

# Environmental Enrichment Reduces the Likelihood of Alopecia in Adult C57BL/6J Mice

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**Barbering (incessant grooming) is an abnormal behavior causing alopecia and commonly affects various strains of laboratory mice, including C57BL/6J. Barbering-induced alopecia is a potential symptom of brain impairment and can indicate a stressful environment. We compared alopecia prevalence and severity in mice housed in enriched or standard cages. Providing an enriched environment delayed the onset and reduced the prevalence and overall severity of alopecia in C57BL/6J mice. Husbandry methods that reduce adult alopecia are likely to promote the wellbeing of the animals. We suggest that environmental enrichment is a simple and economic way to reduce alopecia in mouse colonies.**

**Abbreviations:** EE, environmental enrichment; PND, postnatal day.

Enriching environments for laboratory rodents can influence CNS development and forebrain function<sup>13,21</sup> and improve welfare.<sup>22,25,26</sup> Environmental enrichment (EE) comes in many forms (for example, toys, tunnels, nesting material, larger cages, social), and generally includes anything that is preferred (not avoided) by captive animals and increases species-specific behavior or decreases abnormal behavior.<sup>17,23</sup> Research shows numerous benefits of enriched environments, including enhanced cognitive abilities,<sup>7,24</sup> reduced abnormal behavior,<sup>15</sup> increased resistance to stressors, and reduced pathogenesis and progression of disease.<sup>12</sup> A recent study reports that EE can lead to greater external validity of results as compared with standard housing.<sup>20</sup> In addition, standard captive-housing conditions (for example, housing laboratory mice in small, single-sex cages of low complexity) can induce behavioral frustration, leading to chronic stress,<sup>22,26</sup> whereas enriched environments can reduce stress.<sup>3,23</sup>

Excessive hair-pulling is an abnormal behavior that occurs in a range of species (for example, humans, primates, mice, and dogs), particularly in those subjects confined to captivity.<sup>19</sup> In some laboratory strains, such as C57BL/6J, excessive hair-pulling is thought to cause alopecia (hair loss), appearing as asymmetrical patches primarily on the dorsum; whisker trimming is common also, and together are termed 'barbering'.<sup>4,5,14</sup> For mice, whiskers are an important source of sensory information,<sup>11</sup> making their loss a welfare concern and a potential source of behavioral variation in research data.

Barbering may have additional welfare implications: originally thought to be a form of dominance behavior,<sup>14,16</sup> with the remaining sole untrimmed mouse presumed 'guilty' and dominant, recent research suggests that barbering is an abnormal behavior that models the human hair-plucking disorder trichotillomania.<sup>4,5,10</sup> Humans with this disorder show signs of clinical distress,<sup>19</sup> and they increase their compulsive hair-pulling behavior in stressful environments.<sup>5</sup> Similarly, stressful conditions can promote barbering in laboratory mice.<sup>8</sup> Various husbandry factors associated with reduced stress (such as par-

ticular cage designs<sup>5</sup> and delaying weaning ages<sup>6</sup>) are reported anecdotally to reduce barbering-induced alopecia. Providing various toys such as cat or bird toys, balls, climbing structures and replacing them every 2 wk has previously been reported to reduce alopecia in laboratory mice.<sup>2</sup> However, that study did not indicate the strain(s) of the mice used, mice were housed in large groups ( $n = 10$ ), and statistical analyses were not performed.

We evaluated alopecia in mice housed in enriched and nonenriched environments to investigate the effects of EE on barbering in C57BL/6J mice, one of the most commonly used and frequently affected laboratory strains. Because barbering increases with age,<sup>5</sup> we assessed mice at 4 and 6 mo, to address effects of EE on both onset and progression of alopecia.

## Materials and Methods

**Animals.** C57BL/6J mice ( $n = 108$ ; female, 51; male, 57) were bred at the University of Guelph Central Animal Facility in a single temperature ( $20 \pm 1^\circ\text{C}$ ) and humidity (50% to 60%) controlled room maintained under a 12:12-h diurnal cycle, with dry food pellets (2014 Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Laboratories, Mississauga, Canada) and water (nonsterilized) available ad libitum. These mice were generated for a natural weaning experiment;<sup>1</sup> dams ( $n = 17$ ) and their litters were kept in open-topped standard shoebox cages (Ancare, Bellmore, NY: 13 cm high  $\times$  28 cm long  $\times$  19 cm wide) that always contained corncob bedding, a cotton nesting pad (Ancare), and a red shelter (catalog no. K3327, Mouse Igloo, Bio-Serv, Frenchtown, NJ) until postnatal day (PND) 14.<sup>21</sup> Throughout the experiment, all cages, bedding, food, nesting material, and water were replaced weekly. At PND 14, dams and their litters were moved to a new housing system comprising standard shoebox (S,  $n = 9$ ) or duplex (D,  $n = 8$ ; Thoren Caging Systems, Hazleton, PA; 14 cm high  $\times$  30 cm long  $\times$  14 cm wide) cages. To allocate litters between cage type, litters were classified as small ( $n \leq 6$ ), medium ( $n = 7$  or 8), or large ( $n \geq 9$ ), and cage type counterbalanced by litter size.<sup>1,9</sup> From PND 14 to 35, all offspring had unlimited access to an additional cage, identical in every way to the home cage, by means of a tunnel (PVC tubing; 91.4 cm  $\times$  5 cm) with a diameter too narrow to permit the dam to pass.<sup>1</sup> Offspring were separated permanently (weaned) from their dams at PND 35 and housed with same-sex littermates ( $n$

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= 2 to 3 per cage) in enriched ( $n = 9$ ) or standard ( $n = 8$ ) cages, counterbalanced by early (PND 14 to 35) cage type.

Enriched cages were larger (15 cm high  $\times$  42 cm long  $\times$  23 cm wide) and always contained a shelter and another item (a 9-cm PVC tunnel, an 8-oz plastic container, or a 9-cm nylon dog bone). These items were systematically rotated biweekly, such that each cage experienced every type of enrichment item. Enrichment objects were passed through the cagewasher prior to use. In addition, one of two types of nesting material (tissue [20 cm  $\times$  27 cm] or shredded paper towel [17 cm  $\times$  26 cm]) was provided in alternation, together with a nesting pad. These enrichment devices are used commonly in mouse colony management and were expected to increase cage complexity, thus providing hiding and escape places and additional substrates for gnawing. Standard cages contained only a nesting pad and a shelter. All animal procedures were approved by the University of Guelph Animal Care Committee.

**Alopecia scoring.** Alopecia was scored visually at 4 and 6 mo. Hair loss always appeared in the characteristic asymmetrical patches associated with barbering and was unaccompanied by other changes to the skin (for example, redness, scratches, scabbing). The prevalence, or proportion of mice affected by alopecia, was assessed by using a present (1) or absent (0) scoring system and then calculated as a proportion of the cage affected. In addition, mice were evaluated individually according to a 0 to 3 scoring system (Figures 1 and 2) to assess alopecia severity in affected mice and overall severity (includes all mice, even those unaffected by alopecia). For example, a mouse with a severity score of 2 whose cagemate had a score of 0 together had a mean overall severity score of 1.

**Statistical analyses.** Cage scores were averaged by sex and dam (the independent unit of replication), and then, due to the categorical nature of the data, were compared by using nonparametric (Kruskal–Wallis; Minitab Statistical Software, Minitab, State College, PA) tests. A Kruskal–Wallis test was used to determine whether differences in caging during PND 14 to 35 influenced alopecia in adults. Effects of enrichment on the prevalence, severity (at 6 mo only, because none of the enriched mice were affected at 4 mo), and overall severity of alopecia were analyzed. Because our a priori prediction was that EE would reduce alopecia prevalence and severity, these tests were one-tailed. Statistical significance was set at a  $P$ -value of less than 0.05.

## Results

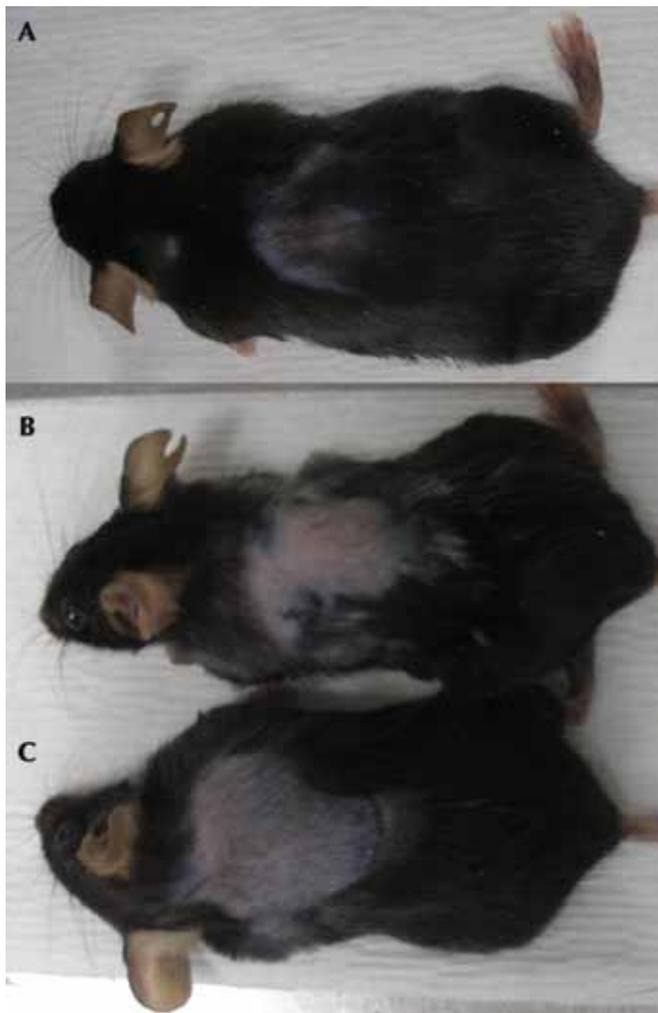
Adult alopecia did not differ between the sexes at 4 mo (Kruskal–Wallis; prevalence:  $P = 1.0$ ,  $H_{1,32} = 0.0$ ; severity:  $P = 0.10$ ,  $H_{1,4} = 2.67$ ; overall severity:  $P = 0.98$ ,  $H_{1,32} = 0.00$ ) or 6 mo (prevalence:  $P = 0.70$ ,  $H_{1,32} = 0.15$ ; severity:  $P = 0.80$ ,  $H_{1,9} = 0.06$ ; overall severity:  $P = 0.70$ ,  $H_{1,32} = 0.18$ ). Therefore, data then were averaged by dam to represent the statistical independent unit of replication.

Mice experienced 2 different cage types (shoebox and duplex) from PND 14 until weaning at PND 35; however, this early housing difference did not influence later levels of alopecia (Kruskal–Wallis; 4 mo: prevalence:  $P = 0.56$ ,  $H_{1,17} = 0.34$ ; severity:  $P = 0.22$ ,  $H_{1,3} = 1.5$ ; overall severity:  $P = 0.61$ ,  $H_{1,17} = 0.26$ ; 6 mo: prevalence:  $P = 0.91$ ,  $H_{1,17} = 0.01$ ; severity:  $P = 0.88$ ,  $H_{1,8} = 0.02$ ; overall severity:  $P = 0.75$ ,  $H_{1,17} = 0.10$ ).

Mice showed less alopecia when maintained under enriched conditions after weaning. At 4 mo, no alopecia was visible in enriched mice; however, one-third of families in standard conditions ( $n = 3$ , or 43%) were already affected and showed alopecia in greater prevalence and overall severity (Figure 3).

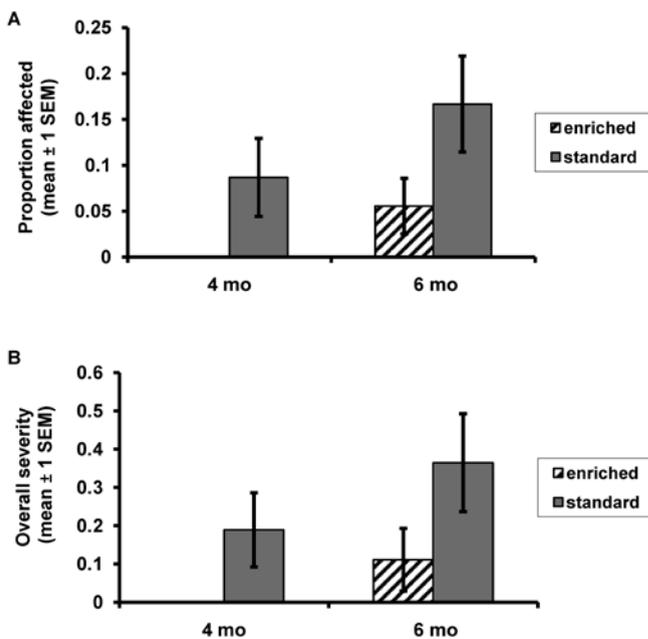
Score	% Alopecia	Whiskers absent?
0	0	no
1	0	yes
1	$0 \leq 10$	no
2	$0 \leq 10$	yes
2	$10 \leq 20$	no
3	$10 \leq 20$	yes
3	$20 \leq 100$	no

**Figure 1.** Alopecia scoring by visual assessment is based on the estimated percentage of area of hair loss relative to body surface area, in conjunction with whisker loss.



**Figure 2.** Examples of barbering-induced alopecia in B6 mice and the categorical score assigned based on visual assessment. All mice pictured have intact whiskers. (A) Alopecia score of 1. (B) Alopecia score of 2. (C) Alopecia score of 3.

Approximately 2 mo later, 38% of the enriched mice ( $n = 3$  litters) were affected by alopecia, compared with 63% of the mice ( $n = 5$  litters) in standard conditions. Although enrichment did not alter the severity of alopecia in affected mice at 6 mo ( $P = 0.53$ ,  $H_{1,8} = 0.38$ ), the prevalence and overall severity of alopecia was lower ( $P < 0.05$ ) in mice housed in enriched compared with standard conditions (Figure 3).



**Figure 3.** Alopecia in mice housed in enriched ( $n = 9$ ) and standard ( $n = 8$ ) conditions. (A) Alopecia was less prevalent in enriched mice at 4 mo (Kruskal–Wallis:  $P = 0.025$ ,  $H_{1,17} = 3.83$ ) and showed a trend toward less prevalent at 6 mo ( $P = 0.053$ ,  $H_{1,17} = 2.6$ ). (B) The overall severity of alopecia was significantly lower in enriched mice at 4 ( $P = 0.025$ ,  $H_{1,17} = 3.81$ ) and 6 ( $P = 0.047$ ,  $H_{1,17} = 2.8$ ) mo.

## Discussion

We examined the hypothesized effects of EE on alopecia in C57BL/6J mice. As predicted, providing EE after weaning reduced the likelihood and slowed the progression of adult alopecia in C57BL/6J mice. The first assessment at 4 mo revealed no alopecia in enriched mice (0%), whereas significantly more standard-housed families (43%) were affected at this age. Mice were reassessed 2 mo later: regardless of housing condition, alopecia increased with age (enriched, 38%; standard, 63%) but was more severe overall and tended to be more prevalent in standard-housed families. Differences in the severity of alopecia were not observed. However, these results are based on only a few families, increasing the likelihood of a type II error. Overall, our findings were consistent with a previous nonpeer-reviewed report<sup>2</sup> of reduced alopecia in mice housed in enriched compared with nonenriched cages.

The observed reduction in alopecia in enriched mice may stem from more normal CNS functioning,<sup>4,5</sup> living in a less stressful environment,<sup>8</sup> or increased subject ability to resist stress, thereby reducing stress-induced hair growth inhibition.<sup>18</sup> The current study does not attempt to elucidate the mechanisms by which EE ameliorates alopecia. However, people with trichotillomania report the behavior to manifest in the context of depression, frustration, boredom, and anxiety.<sup>19</sup> Therefore, if barbering closely models trichotillomania, current results may stem from enriched mice living in a more stimulating, less stressful environment.

Alopecia in laboratory mice is a persistent problem. Our enrichment program did not eliminate alopecia. In addition, the standard early housing conditions (PND 14 to 35) and delayed weaning age (PND 35) did not affect adult alopecia, as the incidence of alopecia in standard-caged families (63%) was comparable to that previously reported (60%).<sup>2</sup> Mice weaned

from standard housing into standard cages may have experienced greater motivation to barber.<sup>8</sup>

We predicted that EE would reduce the likelihood of adult alopecia in laboratory mice, and indeed, we found less alopecia in mice housed with EE. However, our study was small and unblinded and has not been replicated. In addition, our study did not control for stocking density (cage area per mouse) across treatments. Although at least one previous study found that stocking density had no effect on barbering,<sup>5</sup> this factor may be important to consider in future work. Furthermore, future studies might elucidate the mechanism by which EE leads to a reduction in alopecia, and behavioral observations correlating barbering with alopecia would provide insight into whether alopecia progression is due to increased barbering or inhibition of hair growth.

C57BL/6J laboratory mice housed in enriched conditions experienced a delayed onset and overall reduction in adult alopecia when compared with mice housed under standard conditions. Alopecia may influence the suitability of subjects for research. Furthermore, the reduction in alopecia may indicate a less stressful environment. Therefore, husbandry methods that reduce adult alopecia are likely to promote research validity and animal wellbeing. We suggest that EE is a simple and economic way to reduce alopecia in mouse colonies.

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