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THE EFFECTS OF ENVIRONMENTAL ENRICHMENT ON SOCIAL BEHAVIORS IN ISOLATED MICE

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Mice are considered highly social animals, and thus changes in their social environment can have significant impacts to brain structure and function [5]. Zelikowsky et al. (2019) has shown that social isolation stress (SIS) impacts several aspects of the mouse brain state and social behavior (9). This can lead to trends of increased anxiety, social avoidance, stereotypy and other abnormal behaviors [8]. Environmental Enrichment (EE) is defined as providing items that promote motor and sensory stimulation within an animal's housing conditions [1]. In studies conducted on mice in enriched environments, abnormal behaviors are significantly decreased compared to non-enriched mice [7]. To investigate the effects of enrichment and the ability to mitigate effects of isolation, an elevated zero maze, open field test, and resident intruder assay were run on mice separated into group house or isolation conditions, and further separated into enriched and non-enriched groups. Generally, no statistical significance ($p < 0.05$) was found in all three tests. However, mild trends in behavior in the resident intruder assay were found for grooming, self-grooming cornering, and being chased, having potential implications on the usage of environmental enrichment for animal welfare purposes.

Introduction

Mice are the most widely used animal for scientific research, however, much of the information about how housing conditions impact social behavior is relatively unknown [1]. Housing conditions for research animals are designed primarily for economic and user benefits, meaning that mice housing is designed to be as cost effective and moveable as possible [1]. These housing conditions tend to lack outlets for sensory and motor stimulation and previous research indicates mice in such housing conditions tend to display stereotypy, social avoidance, and other abnormal behaviors associated with elevated levels of anxiety [1]. While mice housed in enriched environments tend to display higher levels of aggression than non-enriched mice, levels of play behavior increase as well among the enriched mice [6].

There are many neurological differences that have been recognized between enriched mice and standard housing mice. One of the main differences seen in enriched mice is increases in dendritic spines, synaptic connections, and neural cell size [7]. Enriched mice also have been found to have differences in gene expression, specifically with the regulation of "neuronal structure, synaptic signaling, and brain plasticity" [7]. With the combination of differences in neural structure and gene expression, enriched mice exhibit increased abilities of learning, memory, and the upregulation of proteins in comparison to nonenriched mice [7]. This effect may be due to the decrease in stereotypy and anxious behaviors typically seen in standard housing mice.

There is debate that environmental enrichment may add confounding variables rather than remedy them. Other studies suggest that enrichment should only be used in a way that provides beneficial impacts on animal welfare as opposed to simply putting any object in a cage and referring to these objects as enrichment [1]. Several laboratories have assessed the impact of enrichment on many behaviors in different strains of mice and found that group variability contributed to behavioral variations while enrichment had no effect [7]. Thus, it appears that enrichment provides an optimal experimental environment. Therefore, many labs are now using nesting materials, shelters, mouse wheels, and other forms of enrichment to help provide a more natural environment that reduces the possibility of confounding effects while promoting beneficial practices [1]. These practices include the idea that enrichment promotes intrinsic, species-specific behaviors, which could be significant in making inferences concerning normal and abnormal animal behavior.

Methods

A total of 32 mice were separated equally into either group housing (4 mice per cage) or socially isolated housing (1 mouse per cage). From here, the mice in each condition were further separated into standard or enriched housing. Behaviors were recorded during three tests: open field test, elevated zero maze, and lastly, resident intruder. Once data from the final test

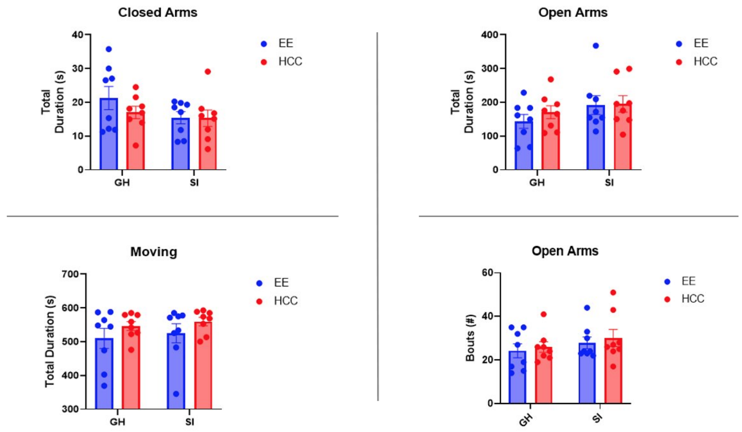


Figure 1 *depicts data from the elevated zero maze, on the environmental enrichment (EE) and home cage control (HCC) groups, which have been further separated into group house (GH) and social isolation (SI) conditions. Total duration in the closed arms, open arms, and total movement was documented.*

(residential intruder) was collected, two mice from each group (8 total) underwent perfusions, and brains were extracted and cryoprotected. Prosocial and avoidance behaviors from the three tests were then scored. Due to available scoring software, the open field test was automatically scored. Due to limitations of the software, the resident intruder behavior and elevated zero maze were hand scored. All scoring consists of assessing behaviors that fall in line with well-defined prosocial and social avoidance behaviors in the literature. Statistical analysis was then conducted using a two-way ANOVA test.

Elevated Zero Maze

For the elevated zero maze, time

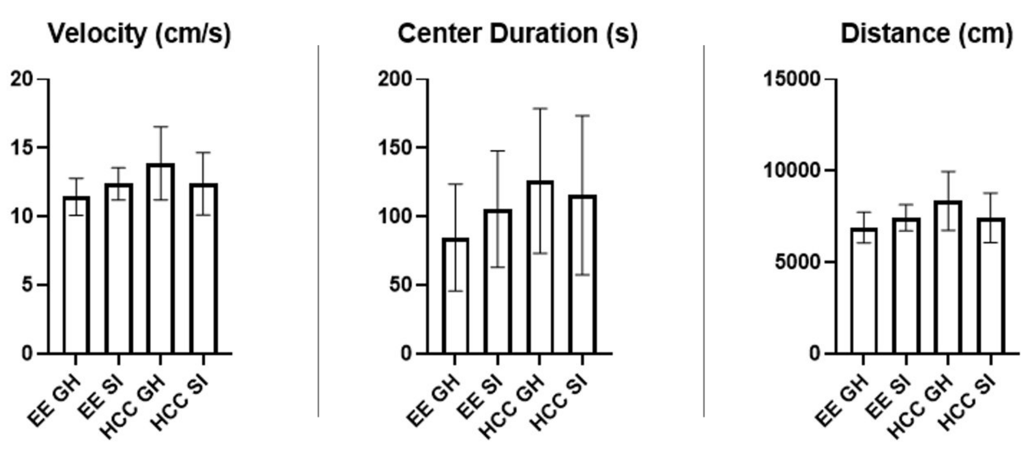


Figure 2 *depicts data captured from the open field test on the environmental enrichment (EE) and home cage control (HCC) groups, which have been further separated into group house (GH) and social isolation (SI) conditions. Velocity (cm/s), central duration (s), and distance (cm) are measured.*

spent in the closed arms, time spent in the open arms portion, and total duration of movement were measured using hand scoring methods. Time spent in the open and closed arms was based on mouse visual perspective, predominant vision of the open arm was counted for open arm duration and predominant vision of the closed arm was scored as closed arm duration.

Open Field Test

For the open field test, velocity in cm/s, duration spent in the center of the field, and distance traveled in cm was recorded. The open field test was scored using a tracking software to detect motion, speed, and location of the mouse throughout the duration of the test. The automated software was verified for validity and reliability.

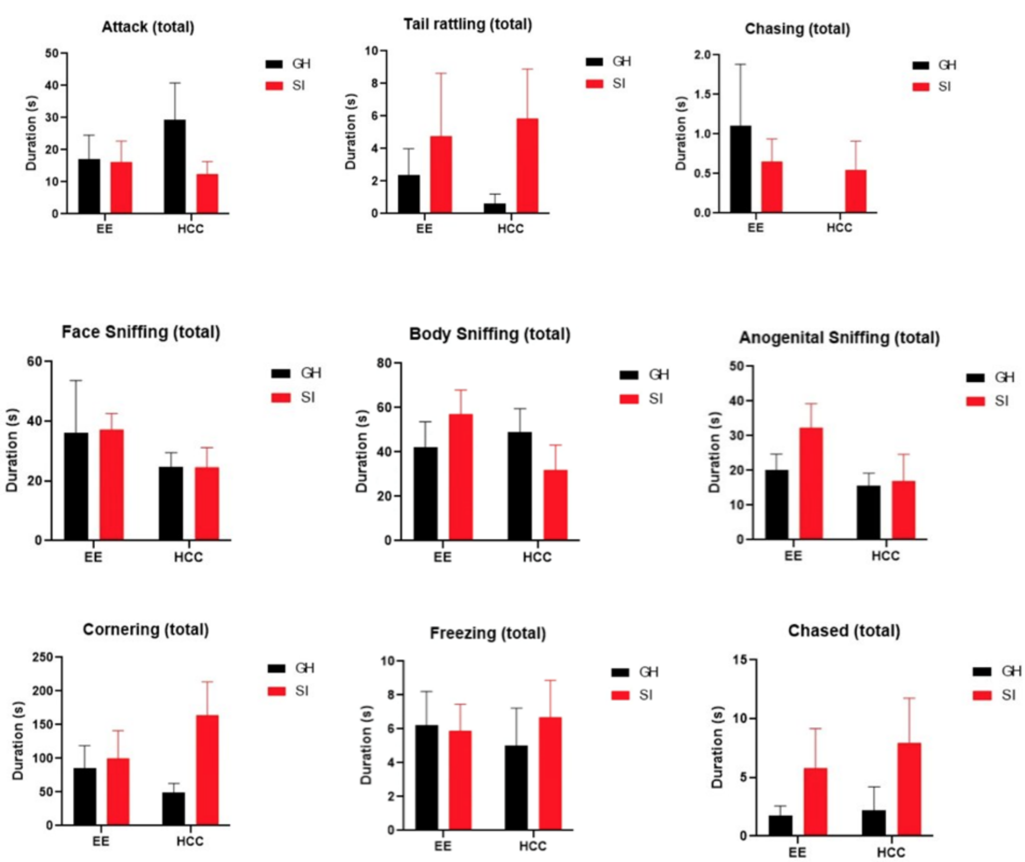
Resident Intruder Assay

For the resident intruder assay, mice were paired with an intruder mouse in their home cage. Prosocial behaviors (face investigation, body investigation, anogenital investigation, grooming, and self-grooming), avoidance behaviors (attack, tail rattling, and chasing), fear behaviors (cornering, freezing, and chased), and miscellaneous behaviors (digging and sideways threat) were documented using hand scoring technique

Results

Elevated Zero Maze

No statistical significance was found for total duration in closed arms, open arms, and total movement.



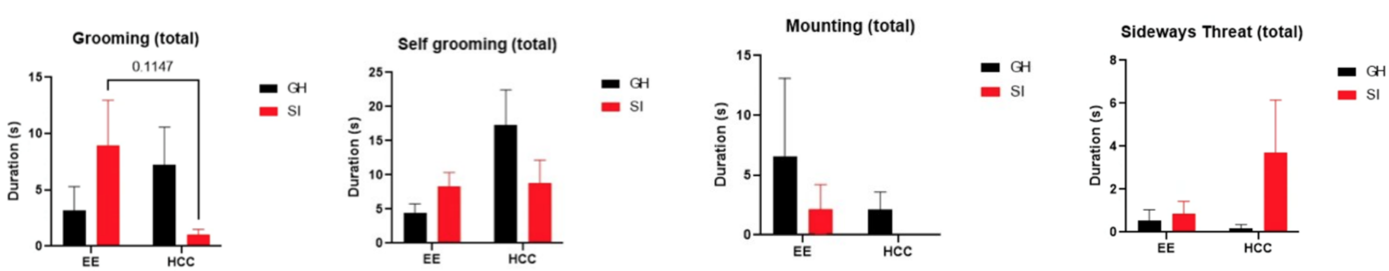


Figure 3 depicts behavior scored during the resident intruder assay on the environmental enrichment (EE) and home cage control (HCC) groups, which have been further separated into group house (GH) and social isolation (SI) conditions. The top row describes avoidance behaviors, which were defined as attacks initiated by the home cage mouse, tail rattling, and chasing initiated by the home cage mouse. The second row describes prosocial investigatory behaviors, which were defined as facial sniffing, body sniffing, and anogenital sniffing. The third row describes fear behaviors, which were described as cornering, freezing behaviors, and the home cage mouse initiating being chased. Lastly, the fourth row describes prosocial grooming behaviors including grooming of the intruder mouse by the home cage mouse and self-grooming. Miscellaneous behaviors are also included on the fourth row, which includes mounting and sideways threat. These behaviors were considered miscellaneous since no clear research establishes the likely reasoning behind these behaviors in mice.

Open Field Test

No statistical significance was found for velocity, center duration or distance.

Resident Intruder Assay

In general, no statistical significance was found for all behaviors scored. However, weak trends in behavior were found. Due to the nature of the two-way ANOVA test, sources including enrichment, housing, and an interaction of the two were analyzed. For housing (group house vs isolated housing), a p-value of 0.0866 was found for chased and a p-value of 0.0883 was found for cornering. For a combined interaction of housing and enrichment, a p-value of 0.0400 for grooming and a p-value of 0.0699 was found for self-grooming. For the enrichment (enriched vs nonenriched), a p-value of 0.0699 was found for self-grooming. The only statistically significant result was grooming from the interacting source.

Discussion

Since behavior was generally not statistically significant ($p < 0.05$), this study plays a role in the discussion on whether environmental enrichment should be included for animal welfare purposes. Because of what was found in the resident intruder assay, potential trends in self-grooming with enrichment could potentially signify what constitutes normal mouse behavior specific to the species.

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