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Early Exposure to Dynamic Environments Alters Patterns of Motor Exploration throughout the Lifespan

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Abstract

We assessed early rearing conditions on aging-related changes in mouse behavior. Two isolated-housing groups, Running Wheel (IHRW) and Empty Cage (IHEC), were compared against two enriched environments, Static (EEST) and Dynamic (EEDY), both of which included toys and other mice. For EEDY, the location of toys and sources of food and water changed daily, but remained constant for EEST. All mice, randomly assigned to one of the four groups at ~4 weeks of age, remained in their respective environments for 25 weeks followed by single housing in empty cages. Beginning at ~40 weeks of age, all mice were tested at monthly intervals in a plus-shaped maze in which we measured the number of arm entries and the probability of entering a perpendicular arm. Despite making significantly more arm entries than any other group, IHEC mice also were less likely to turn into the left or right arm, a sign of motor inflexibility. Both EEDY and EEST mice showed enhanced turning relative to IHRW and IHEC groups, but only EEDY mice maintained their turning performance for up to ~100 weeks of age. EEDY and EEST mice also were unique in showing an increase in expression of the major glutamate transporter (GLT1) in striatum, but a decrease in motor cortex, suggesting a need for further assessment of environmental manipulations on long-term changes in forebrain glutamate transmission. Our behavioral results indicate that early exposure to continually changing environments, rather than socialization or exercise alone, results in life-long changes in patterns of motor exploration.

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Competing interests

The authors declare no conflicts.

Authors' contributions

SLH and GVR conceived and designed the experiments. AMES and SJB carried out the experimental work; SLH, AMES, and SJB analyzed the data; SLH and GVR wrote the manuscript with input from AMES and SJB. All authors gave final approval for publication.

Keywords

enriched environment; isolated housing; plus maze; turning behavior; GLT1 expression

1. Introduction

Mobility has been consistently shown to be an important marker of central nervous system function [1]. Declines in spontaneous motor behavior with advancing age have been observed in virtually every species examined, including *C. elegans*, mice, and humans (see [2] for a review). In particular, motor performance decreases as complexity of a given movement increases. In a mouse model of Huntington's disease (HD), an inherited but also an age-related condition [3,4], we found a decreased likelihood of performing a 90° turn into a perpendicular arm of a standard plus maze [5,6]. In healthy animals, moreover, turning was associated with anticipatory electrophysiological signaling in motor cortex and striatum, suggesting that this spontaneous motor response is an indicator of underlying brain function.

The benefits of environmental enrichment on healthy brain aging and motor performance have been widely observed and consistently replicated [7–11]. In fact, whereas aging impairs cortical and hippocampal circuitry [12], environmental enrichment promotes neurogenesis [13,14] and strengthens synaptic connections [7]. Socialization and exercise have long been considered critical components of this effect. But novelty also could be a major contributor. In fact, exposure to novelty increases the release of forebrain dopamine [15], and a dynamic environment, which includes novel experiences, aids in the maintenance of dopamine transmission and leads to healthy brain aging overall [16,17].

Here, we compared the effects of environmental novelty on aging-related turning behavior in mice previously housed in one of four environmental conditions for 25 weeks beginning shortly after weaning. Two groups lived in isolated housing - running wheel (IHRW) and empty cage (IHEC) - and two in enriched environments - static (EEST) and dynamic (EEDY). Both enriched groups included other mice and the same assortment of toys and sources of food and water, but only EEDY mice had these items changed every day, introducing a daily dose of novelty.

Another aspect of environmental enrichment is its ability to create reserve capacity or promote brain plasticity. The hypotheses of brain [18] and cognitive [19] reserves posit that behavioral and lifestyle patterns (e.g., higher educational attainment) in early adulthood play a strong role in healthy aging. Such reserves are built up and maintained against the effects of aging, leading to better brain health and behavioral outcomes in old age. Supporting the reserve hypothesis, rats raised in an enriched environment have been shown to be less vulnerable than standard-housed rats to brain insults that impair cognitive and motor function [20]. The aforementioned study, however, only tested the acute effects of enrichment in middle-aged animals (110 days-old), and did not test whether the reserve could be maintained even when all animals were returned to isolated housing. Thus, we assessed plus-maze turning long after environmental stimulation was withdrawn to determine the persistence of the behavioral change. Presumably, environmental enrichment

early in the lifespan confers a level of plasticity that resists the effects of aging, even after enrichment has been withdrawn. Our experiment tests this hypothesis.

Improved turning performance in HD mice has been linked to increased uptake of glutamate in striatum mediated by increased expression of glutamate transporter 1 (GLT1) [21], the protein responsible for clearing >90% of this excitatory amino acid from striatal extracellular fluid [22]. Glutamate drives key aspects of neuroplasticity, including age- and environment-related changes in synaptic efficacy underlying learning and motor control [16,23–26]. By modulating the temporal profile of glutamate-induced changes in neuronal excitability, synaptic transmission, and other cellular functions [27], GLT1 is a likely major player in the neuroplastic changes underlying environmental enrichment. Interestingly, GLT1 expression declines with age [28,29], suggesting a potential involvement of GLT1 in any persistent aging-related changes in plus-maze turning induced by early exposure to our environmental manipulations. Thus, upon completion of behavioral testing, all groups were examined for GLT1 expression in striatum and in motor cortex, which not only supplies striatum with glutamate but also receives substantial glutamate input.

2. Methods

2.1. Subjects and groups

Male C57BL/6 mice used for this study were bred in the departmental animal colony at Indiana University Bloomington, which allowed unlimited access to food and water on a 12-hr light-dark cycle (lights on at 07:30). All aspects of breeding and housing were approved by the institutional animal care and use committee, and followed National Institutes of Health guidelines. Mice were randomly assigned to one of the following four groups soon after weaning (~4 weeks of age) and remained in their respective environments for the next 25 weeks (see Fig. 1).

IHEC – Isolated Housing: Empty Cage—This group served as the control condition. Mice were housed in isolation in standard clear-plastic cages (27 cm L × 17 cm W × 13 cm H) with a layer of inert bedding material.

IHRW – Isolated Housing: Running Wheel—This group served as the “exercise” condition. Mice were housed in isolation in clear-plastic cages (26 cm L × 20 cm W × 14 cm H) that could accommodate a plasticized running wheel (11 cm diameter) to permit bouts of spontaneous exercise. Wheel turns were monitored by computer to ensure that the wheel was active and each mouse used it on a daily basis.

EEST – Enriched Environment: Static—This group served as the “exercise + socialization” condition. Mice were housed 12 to a cage (91 cm L × 43 cm W × 41 cm H), which was constructed of stainless steel mesh and rested on an oversize galvanized tray filled with inert bedding material. Bedding was changed weekly by lifting the cage and lowering it onto a new tray with fresh bedding to minimize animal handling. The cage was filled with hard plastic and ceramic toys that provided opportunities for exploring, climbing, running, and escaping. The environment was kept “static” by maintaining the position of the toys as well as food and water sources during the entire 25-week period.

EEDY – Enriched Environment: Dynamic—This group served as the “exercise + socialization + novelty” condition. Mice were housed 12 to a cage, which was serviced and provided with toys as described for EEST mice. To introduce novelty, the toys as well as food and water sources were repositioned daily, and new toys were inserted weekly.

After the 25-week experimental housing period, all mice were moved into isolated housing where they remained for the duration of the experiment. Beginning at ~40 weeks of age, mice were tested for plus-maze turning performance at monthly intervals for one year or longer.

2.2. Plus-maze testing

To measure spontaneous motor performance, mice were placed in a standard plus maze for 30 min while their movements were tracked by force-plate actometer (Fig. 2). The force plate detects the position of the mouse within the maze, allowing us to determine whether the mouse is running in a straight line or is making a left or right turn into one of the arms. Over the 30 min testing period, the number of times the mouse traversed the choice point in the center of the maze was recorded as well as the number of times the mouse turned into a perpendicular arm or went straight into the opposite arm. These plus-maze tests were conducted monthly beginning 8–10 weeks after the 25-week experimental housing period had ended and all mice were placed in isolated housing.

2.3. Western blot

In a randomly selected subset of animals that completed behavioral testing (between 89 and 107 weeks of age), the brain was removed after cervical dislocation and striatum (IHEC $n = 5$; IHRW $n = 6$; EEST $n = 8$; EEDY $n = 7$), and primary motor cortex (IHEC $n = 4$; IHRW $n = 6$; EEST $n = 8$; EEDY $n = 8$) from both hemispheres were dissected. Brain tissue was homogenized in 1:10 (w/v) extraction buffer containing Tris-HCl (25 mM) pH 7.5, and protease inhibitor cocktail containing EDTA (final concentration 2 mM; Roche, Mannheim, Germany). Samples were centrifuged at 38,000 rpm at 4 °C during 35 min and pellets were resuspended in 180 μ L of the same buffer. Samples were boiled for 3 min and electrophoresed at 70 V for 2 hr and then transferred to immobilon-P membrane (Millipore, Bradford MA, USA) for 2 hr in Semi-Dry transfer Cell (Bio-Rad). Membranes were incubated in medium containing 5% dry milk in Trizma-base buffer saline/Tween 20 (TBS-T; Trizma Base 0.1 M pH 7.2, NaCl 0.9%, Tween 20 0.1%) for 1 hr. Membranes were subsequently incubated with GLT1 antibody (1:3000, Millipore) for 12 hr at 4°C, and washed (4 times/5 min) in TBS-T and incubated with anti-guinea pig horseradish peroxidase-conjugated antibody (1:10,000 Jackson ImmunoResearch, USA) for 1 hr. Finally, membranes were washed with TBS-T (4 times/5 min) and incubated with chemiluminescence reagent (ECL™ Amersham Biosciences, Little Chalfont, UK) and exposed to Xray film. Actin (1:3000, Millipore) immunoreactivity was used as a loading control, as previously described [30, 31]. Levels of immunoreactivity were quantified densitometrically by an image analyzer (ImageJ). GLT1 densitometry was corrected relative to the actin content and results were then normalized relative to the values obtained in IHEC controls.

2.4. Statistical analysis

To assess the effects of experimental housing on motor behavior, we used a one-way ANOVA with Tukey post-hoc tests for pairwise comparisons. The ANOVA was conducted on plus-maze data, collapsed across age. To examine the effects of aging following the return to isolated housing, we conducted regression analyses on the relationship between age and number of arm choices and between age and probability of plus-maze turning for each group. These analyses allowed us to assess overall movement (arm choices) and directed motor behavior (turning) following the cessation of experimental housing. The probability of turning is calculated as the sum of right and left turns divided by the total number of arm choices. For western blots, bands were normalized to IHEC controls, and the effect of housing was assessed by one-way ANOVA [30,31]. Post-hoc pairwise comparisons were analyzed by Least Significant Difference (LSD) tests.

3. Results

Both enriched conditions were run twice to rule out any unusual effects associated with each random grouping of mice. Comparisons of data from each run within a condition indicated no significant differences. Between 14 and 20 mice from each group (IHEC = 16; IHRW = 14; EEST = 20; EEDY = 14) provided behavioral data, and some mice from each group were randomly selected for assessment of GLT1 expression at the completion of behavioral testing. In both enriched groups and the IHRW group, some mice were removed from the study during the last week of experimental housing due to illness. In all groups, some mice (no more than 2 in each case) were removed because they failed to navigate the plus maze. Across all groups, the mice ranged in age from 89 to 107 weeks at final testing.

3.1. Arm choices

Fig. 3 shows the total number of arm choices made across all weeks of testing. A one-way ANOVA revealed a significant main effect of housing on total arm choices ($F(3,522) = 19.89$; $p < 0.0001$). Tukey post-hoc tests indicated that IHEC mice made significantly more arm choices than any other group, indicating that they had the highest level of locomotor activity in the maze. In contrast, EEDY mice made more than 50% fewer arm choices than IHEC mice, while the IHRW and EEST groups fell between these extremes.

When we assessed performance as a function of age, all groups typically made between 60 and 90 arm choices on the first day of testing at ~40 weeks of age, but as shown in Fig. 4, these numbers declined to fewer than 40 arm choices one year later for all but IHEC mice. Our regression analysis indicated that IHEC performance was remarkably stable at ~70 arm choices per test. There was no sign of habituation across more than one year of evaluation. All other groups showed a steady decline with age. Regression slopes for all but IHEC mice were significantly different from zero ($p < 0.0001$).

3.2. Turning behavior

Although IHEC mice made the most arm choices overall, they were much less likely to turn into a perpendicular arm than either of the enriched groups. One-way ANOVA indicated a significant main effect of housing ($F(3,522) = 13.82$; $p < 0.0001$) on probability of turning

into a perpendicular arm of the plus maze. Tukey post-hoc tests showed that mice from both the EEDY and EEST groups were significantly more likely to turn than their IHEC and IHRW counterparts. These results are summarized in Fig. 5.

In addition, as shown in Fig. 6, regression analysis of the change in probability of plus-maze turning revealed a significant negative nonzero slope value only for the EEST group ($F(1,166) = 4.681$; $p = 0.0319$), which exhibited a decay in probability of plus-maze turning as a function of age. The slope values for the EEDY ($F(1,124) = 0.204$; $p = 0.4966$), IHRW ($F(1,92) = 3.602$; $p = 0.0609$), and IHEC ($F(1,136) = 0.465$; $p = 0.0319$) groups were not significantly different from zero. Slope values were -0.00026 for EEDY, -0.00101 for EEST, -0.00108 for the IHRW, and -0.00037 for IHEC. Thus, both EE groups show more turning across age than the IH groups, but whereas this effect fades in the EEST group, it persists in EEDY mice.

3.3. GLT1 expression

A significant effect of housing on GLT1 expression was observed in striatum ($F(3,21) = 3.52$; $p = 0.033$) and motor cortex ($F(3,22) = 5.39$; $p = 0.006$). As shown in Fig. 7, however, LSD post-hoc analyses revealed that the direction of the effect of housing was region specific. In striatum, GLT1 expression in EEDY mice was significantly higher ($p < 0.05$) than in IHEC and IHRW groups, whereas in motor cortex, GLT1 expression was significantly lower than the isolated groups in both EEST and EEDY mice. Data represent means $\pm$ SEM for each group.

4. Discussion

Our results not only show markedly different patterns of movement between mice reared in isolated and enriched conditions, but the impact of enrichment is evident for a year or more after the enriched environment is removed. In fact, for EEDY mice, an enrichment period comprising roughly 30% of the lifespan produced effects that were maintained for the remaining 70%. Importantly, the persistence of these effects was not evident in EEST mice, indicating that daily exposure to novel stimuli rather than environmental complexity alone plays a key role in maintaining behavioral plasticity.

4.1. Effect of housing on motor behavior

For IHEC mice, movement in the plus maze, as measured by total arm choices, was not only greater than all the other groups, but also relatively constant across the lifespan. Moreover, despite the increase in arm choices, IHEC mice showed proportionally less turning than other mice and this effect accelerated with age. The decline in turning is difficult to explain as an age-related loss of overall motor ability since there was no decline in the number of arm choices across the lifespan. Thus, IHEC mice failed to habituate to the locomotor activating effects of the maze and the IHEC pattern of movement, favoring the opposite rather than a perpendicular arm, became increasingly repetitive or stereotyped. Repetitive behavior is a common outcome of prolonged isolation [32]. Although IHRW mice were not as active in the maze as IHEC mice, there was evidence of habituation as the number of arm choices declined with age and repeated monthly exposure to the plus maze. Arm choices

also declined with age in the EEST and EEDY groups, further supporting a habituation effect. These findings are consistent with evidence of faster behavioral habituation in enriched animals [33].

Turning was enhanced in both enriched groups, but when we evaluated this behavior as a function of time after removal from enrichment, the EEST but not EEDY group exhibited a significant decline, suggesting that the behavioral flexibility associated with enrichment is more persistent when the enrichment includes daily exposure to novelty. Thus, daily changes in environmental stimuli in early life have added benefits across the lifespan beyond that of socialization. Moreover, although the IHRW group started at a higher level than IHEC mice on the first plus-maze test, performance steadily decayed over time. Even with the opportunity for spontaneous exercise, however, IHRW mice did not turn at a higher rate than mice housed in complete isolation. Thus, to the extent that spontaneous turning to explore all four arms of the maze represents a form of behavioral plasticity, these findings are consistent with evidence that exercise alone, without socialization, does not benefit brain health [34,35].

Collectively, our behavioral results indicate two important outcomes. First, even if there is some benefit of exercise alone, the effect is transient with no capacity for motor reserve. Second, novelty in addition to exercise and socialization leads to a behavioral reserve that is maintained throughout the lifespan, even for months after environmental enrichment is withdrawn. It may not be possible to relate IHEC mice directly to specific human conditions since complete isolation without socialization or even exercise would be rare. The IHEC group, however, provides a baseline level of motor performance that allows for a systematic assessment of the time course of behavioral plasticity for groups withdrawn from different environmental manipulations. Because both IHEC and EEDY mice were housed in the same isolated environmental conditions after ~30 weeks of age, any subsequent differences in maze performance are likely due to earlier rearing conditions. In fact, even small changes in enriched environments early in life can have long lasting effects on behavior throughout the lifespan.

4.2. Effect of housing on GLT1 expression

Environmental enrichment, including daily exposure to novelty, did not have uniform effects on GLT1 expression in the corticostriatal system. In EEDY mice, the level of GLT1 protein was increased in striatum, but decreased in motor cortex, while the only change in EEST mice was a decrease in cortical GLT1. Neither of the isolated groups showed a change in corticostriatal GLT1. Thus, the only group that maintained a high probability of plus-maze turning performance into old age was the group with opposing GLT1 changes in cortex and striatum. The reduction in cortical GLT1 in both EEDY and EEST mice suggests a change in glutamate related to a motor plan based on early exposure to a complex environment, whether it changes or not. At least part of this glutamate change may involve a shift in the dynamics of glutamate input from thalamus allowing for an increase in recurrent cortical excitation [36]. But the ability to maintain the motor plan throughout the lifespan, which occurs only in EEDY mice, appears to require a corresponding increase in striatal GLT1. Interestingly, striatal neurons are responsive to novelty [37,38], which fits with the EEDY

manipulation, but difficult to explain without further information on possible changes in other aspects of glutamate transmission including release, receptor sensitivity, and intracellular signaling. It is even possible that increased striatal GLT1 expression may have a neuroprotective effect given that striatum is particularly vulnerable to glutamate excitotoxicity [39] and that glutamate diffusion beyond the synapse is likely to activate neurodegenerative mechanisms [40].

Our results are consistent with previous findings that aging has different effects on glutamate levels in different brain regions [41]. Altered glutamate levels are a reflection of alterations to a complex balance between release and uptake, as well as interactions with other neurotransmitters [41]. Thus, it is likely that a combination of the aforementioned factors interact with environmental conditions, where a change in GLT1 levels is one component of the overall adaptive process. Additionally, the EEDY manipulation is also likely to involve other forebrain circuits. The spatial learning required to adapt to a constantly changing environment is consistent with hippocampal involvement, a phenomenon observed across a variety of different species [42]. Thus, a change in hippocampal GLT1 is possible, and interestingly, elevated glutamate has been reported in hippocampus of 25-month old Wistar rats exposed to environmental enrichment [10].

Glutamate transmission is controlled by multiple mechanisms and confirmation of glutamate involvement in the neuroplastic changes associated with environmental enrichment would require, at least in part, direct measurements of glutamate release and clearance. It also would be useful to measure GLT1 expression immediately after the changes in environmental rearing are withdrawn to assess the extent to which an environmental change in GLT1 expression is maintained or changes across the lifespan. This information would reveal if the behavioral reserve that decays in EEST mice is related to a parallel change in GLT1 expression. Moreover, regional differences in GLT1 expression also may reflect a change in astrocyte number, which would impact communication in multiple neural circuits, an interesting hypothesis that deserves further research.

5. Conclusions

Our results are consistent with the hypothesis that novelty, perhaps via enhanced dopamine transmission, is the mechanism through which environmental enrichment yields its positive effects on the aging brain [17]. The continued introduction of novel stimuli in a dynamic enriched environment could promote prolonged strengthening [43] of dopamine transmission that along with regional changes in GLT1 expression could underlie the motor reserve in EEDY mice. It now will be important to assess how environmental effects alter dopamine and glutamate interactions across the lifespan. Overall, our results suggest that an important factor in the motor benefits of the EEDY group is an opportunity to engage in a varied range of motor patterns within a changing environment. This interaction between motor function and the environment builds up a capacity to continually adapt and reconstruct motor plans.

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Highlights

Early rearing of mice in enriched environments improves motor flexibility later in life.

This effect is most pronounced when the enriched environment changes daily.

Opportunities for exercise or socialization do not have the same long-term impact.

A dynamic environment produces region-specific changes in forebrain GLT1 expression.

Figure 1. Diagrammatic summary of the experimental conditions and timeline
See text for details.

Figure 2. Dimensions of the plus maze (left panel) and placement of the force plate actometer (right panel) used to track motor behavior of the animals
See text for details.

Figure 3. Effect of housing on the number of arm choices made in the plus maze
Mean (±SEM) number of arm choices made by each group during each monthly plus-maze session beginning at ~40 weeks of age. *p<0.05, ** p<0.01; *** p<0.001.

Figure 4. Total arm choices made by each environmental group as a function of age
 Individual dots represent the mean ($\pm$ SEM) response of each group at each time point indicated (see legend). Linear regression lines are plotted for each group. All regression slopes are significantly different from zero ($p < 0.05$) except for the IHEC regression slope

Figure 5. Effect of housing on the probability of turning in the plus maze

Mean ($\pm$ SEM) probability of an animal making a 90-degree turn during each monthly plus-maze session beginning at ~40 weeks of age. ** $p < 0.01$; *** $p < 0.001$.

Figure 6. Probability of turning within the plus maze as a function of age and housing group
 Individual dots represent the mean ($\pm$ SEM) response of each group at each time point indicated (see legend). Linear regression lines map probability of turning as a function of age. Only the slope of the regression for the EEST group is significantly different from zero ($p < 0.05$).

Figure 7. Protein levels of GLT1 in striatum and motor cortex of IHEC, IHRW, EEST, and EEDY mice

Top: representative western blots showing content of GLT1 and actin, which was used as a loading control. Bottom: bars show the ratio GLT1/actin as a percent optical density of the IHEC control group. Data are presented as mean $\pm$ SEM for 3–8 animals per group. *denotes significant difference ($p < 0.05$) from IHEC; @ denotes significant difference ($p < 0.05$) from IHRW; † denotes significant difference ($p < 0.05$) from EEST.