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SIMPLE SHELTER-STYLE ENVIRONMENTAL ENRICHMENT ALTERS BEHAVIOR IN MICE

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Abstract

Environmental enrichment aims to improve the well-being of laboratory animals and provides an opportunity to improve experimental reliability and validity. Animals raised in more stimulating environments have improved learning and memory as well as more complex brain architecture. However, the effects of environmental enrichment on motor performance, anxiety and emotional development have been poorly studied. Moreover, most investigators studying the effects of enrichment provide extremely large and complex housing conditions to maximize the likelihood of finding effects. These situations are difficult to replicate across animal facilities and are not operationally practical. In this experiment, we investigated how simple, inexpensive disposable shelter-style enrichment items alter behavior in C57Bl/6 and 129S6 mice. Breeding pairs were established in the presence of a Ketchum "Refuge", Shepherd Shack "Dome", or no enrichment. Offspring were assessed neurobehaviorally, either just after weaning (pre-adolescent, P22-P25), or as young adults (P60-P90). Major strain differences were observed in open field activity, elevated maze exploration, and Y-maze activity levels. The presence of the Refuge and/or Dome enrichment shelters significantly altered motor activity, coordination and some measures of anxiety. Mice housed in the presence of shelters were also less dominant than control mice in a tube test assay. Our experiments provide a detailed analysis of the effects of inexpensive and practical methods of housing enrichment on biobehavioral phenotypes in these two commonly used strains of laboratory mice, and suggest that the effects of these shelters on mouse neurobiology and behavior need to be rigorously analyzed before being adopted within vivariums.

Keywords

• Mouse • Refuge • Anxiety • Locomotor • Thigmotaxis • Dominance • Shelters • Coordination • Tube test • Open field

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Introduction

The primary aim of environmental enrichment is to meet the physical and psychological needs of laboratory animals in order to improve their well-being in captivity and generate species-appropriate biological and behavioral responses [1,2]. Environmental factors, including bedding and nesting material, and the presence of objects to explore and/or gnaw upon, affect not only the health and welfare of laboratory mice [3-5], but also experimental results [6-12]. Thus, it is also vital to consider the effects on the biomedical research at hand [13,14]. For example, within neuroscience, environmental enrichment has been shown to affect a wide variety of responses, including exploratory behavior [6,11,15], cognition [6,16-18], emotionality [12,19,20], brain neurochemistry [21,22], gene expression patterns [9], stress resiliency [23], and even responses to addictive drugs such as cocaine [9,24,25]. These data lead to understandable resistance by researchers to adopt even the most rudimentary forms of enrichment, such as nesting materials and shelters.

A limitation of the currently published work on this topic, however, is the fact that when enrichment is employed, laboratories typically use extremely complex environments (also known as "Mouse Disney World") for their enrichment strategy, in order to maximize its effects. These situations are difficult to replicate across facilities and are not operationally practical - it is virtually impossible to utilize such devices consistently through an animal use program. Furthermore, since non-standardized objects (e.g., toys) are often selected for addition to the cages, it is difficult, if not impossible, to compare results across facilities, or in some cases even across laboratories within the same institution.

Many types of simple enrichment devices have been used in the literature and are marketed commercially. We believe that in order for enrichment to be adopted widely, it must be standardized. Also, if any one type of enrichment device is to be used across laboratories and institutions, it needs to be simple, inexpensive and disposable. A recent study examined sleep in mice housed in the presence and absence of a very simple environmental shelter and found increased slow-wave sleep and reduced locomotor activity when a shelter was included with single-housed mice [26]. In the data reported here, we tested to what degree inclusion of simple, inexpensive and disposable enrichment devices (Ketchum "Refuges" and Shepherd Shack "Domes") altered behavior in two common strains of laboratory mice (wildtype 129S6/SvEvTac and C57Bl/6J). In this regard, many knockout and transgenic mice have been created in the 129S6/SvEv background, and are then extensively back-crossed to C57Bl/6J. We hypothesized that

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these devices would influence specific domains of neurobehavioral function, perhaps in a strain-dependent fashion. In fact, we observed significant effects of shelter inclusion, and these effects were often strain-dependent. Our study suggests that great care and caution need to be exercised when selecting shelter-style enrichment devices for laboratory mice being used in neurobehavioral studies.

Experimental procedures

Animals

Mice were housed under standard ventilated housing conditions on a 12 h light/dark cycle (lights on 0600-1800 h) with *ad libitum* food and water in transparent Allentown XJ polycarbonate cages (Allentown Inc., Allentown, NJ, USA). The mice were socially housed on corn cob bedding with paper rolls for nest building ('Enrich-o'Cob', The Andersons, Maumee, OH, USA). Mice housed under standard housing conditions (no enrichment) were kept in isosexual sibling groups ranging from 2-5 animals dependent on litter census in standard laboratory caging with corn cob bedding and nest material. Mice housed under "enriched" conditions were also kept in isosexual sibling groups ranging from 2-5 animals in standard laboratory caging but cages additionally contained either a paper dome ('Dome', Shepherd Specialty Papers, Watertown, TN, USA) or paper hut ('Refuge', Ketchum Manufacturing, Brockville, ON, Canada) at all times. Photographs of the shelters are displayed as Figure 1a and Figure 1b.

A breeding colony for C57Bl/6J (C57, Jackson Laboratories, Farmington, CT, USA) and 129S6/SvEv/Tac (129, Taconic, Hudson, NY, USA) mice was established on-site for each housing condition (n = 42 breeding pairs; n = 14 pairs per condition). After breeding successfully for at least one litter, a second litter was utilized for neurobehavioral testing. Enrichment devices were placed in the cages just prior to breeding. Litters were weaned at 21 days after birth. Isosexual siblings were then housed together with the same style of enrichment device with which they were raised. Two male and two female preadolescent mice from each litter were tested at ~P24-28 (N = 48 total juvenile mice, 24 mice of each strain). The remaining male and female littermates were tested as young adults (N = 99 adult mice; N = 44 129 and N = 55 C57). No sex-dependent effects were observed, so data from male and female mice were combined for the analyses. All experiments were carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) and all procedures were approved by the Animal Care and Use Committee at Vanderbilt University. Body weight data collected from birth to adult testing revealed no effects of enrichment shelter condition (data not shown). Unless otherwise specified, mice were transported from the colony room to the testing room and allowed to habituate for 30 min before any testing occurred. Testing occurred during the light phase. Juvenile testing consisted of the elevated zero maze at ~P22, spontaneous alternation in a Y maze at ~P23, and open field at ~P24. We have previously validated these (and only these) measures in weanling mice [27,28] and they require no training. A separate cohort of young adult mice began testing at ~P60 and a serial battery approach was followed with several days separating each test in this order: elevated zero maze, open field, Y-maze, light-dark, rotarod, marble burying, tube test, and forced swim.

Elevated zero maze

Mice were first tested on an elevated maze as a measure of anxiety-related behaviors around P24 for preadolescent mice and around P60 for young adult mice. The elevated zero maze (EZM) is a modification of the elevated plus maze and use of the circular maze removes any ambiguity in data interpretations as there

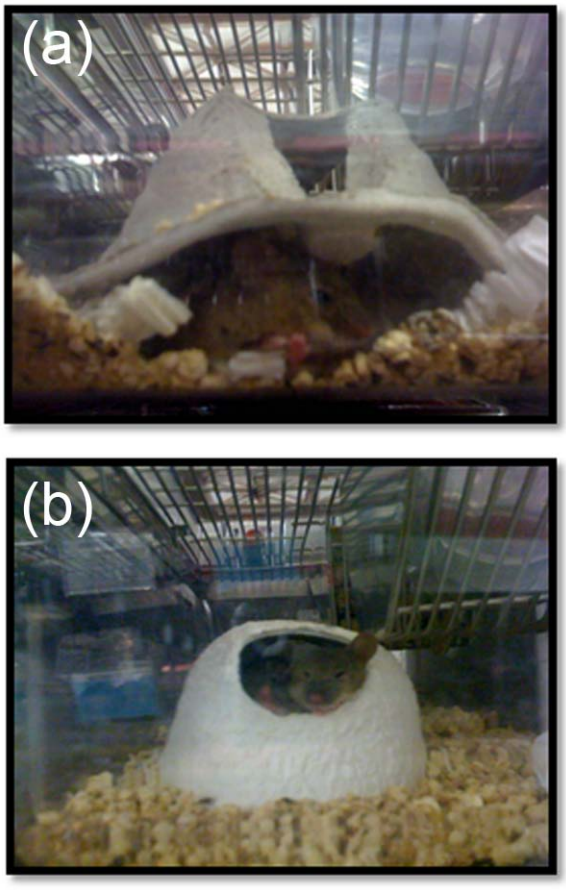

Figure 1. Photograph of housing cages containing Otto Environmental cardboard "refuge" (a) and Shepherd "dome" (b). These are two of the most common commercial shelters used in laboratory vivariums at this time.

is no center zone [29-31]. The elevated circular platform (40 cm off the ground, 50 cm in diameter) had two enclosed areas opposite each other (5 cm wide with 15 cm high walls) and two open areas (5 cm wide). At the start of the test, each mouse was lowered by its tail into an open sector of the maze and allowed to explore the maze for 5 min under red light conditions [28,32]. Activity of the mouse was monitored via an overhead camera connected to a computer in a separate room using video acquisition and ANY-maze analysis software (Stoelting, Wood Dale, IL, USA). Data analyzed included the percentage of time spent in the open versus closed arenas and the total distance traveled in the maze.

Activity monitors / Open field

Locomotor activity was measured using commercial open field activity chambers (Med Associates, 27 × 27 × 20.5 cm) that were contained within light- and air- controlled environmental chambers (Med Associates, St. Albans, VT, USA; 64 × 45 × 42 cm; illumination ~60 lux) around P26 or after P60. Location and movement were detected by the interruption of infrared beams by the body of the mouse (16 photocells in each horizontal direction, as well as 16 photocells elevated to measure rearing) and were measured by the Med Associates Activity Monitoring program for 60 min [27,28,33]. Center and surround zones, each encompassing 50% of the chamber area, were defined for thigmotaxis analyses.

Y-maze

The Y-maze assesses spatial working memory as animals tend to alternate between arms based on their memory of the previously visited arms. Juvenile mice began testing around P23 and young adult mice shortly after P60. The Y-mazes used for the juvenile and adult mice varied in size. Each Y-maze contained three clear arms joined in the center and was placed on an opaque table about 91 cm above the ground in a room containing several large immovable objects to use a spatial cues. For adult mice, each arm of the maze was 35.5 × 10.2 cm and there was no lid. For juvenile mice, a smaller maze was used (34.5 × 5.2 cm) and each arm had a semi-circular covering. Three uniquely colored-patterned construction paper sheets were also placed beneath each arm of the maze during juvenile testing as a secondary spatial cue. In the absence of these cues, juvenile mice struggle with this task (unpublished observations). At the start of the test, each mouse was lowered by its tail into one of the arms facing the center and allowed to explore the maze for 6 min. The same starting arm was used for each mouse. Activity of the mouse was monitored via overhead camera connected to a computer in a separate room using ANY-maze. The sequence of individual arm entries was scored in real time and used to calculate the percentage of spontaneous alternations for each animal (consecutive entry into each of the three arms) as previously described [28,34]. Chance performance is 22.2% in this paradigm.

Light-dark preference

The light-dark preference test uses an opaque insert placed within one-half of the Med Associates Open Field chambers [35,36]. The dark insert measures 27 cm in length, 14 cm in width, and 20.5 cm in height, with a single door for entering/exiting a darkened area and is placed on the left side of the activity chamber. Movement and location are again detected by the interruption of infrared beams during a 10 min session and the time spent in each compartment (light and dark) was monitored.

Marble burying

Mice were individually placed in Plexiglass cages in which 20 black glass marbles (14 mm diameter) were distributed in a 4 × 5 layout with 1.5 cm between each marble on top of 2.5 cm Diamond Soft Bedding (Harlan Teklad, Madison, WI, USA). The amount of marble burying was recorded over a 20 min interval. The mice were then removed from the cages, and the number of buried marbles was counted using a criterion of greater than 2/3rd covered by bedding.

Rotarod

Motor coordination and balance were measured using a commercially available accelerating rotarod apparatus (Ugo Basile model 7650, Ugo Basile Srl, Varese, Italy) as previously described [34,36]. Mice were placed on the rotating cylinder (3 cm in diameter) and confined to a section approximately 6.0 cm long by gray plastic dividers. The rotational speed of the cylinder was increased from 5 to 40 rpm over a 5 min period. Latency at which mice fell off the rotating cylinder was measured. Each mouse was given three trials per day over a period of 3 days, with a 15 min inter-trial interval.

Tube test

The apparatus is a 30-cm-long, 3.5-cm-diameter clear acrylic tube with small acrylic funnels added to each end to facilitate entry into the tube [37]. On two separate days before testing, each mouse was exposed to the tube, with progress through the tube resulting in the mouse being returned to the home cage. For the tube test bouts, male mice from the same strain, but from distinct enrichment conditions were placed at the opposite ends of the tube and released. A subject was declared a "winner" when its opponent backed out of the tube. Each mouse was tested against four to five individuals from other cages, with counterbalancing of which mouse was at each end to avoid position bias.

Forced swim test

Behavioral despair was assessed in the forced swim test using plastic cylinders (14.5 cm in diameter, 21 cm in height) filled approximately ¾ full with room temperature water [34,36]. Mice were individually placed into the cylinder for a 6 min test and were recorded on video for the duration of the test. After testing, the mice were placed into a heated cage to dry before returning to the home cage. The water was changed between tests and the temperature of the water was recorded. Videos were later analyzed for time spent immobile for each mouse by a blinded observer.

Statistical analyses

Strain and enrichment differences were assessed by one-way or two-way ANOVA as appropriate with significance defined as two-tailed $p < 0.05$ using GraphPad Prism (La Jolla, CA, USA). Individual values greater than 3 standard deviations from the mean were removed from analyses as outliers (this resulted in elimination of at most 1 data point

per measure and were not associated with any particular enrichment condition or strain). *Post-hoc* comparisons used Bonferroni's multiple comparisons tests. In most cases we present one-way ANOVAs within each strain and age in order to maximize power to detect effects of the enrichment shelters. Tube test data were analyzed by chi-square test.

Results

Elevated zero maze

The results of elevated maze testing at each age (juvenile and adult) and strain (C57 and 129) are displayed in Figure 2. The inclusion of enrichment shelters produced a significant increase the percent time spent in the open zones of the EZM in juvenile 129 mice (Figure 2b; $F_{2,47} = 7.81$, $p = 0.021$). The Dome shelters were significantly different from controls by *post-hoc* test (Bonferroni corrected *t*-test $p < 0.05$). A similar trend persisted in adult 129 mice, but was not statistically significant (Figure 2d; $F_{2,40} = 2.58$, $p = 0.089$). Performance of C57 juvenile mice was unaffected by enrichment condition, but enrichment decreased percent open time in adult C57 mice (Figure 2c; $F_{2,51} = 4.52$, $p = 0.016$). In this case, the Refuge shelter appeared to have the largest contribution to this apparent increase in anxiety response (Bonferroni corrected *t*-test $p < 0.05$). The number of open arm entries was utilized as a locomotor measure. Juvenile C57 mice displayed increased arm entries due to Refuge-style enrichment (Figure 2e; $F_{2,65} = 7.87$, $p = 0.001$). With regard to strain, 129 mice entered more arms than did C57 mice at both ages ($F_{1,112} = 10.00$, $p = 0.002$ for juveniles; $F_{1,92} = 26.69$, $p < 0.001$ for adults).

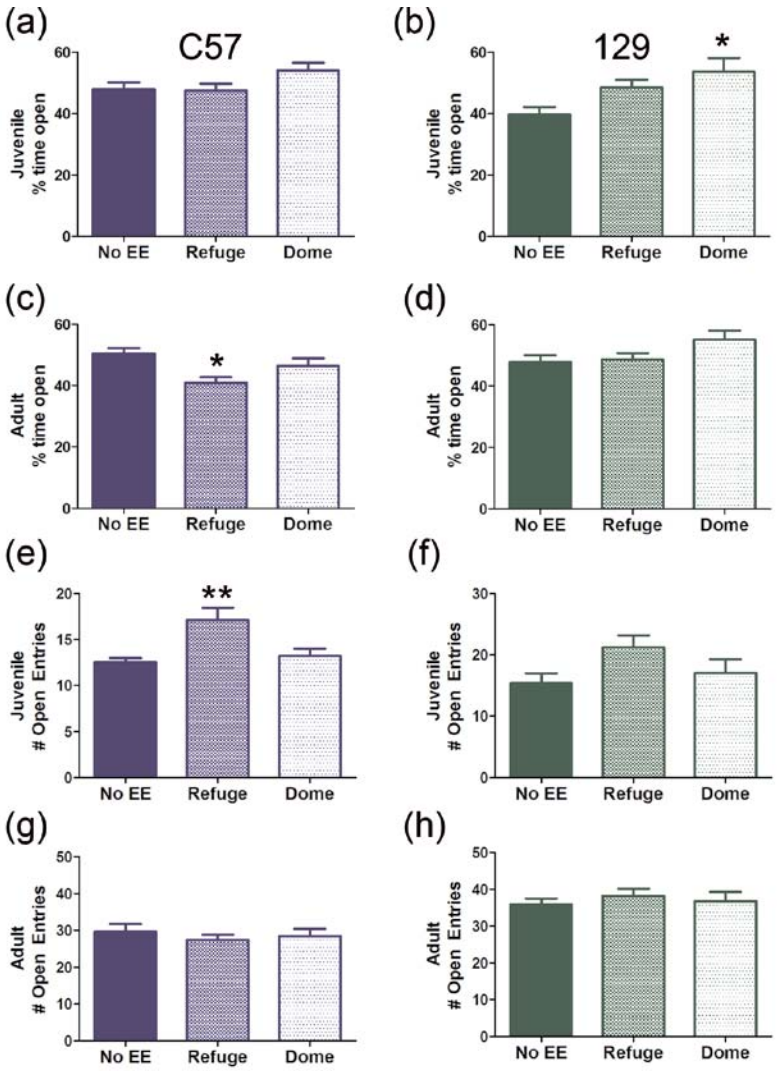

Figure 2. Graphs display performance of C57 (a, c, e, g) and 129 (b, d, f, h) mice on an elevated zero maze. Data depicted are percent time spent in an open arm (a, b, c, d) as an index of anxiety state, and total open arm entries (e, f, g, h) as an index of locomotor activity. As juveniles (~P24) 129 mice housed in the presence of a Dome shelter displayed increased time in the open arms, suggesting a reduction in anxiety. In contrast, adult C57 mice (~P60) housed in the presence of a Refuge shelter displayed decreased time in the open arms, suggesting increased. Juvenile C57 mice also displayed increased arm entries due to Refuge-style enrichment (* = $p < 0.05$; ** $p < 0.01$ by Bonferroni multiple comparison test). n = 14-24 mice per group.

Open field

As expected, strain effects were observed for locomotor measures in the open field such that C57 mice expressed greater ambulatory distance and motor stereotypies than 129 mice (Figure 3; ambulatory distance $F_{1,112}$ = 39.35, $p < 0.001$ for juveniles; $F_{1,91}$ = 133.9, $p < 0.001$ for adults; stereotypies $F_{1,114}$ = 143.8, $p < 0.001$ for juveniles; $F_{1,92}$ = 266.2, $p < 0.001$ for adults). Enrichment shelter had significant effects in juvenile C57 mice both with regards to ambulatory distance and stereotypies (Figure 3a, distance $F_{2,65}$ = 10.70, $p < 0.001$; Figure 3e, stereotypies $F_{2,65}$ = 15.18, $p < 0.001$). *Post-hoc* comparisons indicated that these effects were largely driven by increased activity in mice housed in the presence of Refuges (Bonferroni corrected *t*-test $p < 0.001$). These effects normalized by adulthood (Figure 3c and Figure 3g). Analyses of data split into 5 min epochs revealed no additional effects of strain or shelter type (data not shown).

Anxiety was further measured by thigmotaxic behavior, as indicated by the percentage of time the mice spent in the center of the open field arena. Once again, a strain effect was observed with 129 mice exhibiting a greater propensity to avoid the center area ($F_{1,106}$ = 95.99, $p < 0.001$ for juveniles; $F_{1,88}$ = 88.22, $p < 0.001$ for adults). In juvenile C57 mice, enrichment shelters increased center time, potentially indicative of a reduction in anxiety (Figure 4a, $F_{2,65}$ = 5.10, $p < 0.001$), but had no effect in adulthood. The juvenile effect was largely due to the Refuge condition (Bonferroni corrected *t*-test $p < 0.01$). In sharp contrast, both enrichment shelter styles resulted in reduced center time in 129 mice ($F_{2,45}$ = 4.39, $p < 0.018$ for juveniles; $F_{2,40}$ = 4.05, p = 0.025 for adults), suggesting enrichment-induced *increases* in anxiety.

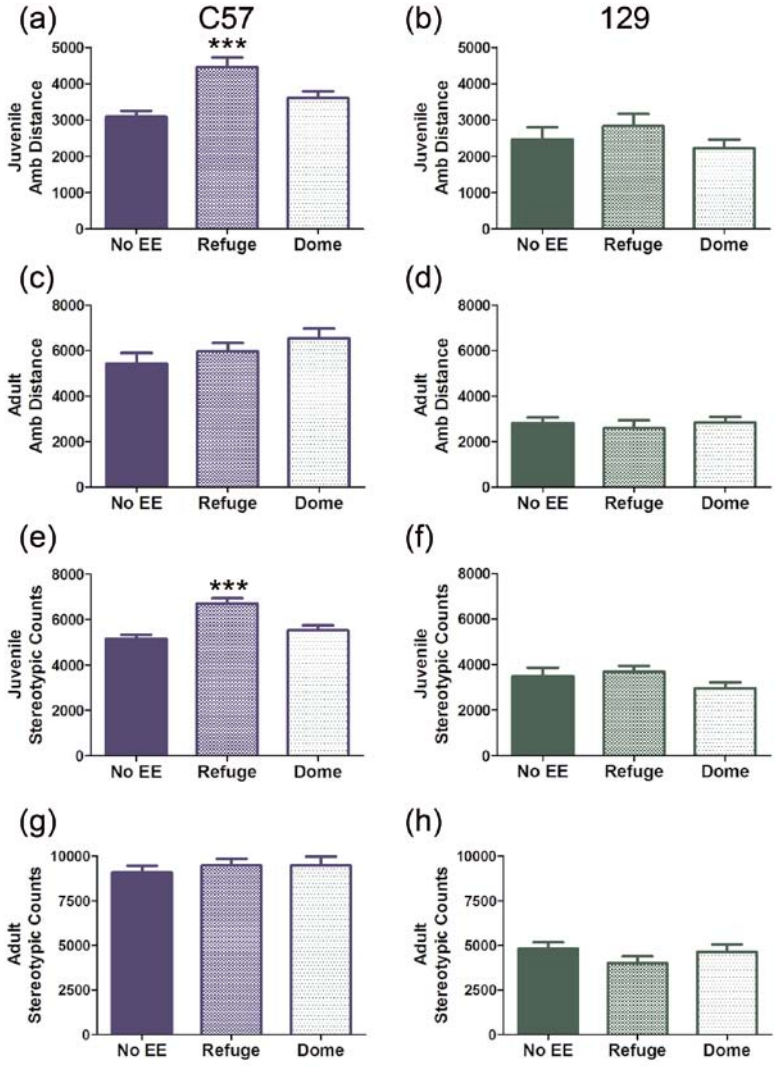

Figure 3. Graphs display behavior of C57 (a, c, e, g) and 129 (b, d, f, h) mice in an open field. Data depicted are total ambulatory distance (a, b, c, d) and stereotypies (e, f, g, h) during a 30 min session. Refuge shelters increased both ambulatory distance and stereotypies in juvenile (~P25) C57 mice (*** = $p < 0.001$ by Bonferroni multiple comparison test). n = 14-24 mice per group.

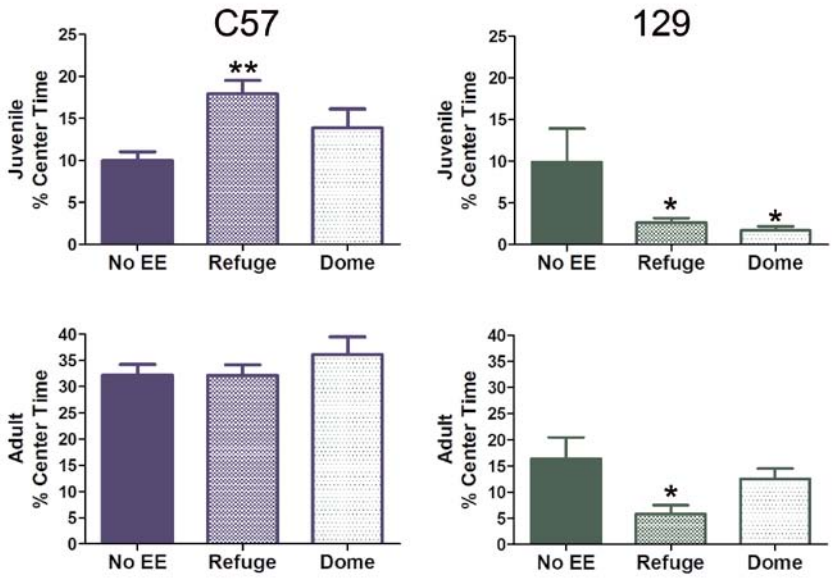

Figure 4. Graphs display thigmotaxis of C57 (a, c) and 129 (b, d) mice in an open field as an additional measure of anxiety behavior. ANOVAs reveal significant effects of enrichment in both strains and at both ages, but they were strain dependent. In C57 mice, post-hoc tests indicate significance for Refuge shelters in juveniles (** $p < 0.01$ by Bonferroni multiple comparison test). In 129 mice, however, enrichment shelters reduced center time even from the already low baseline for 129 mice (* = $p < 0.05$ by Bonferroni multiple comparison test). n = 14-24 mice per group.

Y-maze

Spatial working memory was assessed using spontaneous alternation in a Y maze (Figure 5). Juvenile C57 mice displayed significantly a lower percentage of spontaneous alternations when reared with environmental shelters (Figure 5a; $F_{2,65} = 3.62$, $p = 0.032$). The Dome shelters were significantly different from controls by post-hoc test (Bonferroni corrected *t*-test $p < 0.05$). This effect did not persist into adulthood (Figure 5c), and was not seen in 129 mice at either age (Figure 5b and Figure 5d). However, 129 mice with the refuge shelter did have a modest decrease in total arm entries in the Y maze (Figure 5h; $F_{2,42} = 3.43$, $p = 0.042$). With regard to strain, juvenile 129 mice showed decreased spontaneous alternation rates than did C57 mice ($F_{1,111} = 24.83$, $p < 0.001$). The two strains did not differ in adulthood, however, suggesting that any deficit in the 129 line had been compensated for.

Rotarod

The ability to navigate an accelerating rotarod without falling off is a measure of motor coordination and motor learning. Somewhat surprisingly, this assay is where we observed some of the most robust alterations induced by the presence of enrichment shelters in the home cage (Figure 6). C57 mice showed significant positive effects of both enrichment condition ($F_{2,147} = 12.68$, $p < 0.001$) and day of training ($F_{2,147} = 5.22$, $p = 0.006$) (Figure 6a). Similarly, 129 mice also expressed significant effects of both enrichment condition ($F_{2,123} = 5.83$, $p = 0.004$) and day of training ($F_{2,123} = 24.66$, $p < 0.001$) (Figure 6b), although 129 mice exhibited strikingly poor performance on this task, regardless of their enrichment condition. When performance across all trials was collapsed, this resulted in primary effects of both strain ($F_{1,90} = 108.0$, $p < 0.001$) and enrichment ($F_{2,90} = 8.69$, $p < 0.001$), as well as a modestly significant interaction ($F_{2,90} = 3.21$, $p < 0.045$) (Figure 6c).

Light-dark

A light-dark preference assay was used as an additional measure of anxiety in adult mice (Figure 7a and Figure 7b). There were no effects of enrichment condition on this task, although 2-way ANOVA revealed a significant effect of strain ($F_{1,92} = 9.59$, $p = 0.003$), with 129 mice displaying increased dark preference with respect to C57s.

Marble burying

The propensity of rodents to bury unknown objects, such as marbles, underneath cage bedding has been used previously as a screen for anxiety state and compulsive behaviors [38]. We therefore next assessed marble burying in adult C57 and 129 mice reared in the presence of enrichment shelters, but observed no significant effects of shelter enrichment in either strain (Figure 7c and Figure 7d).

Tube test

Our next paradigm explored dominance relationships in a tube test. Mice were paired against mice from the same strain but different enrichment conditions. Within each strain, mice from the no enrichment condition were more likely to be the dominant animal in each dyad (Figure 8, $p < 0.001$ by chi-square; 57-64% "wins" as compared to chance 50%). A more complex relationship was evident when mice from the two styles of shelters were tested against one another. Within the C57 strain, the Dome style of enrichment resulted in increased dominance over Refuges, but in 129 mice the converse was observed with Refuge-housed mice winning the encounters 95% of the time (Figure 8).

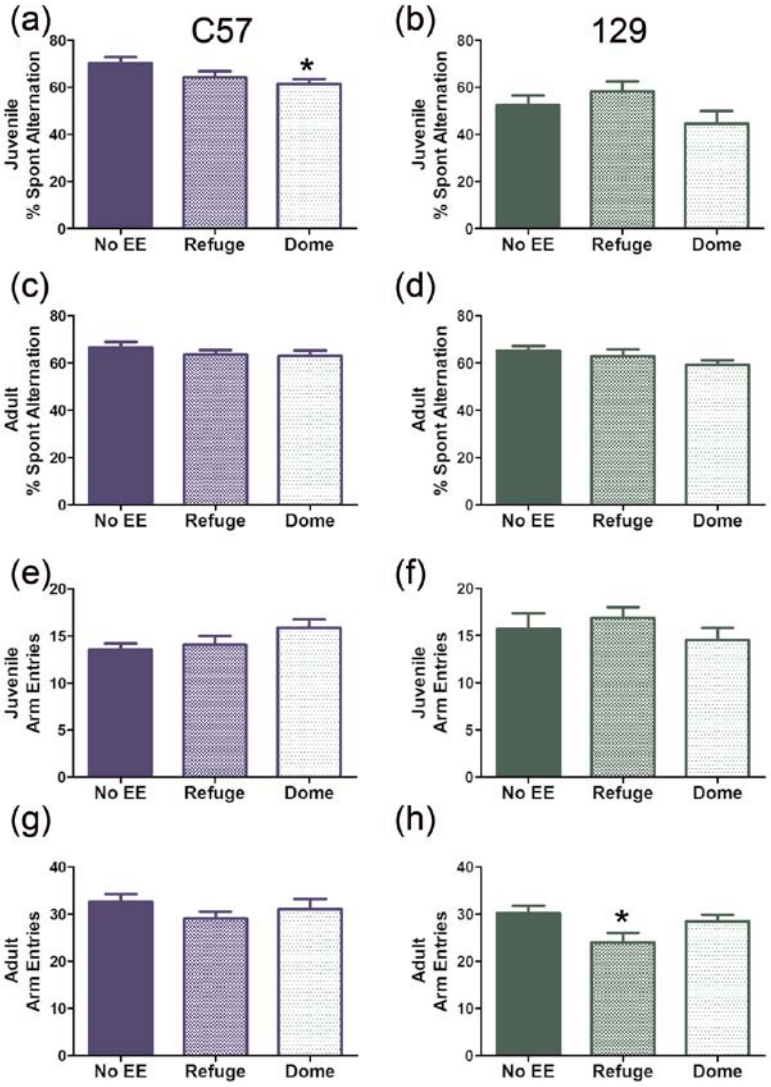

Figure 5. Graphs display behavior of C57 (a, c, e, g) and 129 (b, d, f, h) mice in a Y maze. Data depicted are percent spontaneous alternations (a, b, c, d) as an index of spatial working memory), and total number of arm entries (e, f, g, h) as a measure of locomotor activity. Dome shelters decreased spontaneous alternation rate in C57 juvenile mice (* = $p < 0.05$ by Bonferroni multiple comparison test) and Refuge shelters decreased arm entries in adult 129 mice. n = 14-24 mice per group.

Forced swim test

Our final paradigm examined depressive-like behavior in a forced swim test (Figure 9). There were no effects of enrichment condition on this task, although 2-way ANOVA again revealed a significant effect of strain ($F_{1,65} = 12.57$, $p < 0.001$), with 129 mice displaying increased immobility as compared to C57Bl/6 mice.

Discussion

We tested to what degree inclusion of simple, inexpensive and disposable enrichment devices in housing cages might alter behavior in two common strains of laboratory mice. We observed shelter device-induced modulation of behavior, often in age- and strain-dependent manners. Our study suggests that care and caution need to be exercised when selecting shelter-style enrichment devices for laboratory mice being used in neurobehavioral and neurobiological studies. Although in some cases the devices improve the dynamic range of assays, in other situations they may impede study design and may even not actually be beneficial for animal welfare. Based on these data, these relationships depend on the domains to be studied and the genetic background of the mice being assessed.

Our studies utilized a variety of measures of locomotor behavior and motor functions. Maze-based tasks in our current study revealed subtle and task-specific impacts of housing enrichment. For example, juvenile C57 mice housed with Refuges had substantially greater locomotor behavior on an elevated zero maze, but adult 129 mice housed with Refuges instead displayed decreased entries in a Y maze. In the open field task,

juvenile C57 mice again exhibited increased locomotion, both as revealed by increases in ambulatory distance and in the number of detected motor stereotypies. These changes normalized by adulthood, perhaps through some compensation in neurodevelopmental trajectory [39-41]. In contrast, a study using more elaborate enrichment (large cages, wheels, swings, aspen houses, ladders, etc.) found increased locomotion in both C57 and 129 mice when tested as adults [42].

Motor coordination was assessed in adult mice using a rotarod. In C57 mice, the presence of either Refuge or Dome shelters produced subtle but significant improvements in baseline performance and motor learning across trials. This was somewhat unexpected – we had predicted the most salient effects of shelters to be emotional and social behaviors. Nevertheless, the inclusion of shelters resulted in improved and asymptotic performance of C57 mice by the second day of training (although performance on day 3 was excellent in all conditions). Thus, if one was characterizing genetic or environmental facilitation of motor learning in C57 mice, the inclusion of enrichment shelters would make detection of significant beneficial effects very difficult. In contrast, the inclusion of the shelters did not facilitate motor learning in the 129 strain – even though those mice began from a significantly lower performance level on day 1.

For anxiety measures, effects observed in this study were again dependent on age and strain. The elevated zero maze indicated reduced anxiety in juvenile mice (especially 129s) receiving enrichment shelters, as evidenced by increased open arm time. However, thigmotaxis in the open field suggested the opposite phenotype, with 129 mice receiving shelters expressing an anxiogenic phenotype. This dissociation between the zero maze and the open field is difficult to reconcile. Juvenile C57 mice housed with Refuges had greater center time in the open field but no change in EZM. Adult C57 mice expressed a modest reduction in open arm time in the EZM, but no change in thigmotaxis. Given that marble burying, light-dark assay, and forced swim detected no enrichment-induced phenotypes in adult mice of either strain, it is again difficult to reconcile

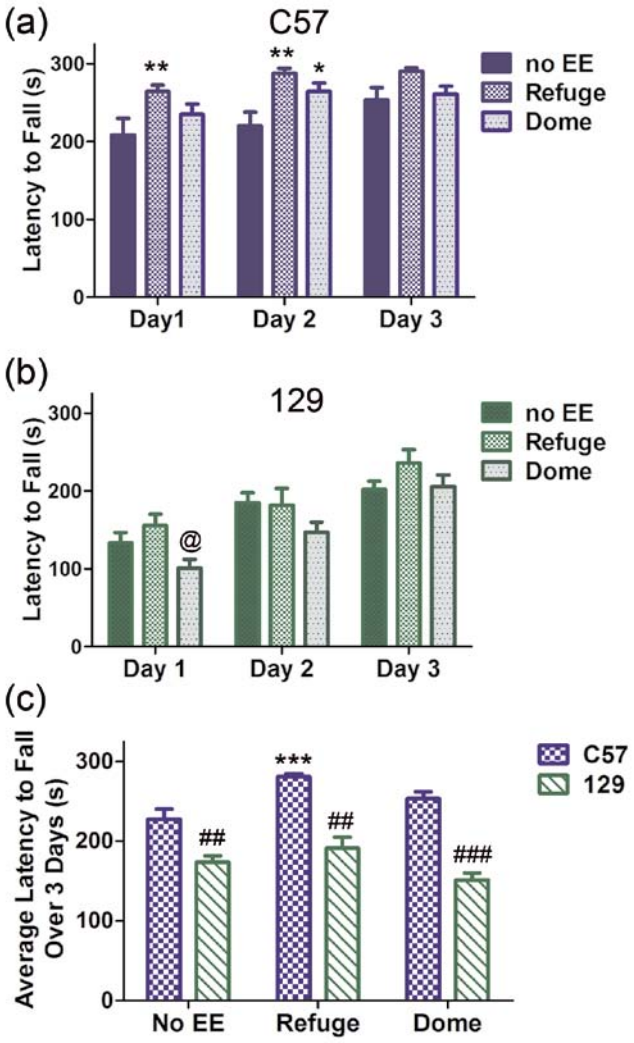

Figure 6. Latency to fall off an accelerating rotarod was measured over 3 days. C57 mice (a) exhibited increased latency to fall off on days 1 and 2 when housed in the presence of shelters (* = $p < 0.05$ and ** = $p < 0.01$ by Bonferroni multiple comparison tests). 129 mice performed this task quite poorly (panels b and c) and Dome shelter housing further impaired baseline performance at day 1 (@ = $p < 0.05$ as compared to Refuge). Analyses of all trials together again supported inferior performance by 129 mice as compared to C57 (## = $p < 0.01$), and also confirmed beneficial effects of Refuge shelters on performance in C57 mice (*** = $p < 0.001$). n = 14-24 mice per group.

these somewhat disparate findings. Several previous studies have described enrichment-induced decreases in anxiety [21,42-46], but it should be noted that enrichment procedures, genetic background and social housing all vary across these studies. We conclude that there are highly complex effects of the shelters on the expression of anxiety that appear to be assay specific and in some cases suggest enrichment-induced increases in anxiety, which has also been reported by at least two other groups [47,48]. Our data thus do not support the conclusion that shelter enrichment uniformly decreases anxiety in laboratory mice. Future work should consider even broader assessments of emotional reactivity, anxiety and anhedonia.

Spatial working memory was assessed using spontaneous alternation in a Y-maze. The lack of training needed and short duration of this task allowed us to employ it both in juvenile and adult cohorts. Somewhat surprisingly, juvenile C57 mice displayed a significantly lower percentage of spontaneous alternations when

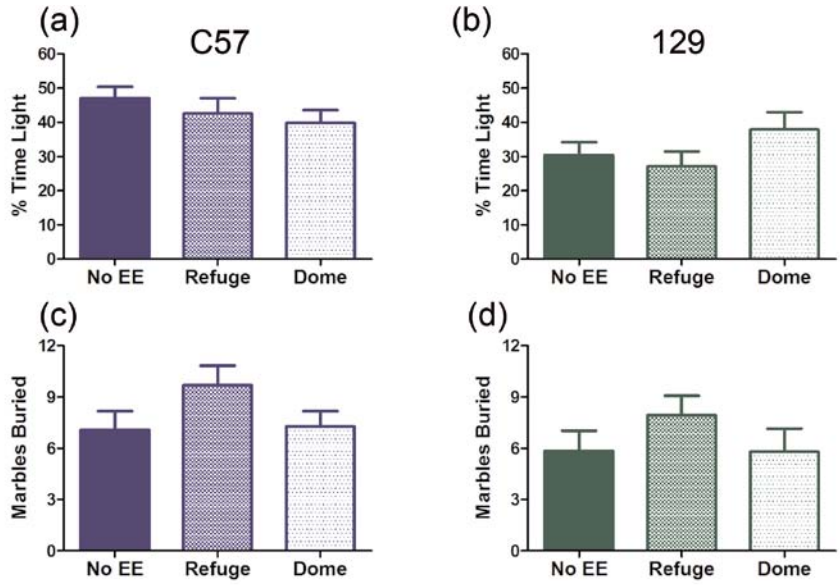

Figure 7. Graphs display behavior of C57 (a, c) and 129 (b, d) mice in a light-dark assay (a, b) and in marble burying (c, d). No significant differences of shelter were observed, although once again a strain difference was evident. n = 14-21 mice per group.

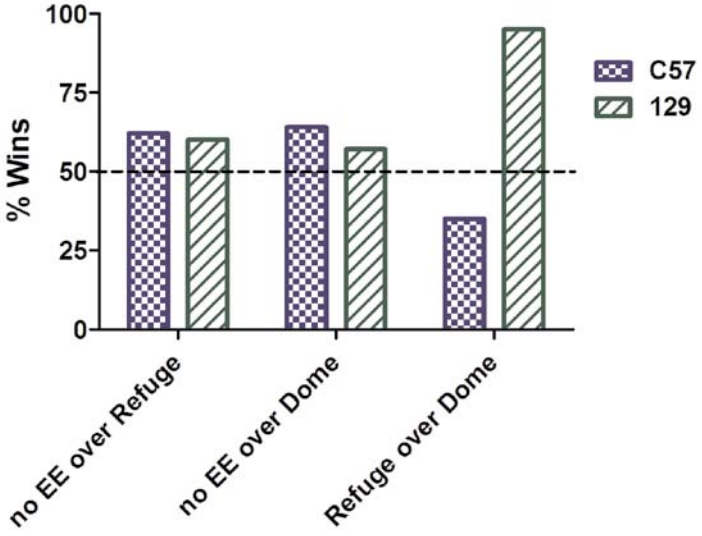

Figure 8. Tube test of dominance indicates that mice housed without shelter enrichment were more dominant than those housed with enrichment ($p < 0.05$). n = 11-14 pairs of mice for each comparison. C57 mice housed with a Dome were more dominant over those with a Refuge, but in 129 mice the Refuge style led to dramatically increased dominance, demonstrating complex interactions between strain and exact enrichment style.

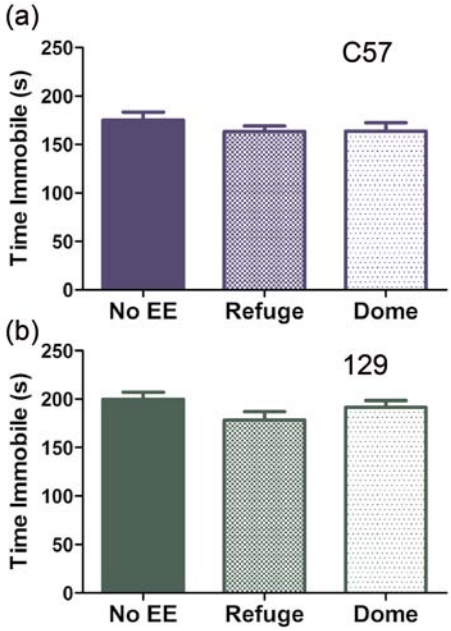

Figure 9. Graphs display immobility of C57 (a) and 129 (b) mice in the forced swim test. No significant differences of shelter were observed, although once again a strain difference was evident, with 129 mice showing greater depressive-like behavior. n = 11-12 mice per group.

reared with environmental shelters, especially the Dome style. Although performance in this task can be affected by a variety of domains [49-51], these data suggest that enrichment shelters may modestly impair spatial working memory in this task. This may be unique to these shelters, because a substantial literature suggesting that complex environments contribute to increased learning and memory [6,16-18, 52]. It is thus likely that these shelters are not complex enough to support cognitive enhancements.

Social dominance assays also produced intriguing and unexpected results, with mice raised with no enrichment shelters being slightly more dominant over mice raised with the shelters (57-64% "wins" as compared to chance 50%). In the C57 strain, mice housed with the Refuges were less dominant than those with Domes (35%), but in 129 Refuge-housed mice were dominant 95% of the time over Domes. A recent study using a much more complex enrichment procedure suggested increases in sociability and decreases in aggression in NMRI mice [53]. Our data are consistent with those findings. Additional work should consider focusing on social interaction and social preference following shelter enrichment, as other studies have found that certain types of environmental enrichment shelters can increase aggression in mice [54-57].

It is worth noting that our experimental design included the shelters throughout the lifetime of the experimental mice – from when their mothers were bred. We chose this procedure because for laboratories working in genetic models, it is likely that their cages always contain shelters, if they are being used at all. However, this does not capture the situation where mice may be acutely ordered in from a commercial vendor, and then housed in an environment quite distinct from earlier in life. Future studies will need to address factors such as whether there is a sensitive period for these types of effects [58,59].

In conclusion, we performed comprehensive neurobehavioral testing on C57 and 129 mice that were socially housed in the presence or absence of two specific styles of environmental shelters. Not surprisingly, we observed large strain differences between C57 and 129 mice [42,60-64]. More remarkably, we observed significant effects of these simple devices on motor behavior and learning. The effects were more prevalent in juvenile mice, but several differences persisted into adulthood, such as a facilitation of motor performance in C57 mice on a rotarod. Many of the shelter effects were specific to one versus the other shelter, despite the fact that the Shepherd "Dome" and Ketchum "Refuge" look very similar to human observers. We recommend that investigators use care when deciding what types of enrichment to include in their studies, to use them consistently within colonies, and to expect genetic strain-dependent outcomes [42,65].

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