDR; a measure of stop-and-go motion) (Fig. S2). To determine whether the above three variables could be integrated and summarized as a single metric, we performed a principal components analysis using the three variables. Only the first principal component had an eigenvalue greater than 1, and it explained 62% of the variance in the data. The component matrix for the three individual behaviors was as follows: distance moved, −0.824; inner exploration, 0.791; and MSDR, 0.729. We designated the first principal component as "darting exploration"; individuals with high scores tended to move shorter distances overall but in stop-and-go motions that tended to include the inner zone of the open field.

The adult nutritional environment had little effect on darting exploration (adult food: $F_{1,93} = 0.59$, $P = 0.44$; all adult food interactions: $P > 0.20$), and we therefore focused our investigations on the question of early larval adversity and its consequences for adult outcomes. We found that darting exploration was significantly higher in sitter adults reared in larval nutritional adversity compared with those reared in standard food, whereas darting exploration in rovers did not differ significantly between treatments (variant·larval nutrition: $F_{1,158} = 4.4$, $P = 0.024$; post hoc, sitters: $F_{1,84} = 26.3$, $P < 0.001$; post hoc, rovers: $F_{1,74} = 2.6$, $P = 0.11$; Fig. 1*A*). The three components of darting exploration showed the same general pattern. Distance walked was significantly lower in flies of both variants from early nutritional adversity (variant·larval nutrition: $F_{1,158} = 0.8$, $P = 0.38$; post hoc, sitters: $F_{1,84} = 24.0$, $P < 0.001$; post hoc, rovers: $F_{1,74} = 9.9$, $P = 0.002$; Fig. 1*B*). Inner zone exploration was significantly higher in

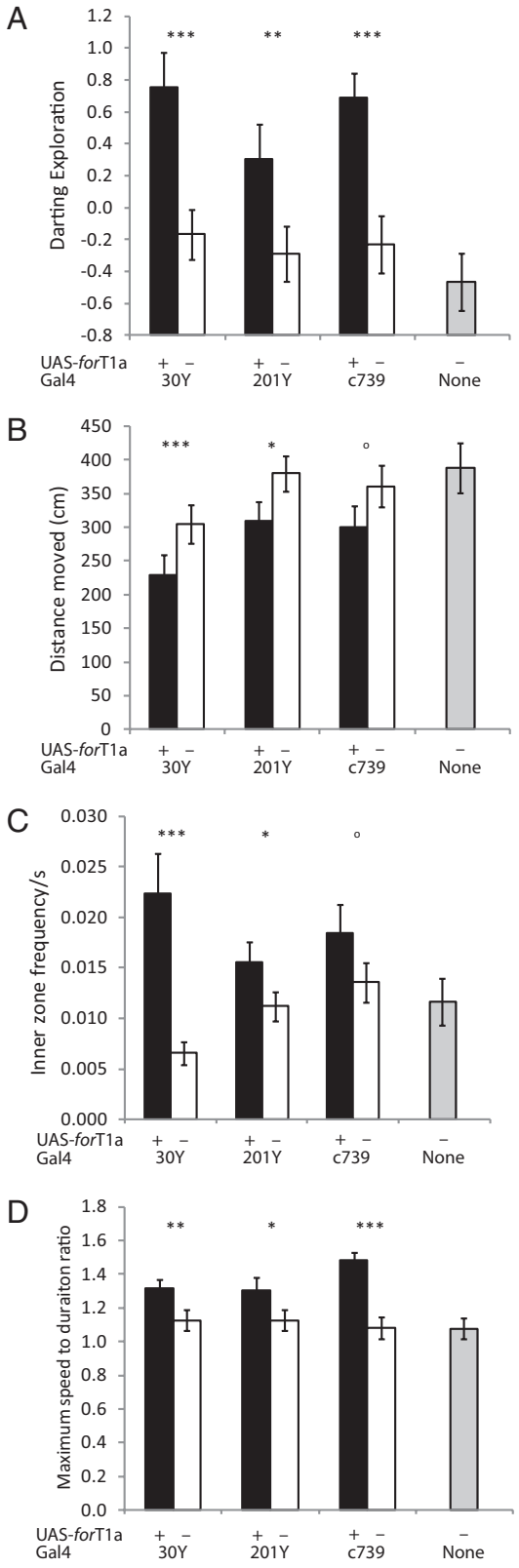

Fig. 2. Increased *for* expression in MBs, using the UAS-Gal4 system in a sitter genetic background, recovered rover exploratory behavior in female flies. All three drivers are expressed in the α- and β-lobes, 30Y and 201Y are expressed in the γ-lobes, and 30Y is also expressed in the neurons that project to the α′- and β′-lobes (22). Black bars represent the UAS-Gal4 experimental crosses, white bars represent the Gal4 control crosses, and gray bars represent the UAS control crosses. (*A*) Darting exploration was greater in all three MB drivers. (*B*) Distance moved was significantly lower in the 30Y-Gal4 and 201Y-Gal4 driver lines and marginally lower in the c739-Gal4 driver line. (*C*) Inner zone exploration was significantly higher in the 30Y-Gal4 and 201Y-Gal4 driver lines and marginally higher in the c739-Gal4 driver line. (*D*) MSDR was significantly higher in all three MB driver lines. The symbols above the bars show the significance of a planned contrast between a given UAS × Gal4 line and the two corresponding controls (Gal4 × w^1;for^5 and w^1;for^5 × UAS) in the ANOVA, corrected for multiple comparisons by the Holm–Bonferroni method. Error bars represent SEM. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; °$P < 0.10$.

sitters from early adversity but did not differ between treatments in rovers (variant·larval nutrition: $F_{1,158} = 7.0$, $P = 0.009$; post hoc, sitters: $F_{1,84} = 23.9$, $P < 0.001$; post hoc, rovers: $F_{1,74} = 0.3$, $P = 0.58$; Fig. 1*C*). The MSDR was significantly higher in sitters from early adversity but did not differ between nutritional environment treatments in rovers (variant·larval nutrition: $F_{1,158} = 3.2$, $P = 0.077$; post hoc, sitters: $F_{1,84} = 6.0$, $P = 0.016$; post hoc, rovers: $F_{1,74} = 0.1$, $P = 0.89$; Fig. 1*D*). These differences between rovers and sitters could not be explained by body mass differences. Rovers and sitters reared in standard larval food did not differ in dry body mass [mean mass per fly (SD): rovers = 0.41 (0.02) mg, sitters = 0.42 (0.02) mg; $F_{1,22} = 1.3$, $P = 0.26$], whereas rover body mass was affected to a greater degree than sitter body mass when reared in early nutritional adversity [mean mass per fly (SD): rovers = 0.28 (0.03) mg, sitters = 0.32 (0.01) mg; $F_{1,20} = 8.8$, $P = 0.008$]. We observed greater plasticity in rovers compared with sitters in body mass, but the opposite was found for exploratory behavior.

Early nutritional adversity and the differences in plasticity between rovers and sitters produced a GEI that led to individual variation in exploratory behavior in *D. melanogaster* lasting into adulthood. Interestingly, Carere et al. (42) showed differences in plasticity in response to food deprivation on lines of great tits (*Parus major*) selected for "fast" or "slow" exploration of novel environments. As in our study, food-deprived chicks in the slow explorer lines became fast explorers of a novel environment, whereas there was little effect of the food manipulation in fast explorers.

Individual differences in exploratory behavior affect both risk and reward in natural environments. There can be a tradeoff between increased resource acquisition, such as food or oviposition substrate, in new environments and the risk for mortality from predators and other unknown dangers (43). The specific characteristics of behavior may influence how these risks and rewards accrue. The darting behavior we observed appears analogous to "staccato" or "skittering" behavior in fish (44). The periods of inactivity between darting movements resemble the freezing behavior shown by many taxa in the presence of predators (45, 46). Freezing probably decreases attention conflicts by allowing an animal to focus on predator detection; it also increases how well hidden an animal is, and thus decreases an animal's chance of being detected by a predator (47). Visual predators of *Drosophila*, such as mantids, have greater difficulty detecting and successfully attacking inactive flies (48). Differences in behavior caused by larval nutritional environment could thus lead to important differences in predator avoidance.

However, darting exploration does not fit neatly into a simple description of exploration vs. antipredator behavior. Sitters from standard conditions moved greater distances but did not tend to move away from the relative safety of the wall into the potential danger, out in the open, in the inner zone. Movement in the outer zone or along the walls of the open field is considered an antipredator strategy because it likely decreases the probability of encountering predators (49). Rovers from both early nutritional adversity and standard conditions and sitters from early

nutritional adversity have an increased propensity to explore the potentially risky inner zone but decrease risk through lower movement distances and increased MSDR. How the risk and rewards of these behaviors balance out for *Drosophila* will depend on the specific conditions they encounter in nature. Our data are consistent with the idea that early life nutritional adversity has important fitness consequences, through resource acquisition and predator avoidance, in adult sitters.

Mushroom Bodies. We next investigated a role for *for* in the mushroom bodies (MBs) in darting exploration. *for* is expressed in a number of regions in the adult brain, including the MBs (22, 50). The MBs are a candidate brain region for determining exploratory behavior. They are bilateral structures in the *Drosophila* brain that are composed of five lobes (α, α′, β, β′, and γ) with apparent functional specializations (51, 52). The MBs are centers of learning, memory, and sensory integration (52), and they influence both exploratory behavior (37, 53) and locomotion (54) in *Drosophila*.

We used the upstream activating sequence (UAS)-Gal4 system to increase RNA expression of *for* in the MBs of sitter flies (55, 56) reared in standard conditions as larvae. This manipulation recovered the exploratory behavior of rovers, demonstrating that increasing *for* expression in the MBs alone was sufficient to increase darting exploration in sitters significantly. As seen in Fig. 2*A*, increasing the expression of *for* in the MBs using the 30Y, 201Y, and c739 Gal4 drivers resulted in increased darting exploration. The individual components of darting exploration also followed this pattern, with decreased distance moved, increased movement into the central zone, and increased MSDR (Fig. 2 *B–D*, respectively). Although the stronger results using the 30Y and 201Y driver lines suggest that the γ-lobes could be important, our results do not allow us to differentiate the roles of each MB lobe in exploratory behavior. Instead, the pattern of effect from each MB driver suggests that differences in exploratory behavior may result from interactions between the MB lobes. We also examined the effects of increasing the expression of *for* in sitter flies reared in nutritional adversity as larvae but in standard conditions as adults, using the 30Y driver. We did not detect any differences in exploratory behavior in this treatment compared with the controls (planned contrast, $P = 0.39$). Thus, *for* expression in the MBs of sitters is sufficient to change exploratory behavior when they are reared in standard conditions but not under early nutritional adversity. This suggests that the genetic contributions and/or tissues involved with exploratory behavior are changed by early food deprivation (or that a ceiling effect on exploratory behavior has been reached). In this regard, a sitter fly reared in early nutritional adversity may be a "different animal" than one reared in standard conditions. For example, manipulation of exploratory behavior in sitter flies with early nutritional deprivation may require *for* expression outside the brain, such as in body tissues that signal the nutritional status of the fly. The involvement of *for* in the relationship between adult physiological or behavioral responses and early nutritional adversity deserves further investigation.

Reproductive Output. We determined whether early life nutritional adversity would generate a similar pattern of differences in plasticity between rovers and sitters in an adult phenotype directly linked to fitness, fecundity. We reared females of the two genotypes in either early nutritional adversity or standard conditions and then assayed fecundity of adults placed in standard conditions for the first 6 d of life. This period is within the same time frame as our behavioral assays. There was a significant interaction between variant and larval food quantity in reproductive output (variant·larval nutrition: $F_{1,56} = 4.6$, $P = 0.037$; Fig. 3). Sitters reared in early adversity oviposited fewer eggs, over the first 6 d of adult life than in those reared in standard conditions (post hoc, sitters: $F_{1,\ 28} = 6.7$, $P = 0.015$); however, fecundity in rovers was not significantly different between larval nutrition treatments (post hoc, rovers: $F_{1,28} = 0.1$, $P = 0.77$). Like the behavioral traits we have studied, these data show that there are GEIs in fitness-related traits generated by early life nutritional environments and greater plasticity in sitters than in rovers. Moreover, they suggest that GEIs in those traits underlying egg production have fitness consequences.

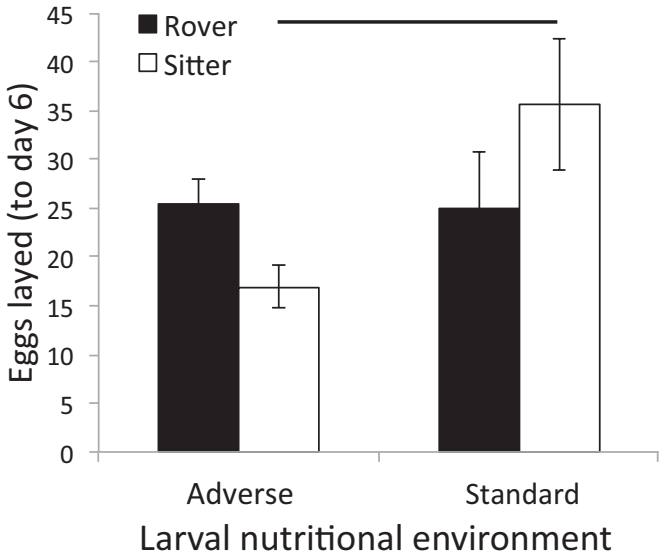

Fig. 3. Female sitters reared in nutritional adversity as larvae laid fewer eggs than sitters reared in standard conditions (post hoc ANOVA, $P = 0.015$), but no effect of nutritional environment was detected in rovers ($P = 0.768$). The line above the bars indicates a significant difference between groups at the terminus of the line. Error bars represent SEM.

General Discussion. Nutritional adversity is common in human populations and is frequently linked to low socioeconomic status (57, 58). Within the study of early childhood development, the biological sensitivity to context hypothesis (59) and the convergent differential susceptibility hypothesis (60) have used reasoning from evolutionary biology to propose explanations for individual variation in how humans react to their environment. Some genotypes may be more sensitive to environmental conditions than others. This can be manifested as differences in behavior or health outcomes within low- and high-adversity contexts (61–63). The adversity exposures characterizing low socioeconomic status have been linked to a variety of developmental risk factors, including heightened sympathetic and adrenocortical reactivity, in some children but not in others (64, 65). Interactions between genes, such as the *for* homolog in mammals, PRKG1, and early experience could potentially contribute to these individual differences. Our study contributes to the view that the effects of early life adversity on adult traits, in humans or other animals, are best understood as arising from the interplay of genes and environment (66).

This study establishes the fly as a model for chronic nutritional deprivation. One key theme of our Sackler Colloquium was the examination of critical periods of plasticity. Although we have identified the importance of the larval period in our experiments, the myriad of genetic tools available with fruit flies allows us to break this episode down into more precise intervals of time or sensitive periods, where we can manipulate tissue-specific gene expression and perform environmental enrichment interventions in flies (67, 68). Identification of the precise biological mechanisms underlying when and how early

adversity "gets under the cuticle" is increasingly accessible using *D. melanogaster*.

An important question, however, remains unresolved: What is the generality of the differences in plasticity that we observed? Are sitters more plastic than rovers across environmental contexts, or does it depend on the type of adversity (e.g., nutritional, social) the individual experiences, and the timing of that adversity (chronic acute) during development? Previous studies have shown that sitters are more resistant to acute abiotic stressors, such as heat and hypoxia, than are rovers (69–71). The type of exposure used, chronic or acute, may explain which variant exhibits plasticity. Alternatively, the greater plasticity observed among sitters in the present study could be specific to the type of adversity we investigated: early nutritional adversity. The strength, timing, and duration of exposure to adversity during development, along with the molecular mechanisms of action within candidate tissues, are key elements to be addressed in future experiments before drawing conclusions about the generality of plasticity differences in rovers and sitters. Many challenges remain, including how early interactions with the environment at one point in development can change later interactions with the environment, whether or how these interactions are adaptive, and how interventions might reverse the effects of early nutritional adversity.

Methods

Fly Variants and Food Media. Rover and sitter natural variants are described by Fitzpatrick et al. (29). Fly populations were maintained in 50-mL plastic vials at 23 °C on a 12:12 light/dark cycle, with 10 mL of food medium. To generate flies from "nutritional adversity" and "standard conditions," flies laid eggs on an agar-grape juice medium and these eggs were transferred to 50-mL plastic vials with 10 mL of food medium. The food medium in standard conditions was composed of 50 g of dry yeast, 100 g of sucrose, 16 g of agar, 8 g of $KNaC_4H_4O_6$, 1 g of KH_2PO_4, 0.5 g of $CaCl_2$, 0.5 g of NaCl, 0.5 g of $MgCl_2$, 0.5 g of $Fe_2(SO_4)_3$, and 5 mL of propionic acid for 1 L of water. The food medium in the early nutritional adversity treatment contained the above amounts of all ingredients, with the important exception of having lower levels of yeast and sucrose (i.e., protein, carbohydrate) compared with the standard condition treatment (75% and 80% reduction in both yeast and sucrose for the open-field and reproductive output experiments, respectively). Dry body mass of flies from each treatment was measured in groups of 10 flies. To control for adult experience, larvae pupated on strips of filter paper that were then placed in fresh food vials. In the exploratory behavior experiments, these strips were transferred to fresh vials with either food from the nutritional adversity treatment or food from the standard conditions treatment the day before eclosion. Thus, the exploratory behavior study used a full-factorial experiment with all four combinations of nutritional adversity and standard conditions for larvae and nutritional adversity and standard conditions for adult flies. In the reproductive output experiments, larvae from both nutritional adversity and standard conditions were exposed to only standard conditions as adults.

Open-Field Tests. Flies (2–3 d of age) were lightly anesthetized with CO_2 anesthesia to collect mated females. These flies were kept in groups of 30 females in vials with 10 mL of food from the nutritional adversity or standard food conditions treatment until they were tested at 5–7 d of age.

The open-field apparatus was a 14-cm-diameter Petri dish with sidewall height of 4 mm and a clear plastic lid (Fig. S1*A*). Individual flies were gently aspirated into the open field, the lid was shifted to cover the whole open field, and the flies were then video-tracked with Ethovision 7 software (Noldus Information Technology) for 10 min. Lighting was provided by an electroluminescent sheet underneath the apparatus. The short height of the apparatus prevented flying; thus, all observations are of walking flies.

The *x*,*y* coordinates provided by the video-tracking software, along with the time stamp for each set of coordinates, allowed the calculation of several behavioral phenotypes previously identified as important in open-field behavior (36, 72, 73). Parameters of particular importance were as follows: distance moved during the whole test, speed, whether the fly was moving or not, and position in the open field. To define these parameters, flies were video-tracked at five data points per second. Moving vs. not moving was defined according to the method of Martin (36): when a fly's speed went below 2 mm/s averaged over five consecutive data points, the fly was considered to have stopped, and it was considered to have started moving again when its speed went above 4 mm/s, averaged over five consecutive data points (Fig. S1*B*).

Three behaviors were calculated from the above parameters: distance moved, inner zone exploration, and MSDR. Inner zone exploration was calculated as the number of times the flies crossed into the inner zone (the inner 10-cm-diameter circle within the 14-cm-diameter arena; Fig. S1*A*), corrected for activity level by dividing by the total time spent moving during the 10-min test. MSDR is a measure of stop-and-go motion, and it was calculated by taking the maximum velocity during each period of movement and dividing that by the duration of that period of movement. The mean value of MSDR for all movement periods for an individual was calculated if there were at least three movement periods during the test. Individuals with fewer than three movement periods were not included in analyses.

Gal4 Lines. To determine the effect of *for* expression in the MBs on open-field behavior, we used UAS and Gal4 constructs (56) crossed into a *white*^{1} (*w*^{1}) sitter (*for*^{s}) genetic background. The three Gal4 driver lines were as follows: 30Y-Gal4, which expresses in all MB lobes (α, α′, β, β′, and γ); c739-Gal4, which expresses in the α- and β-lobes and Kenyon cells; and 201Y-Gal4, which expressed strongly in the γ-lobes and more weakly in the α- and β-lobes (21, 22, 55). Transgenic expression of *for* was accomplished with a *w*^{1};*for*^{s};*UAS-forT1a* line (21). We crossed this *UAS-forT1a* line with the Gal4 lines and tested the female progeny in the open field. Negative controls were produced by crossing the *UAS-forT1a* line with a *w*^{1};*for*^{s} line and each of the Gal4 lines to the *w*^{1};*for*^{s} line.

Reproductive Output. Individual virgin female adult flies, reared as larvae in either nutritional adversity or standard conditions, were placed in standard condition vials with a single male fly from the standard nutritional treatment. Female fecundity was measured at days 2 and 6 of adult life. Three hours before the end of the light cycle, each female and male pair was transferred into a new vial. Sixteen hours later, the pairs were moved to a new vial and the emptied vial was frozen for later egg scoring.

Statistical Analysis. In the comparison of rovers and sitters, each phenotype was analyzed with a full-factorial two-way ANOVA, in which variant and larval nutritional environment were the main factors. Post hoc ANOVAs of effects of larval nutritional environment within each variant were performed to inform on the patterns behind statistical interactions. Post hoc tests were Bonferroni-corrected for the two comparisons, such that statistical significance was only reached at $P < 0.025$. Analysis of Gal4-UAS experiments was conducted with planned contrasts in one-way ANOVAs.

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