

1 **Environmental enrichment delays the development of stereotypic behavior and reduces**
2 **variability in behavioral experiments using California mice (*Peromyscus californicus*).**

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16

17 **Abstract**

18 Domesticated mice and rats have shown to be powerful model systems for biomedical research,
19 but there are cases in which the biology of species is a poor match for the hypotheses under
20 study. The California mouse (*Peromyscus californicus*) has unique physiological and behavioral
21 traits and has emerged as a powerful model for studying sex differences in the biology of
22 psychiatric disease, which is particularly relevant considering the new NIH guidelines that
23 require the inclusion of sex as a biological variable. Despite its growing role in preclinical
24 research, there is a lack of studies assessing species-specific housing needs, which presents a
25 challenge for research facilities seeking to ensure good welfare and obtaining high-quality
26 experimental data. Indeed, captive California mice present a high prevalence of stereotypic
27 backflipping behavior, a common consequence of suboptimal housing and a potential source of
28 experimental outcome variability. Using three different cage systems, the present studies show
29 that increasing housing space as well as social and environmental complexity can delay the
30 development of stereotypic behavior in male and female California mice. Critically, this reduction
31 in stereotypy is accompanied by increased effect sizes of stress in an established model for
32 social anxiety. These results suggest that increased cage size and enrichment could enhance
33 welfare in California mice while simultaneously increasing the quality of behavioral experiments.

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57 **Introduction**

58 The California mouse (*Peromyscus californicus*) possesses unique characteristics that
59 make it a powerful model species for testing hypotheses that are difficult or impossible to test
60 with standard rodent lines. First, California mice are monogamous, biparental, and both males
61 and females show aggression towards intruders of both sexes (Ribble and Salvioni, 1990;
62 Rieger et al., 2019). Male parental behavior and female aggression are present in humans but
63 are very rare in common laboratory rats and mice (Kleiman, 1977; Kleiman and Malcolm, 1981),
64 making the California mouse a great model to study the sex-specific mechanisms underlying
65 social behaviors relevant to human behavior. This is particularly important considering that the
66 new NIH guidelines require consideration of sex as a biological variable in research. Second,
67 California mice are prone to developing insulin resistance and hyperlipidemia independent of
68 obesity, which makes this species an ideal model for the study of the early stages of metabolic
69 syndrome (Krugner-Higby et al., 2011, 2006, 2000), one of the major health hazards of the
70 modern world (Saklayen, 2018). Together, this suggests that the use of the California mice as a
71 preclinical model will increase in the coming years, as it has been for the past decade (source:
72 Web of Science).

73 While the unique physiology and behavior of California mice provide great opportunities
74 for research, they can also present unique challenges for husbandry. Although housing needs
75 can greatly vary among rodent species (Baumans, 2005), there are no species-specific
76 guidelines for California mice (National Research Council (US) Committee for the Update of the
77 Guide for the Care and Use of Laboratory Animals, 2011), which can result in suboptimal
78 housing conditions for this species. Indeed, California mice colonies can present a high
79 prevalence of stereotypic back-flipping (Greenberg et al., 2014), a common consequence of
80 suboptimal housing (Garner, 2005; Gross et al., 2012), and a potential indicator of poor welfare
81 (Mason and Latham, 2004). Importantly, while most individuals in California mouse colonies
82 display this stereotypic behavior at some point, there is great inter-individual variability in the
83 frequency at which this behavior is performed. Stereotypic back-flipping has been reported in
84 multiple species (Mason and Latham, 2004), and it is a problem because stereotypic behavior
85 can be accompanied by altered physiology (McBride and Parker, 2015), cognition (Garner and
86 Mason, 2002), and affective state (Novak et al., 2016). increasing the variability of experimental
87 outcomes (Garner, 2005). We were therefore highly motivated to find housing alternatives that
88 could minimize stereotypic behaviors. With this aim, here we tested three different housing
89 conditions on backflipping behavior, the social interaction test, and welfare indicators in male
90 and female California mice. The housing conditions were a) standard, b) larger cages and

91 increased social complexity (large), and c) larger cages, increased social complexity, and
92 environmental enrichment (large+EE). We designed the enrichment program based on
93 observations of California mice in the wild, who build complex nests in a variety of contexts
94 including tree cavities, leaf litters, and creek banks, and spend a significant amount of the day
95 climbing (Dalquest, 1974; Gubernick and Alberts, 1987). Based on findings in other species
96 (Bayne, 2018; Bayne and Würbel, 2014; Bechard et al., 2016; Shyne, 2006), we hypothesized
97 that large+EE would be the most effective at reducing stereotypic behavior and that this would
98 in turn help reduce variability in the social interaction test.

99

100 **Materials and Methods**

101

102 Animals and housing: All mice were bred in our colony (University of California, Davis,
103 Department of Psychology). At weaning (post-natal day 30, P30) animals were ear punched for
104 identification and assigned to one of three conditions (fig.1A,B): 1. standard (15cm x25cm
105 x12cm cage, 2 same-sex individuals), 2. Large (10.5in x19in x6in cage, 4 same-sex individuals),
106 or 3. large+EE (4 same-sex individuals). All cages were made of clear polypropylene and all
107 treatment groups were provided with Sani-chip bedding, cotton nestlets, and enviro-dri (Newco
108 Distributors). The enrichment group was additionally provided with a crawl ball™ (Bio-Serv®), a
109 stainless-steel loft with holes (Otto environmental®), and a 4x6inch stainless-steel tube (Otto
110 environmental®). For all animals, water and food (Harlan Teklad 2016; Madison, WI) were
111 provided ad libitum. All treatment groups were housed in the same room under a 16L:8D
112 light:dark cycle. The room temperature was kept at (20-23°C). All behavior experiments were
113 carried out during the dark cycle (14:00-17:00), the time at which California mice are most
114 active. Experimenters used red light (3 lux) headlamps to help with experimenter vision but
115 minimize potential effects of light exposure on the mice circadian rhythm (Hattar et al., 2003;
116 Provencio and Foster, 1995; Yoshimura and Ebihara, 1996).

117

118 In-cage behavior: Individual in-cage behavior was assessed using one 5 minute observation at
119 3 developmental stages: P30, P50, and P90 (fig. 2A). Every 15 seconds, the presence/absence
120 of back-flipping was recorded. No other stereotypic-like behaviors were observed. In the
121 large+EE group, the use of enrichment was also recorded.

122

123 Individual welfare status assessment: For a subset of the animals, individual welfare status was
124 assessed every 7 days since weaning (P30) until one week before behavioral assessments

125 started (P86) using a protocol adapted from Spangenberg and Keeling (Elin MF Spangenberg
126 and Linda J Keeling, 2016). Briefly, weight, body condition (scale 1-5, table 1), presence of
127 injuries, coat condition, and response to handling (urination, defecation) were recorded (table 2).

128
129 Social defeat stress: Once mice reached adulthood (3 months old), they were exposed to social
130 defeat stress as previously described (Trainor et al., 2011). Briefly, the focal mouse was
131 introduced into the home cage of a territorial same-sex conspecific for a duration of 7 minutes or
132 until it was attacked 7 times (whichever occurred first). Immediately after each defeat episode
133 was completed, the mice were returned to their home cages. Social defeat resulted in no
134 physical wounds.

135
136 Social Interaction test: Animals were tested in the social interaction test (Trainor et al., 2011)
137 before (7 days prior) and after (7 days) exposure to social defeat stress (fig. 3A). Briefly, the
138 social interaction test consisted of three 3 minute phases during which the behavior of the focal
139 mouse was recorded and automatically scored using Any-maze (Stoelting): open field,
140 acclimation, and social interaction. During the open field phase, the mouse was placed in the
141 center of an empty testing arena (80 x 63 x 60cm) and was allowed to freely explore. During this
142 phase, automatic scoring included total distance traveled to assess locomotor activity and time
143 spent in the center of the arena (within 8cm of the sides and 14cm of the ends), which was used
144 as a measure of anxiety-like behavior. During the acclimation phase, an empty wire mesh cage
145 was placed against one of the walls of the arena. During the interaction phase, the wire mesh
146 cage was replaced by another identical one containing a novel same-sex conspecific (target
147 mouse). During both the acclimation and interaction phase, automatic scoring included time
148 spent within 8cm of the mesh cage (interaction zone) and time spent in corners of the arena.
149 Immediately after the interaction phase was complete, the animals were returned to their home
150 cage.

151
152 Statistical analyses

153 All analyses were done using Prism GraphPad®. Frequency of backflipping behavior was
154 assessed using 1-way nested ANOVAs to compare housing conditions between groups at a
155 given age (P30,P50,P90), and 2-way ANOVAs to assess effects of age on this behavior across
156 groups. For the social interaction test, 1-way nested ANOVAs were used to compare time spent
157 in the open field and social approach between housing conditions (pre and post stress were
158 assessed separately), and 2-way ANOVAs to assess the effects of stress on this behavior

159 across groups. For body weight and body condition, 2-way ANOVAs were used to assess this
160 variable throughout development across groups. Finally, urination in response to handling was
161 compared using nested 1-way ANOVA. Planned comparisons were performed if ANOVAs
162 showed a significant effect, using Tukey for nested analyses and Sidak for 2-way ANOVAs to
163 correct for multiple comparisons. Statistical significance was established at $\alpha < 0.05$ for all
164 tests. An F-test was used to compare variances between groups.

165

166 **Results**

167

168 Backflipping behavior

169 In males, backflips were significantly affected by age in a housing-dependent fashion
170 (Fig. 2B, repeated measures 2-WAY ANOVA main effect of age $F_{2,90}=20.51$, $p < 0.0001$, the
171 main effect of treatment $F_{2,45}=4.23$, $p=0.02$). Backflipping frequency increased from P30 to
172 P50 in standard ($d=2.1$, $p < 0.0001$) and large housing ($d=0.6$, $p=0.03$), but not in large+EE,
173 which showed an increase from P30 only by P90 ($d=0.6$, $p=0.03$). When comparing backflipping
174 behavior within age groups there was a trend for treatment to have an effect at P50 in males
175 (Fig.2D, nested 1-way ANOVA $F_{2,12}=3.2$, $p=0.07$). Post-hoc comparisons showed that
176 larger+EE cages reduced backflip frequency compared to standard housing ($d=0.96$ $p=0.03$).
177 No effects of housing were found in males at P30 or P90. Similar to males, in females,
178 backflipping behavior was affected by age depending on housing conditions (Fig. 2F, mixed-
179 effects model main effect of age $F_{2,75}=14.83$, $p < 0.0001$, the main effect of treatment
180 $F_{2,45}=18.16$, $p < 0.0001$). Backflipping frequency increased from P30 to P50 in standard
181 ($d=1.21$, $p=0.009$) and large+EE ($d=1.56$, $p=0.03$), but not in large cages, which showed an
182 increase only by P90 ($d=1.56$, $p=0.007$). Comparisons within age groups revealed a main effect
183 of housing conditions at P30 (Fig. 2G, nested 1-way ANOVA $F_{2,30}=3.9$, $p=0.03$) and at P50
184 (Fig. 2H, nested 1-way ANOVA $F_{2,30}=5.4$, $p=0.01$). Planned comparisons revealed that large
185 cages, but not large+EE, significantly reduced backflipping behavior compared to standard at
186 both P30 ($d=1.13$, $p=0.03$) and P50 ($d=1.26$, $p=0.003$).

187

188 Open field

189 There were no significant differences between housing conditions in the time spent in
190 the open field when comparing housing conditions before (Fig. 3 B,E) or after (Fig.3 C,F) stress
191 in males or females (nested ANOVAs). When comparing the behavior before and after stress
192 within groups (repeated measures 2-way ANOVAs), only in females there was a main effect of

193 stress (Fig. 3G, $F_{1,39}=12.78$, $p<0.001$). Planned comparisons showed that stress significantly
194 reduced time spent in open field in females housed in large ($d=0.5$ $p=0.03$) and large+EE
195 ($d=0.52$ $p=0.02$), but not standard cages.

196

197 Acclimation

198 There were no effects of housing conditions or stress on time spent investigating an
199 empty cage in males (fig.4B,C,D). Housing conditions did affect this behavior in unstressed
200 females (Fig. 4E, nested one way ANOVA $F_{2,40}=5.478$): standard cage females showed
201 significantly less time exploring the cage compared to large ($d=0.8$, $p=0.042$) and large+EE
202 ($d=1.5$, $p=0.004$) housed females. There were no differences between housing conditions in
203 females after stress (fig. 4F). Interestingly, when comparing the effects of stress on time
204 exploring an empty cage (fig. 4G, repeated measures 2-way ANOVA main effect of stress
205 $F_{1,42}=5.75$, $p=0.02$), we found that stress increased time exploring the object in standard
206 ($d=1.34$, $p=0.005$), but not large or large+EE housed females.

207

208 Social interaction behavior

209 In males, there were no effects of housing conditions on social approach behavior. All
210 males showed high levels of social interaction both 7 days before and 7 days after stress (fig.4
211 B,C,D). On the contrary, pre-stress females showed different levels of social interaction
212 depending on housing conditions (Fig. 5E, nested ANOVA main effect of treatment $F_{2,12}=4.05$,
213 $p=0.04$), with females in standard cages showing significantly less time in social approach than
214 females in large+EE cages ($d=1.1$, $p=0.02$). Importantly, an F-test revealed that, compared to
215 females housed in standard housing, females in large+EE conditions showed significantly less
216 interindividual variance in the time spent in social approach (standard deviation 51.6 vs. 24.5,
217 $p=0.006$). Thus, we repeated the analysis after a square root transformation to equalize
218 variances, but the main conclusions did not change (main effect of treatment $p=0.048$, standard
219 vs large+EE $p=0.038$). There were no differences between groups in females post-stress.
220 Critically for our research program, social defeat stress reduced time spent in social approach in
221 females of all groups (Fig. 5G, repeated-measures 2-way ANOVA main effect of stress
222 $F_{1,40}=51.98$, $p<0.0001$). This replicates previous findings showing that females, unlike males,
223 are susceptible to showing long-term social deficits after exposure to social defeat stress
224 (Duque-Wilckens et al., 2020, 2018; Steinman et al., 2016; Trainor et al., 2011), and shows that
225 the effect is robust and persists regardless of housing conditions. Interestingly, the effect of

226 stress on social approach was the largest in females housed in large+EE ($d=2.2$, $p=0.0001$),
227 followed by large ($d=1$, $p=0.0003$), and standard housing ($d=0.83$, $p=0.002$).

228

229 Use of enrichment

230 All animals except 3 males used the enrichment item at least once during the three 5
231 minutes in cage observations. There was a main effect of age on use of enrichment in males (1-
232 way ANOVA main effect of age $F_{2,47}=5.8$, $p=0.005$, fig. 5A) and females (1-way ANOVA main
233 effect of age $F_{2,32}=6.0$, $p=0.006$, fig. 5E), although in opposite directions: in males, the use of
234 enrichment gradually increased with age, with use of enrichment at P30 being significantly lower
235 that use of enrichment at P50 ($d=1.0$, $p=0.03$) and P90 ($d=1.1$, $p=0.006$). In females, on the
236 contrary, the use of enrichment reduced from P30 to P90 ($d=1.5$, $p=0.004$).

237

238 Use of Enrichment

239 In males, the use of enrichment items increased throughout development (Fig. 6A, 1-
240 way ANOVA $F_{2,47}=5.8$, $p=0.005$). Compared to P30, males used enrichment more frequently
241 both at P50 ($p=0.03$) and P90 ($p=0.006$). Interestingly, females showed the opposite: frequency
242 of enrichment use decreased with time (Fig. 6B, 1-way ANOVA $F_{2,32}=6$, $p=0.006$), compared to
243 P30, females used enrichment less frequently at P90 ($p=0.004$).

244

245 Welfare and body condition analyses

246 We did not see the effects of housing conditions on body weight (Fig. 6B,F) or urination
247 frequency (Fig.6D,H) in response to handling in males or females. In females, housing affected
248 body condition but only in older mice (Fig. 6G, 2-way ANOVA $F_{14, 70}=2.83$, age x housing
249 interaction $p=0.002$). Body condition scores were higher in females housed in large cages
250 compared to standard caging at PN 49 ($p=0.0002$) and marginally higher at PN 56 ($p=0.06$). In
251 males, housing conditions had no significant effect on body weight or body condition across
252 development (all p 's > 0.26). We also did not detect any presence of injuries or differences in
253 coat condition. Animals from all groups showed a similar frequency of urination in response to
254 handling (Fig. 6 D,H). No defecation in response to handling was recorded.

255

256 **Discussion**

257 The present studies show that increasing housing space as well as social and
258 environmental complexity in California mice can delay the development of stereotypic behavior.
259 Critically, this reduction in stereotypy is accompanied by increased effect sizes of stress in an

260 established model for social anxiety. These results suggest that increased cage size and
261 enrichment enhances welfare for mice while simultaneously enhancing the quality of behavioral
262 experiments.

263

264 *Housing conditions on expression of stereotypic behavior*

265 We found that the effects of housing on stereotypic behavior are age and sex-
266 dependent. In males of all housing conditions, backflipping frequency was very low at P30 and
267 significantly increased at P50 in standard and large housing groups, but not in large+EE. In this
268 group, the backflipping behavior did increase, but only by P90. A similar effect has been
269 reported in deer mice, where increased space and environmental complexity delayed the
270 appearance of stereotypic behavior compared to standard housed animals (Powell et al., 1999).
271 This suggests that, although increased space and social/environmental complexity had a
272 positive impact on stereotypic behavior during earlier stages of development, it was not
273 sufficient to inhibit its appearance later in life. The results in California mice could be partly
274 explained by the fact that this species initiates dispersal from their parents' nests between P75-
275 85 in the wild (Ribble, 1992). Thus, between P50 and P90, a motivational shift could render the
276 available space and environmental complexity in large+EE insufficient for the individuals to
277 perform age-specific behaviors essential to survival and reproduction in the wild, resulting in
278 increased backflipping (Garner, 2005).

279 A similar housing-dependent delay of stereotypic behavior was seen in females.
280 Nonetheless, in this case large, but not large+EE, shifted the increase from P50 to P90
281 compared to standard housing. This suggests that, while increased space and social complexity
282 is effective at delaying stereotypies in females, the addition of environmental enrichment
283 somehow negates this effect. It is possible that added enrichment items increased intra-cage
284 competition, although this was not measured in the present study. Some studies in C57 mice
285 have previously reported that the addition of environmental resources can result in increased
286 aggression (Barnard et al., 1996; Haemisch and Gärtner, 1997). If this is the case, it would be
287 intriguing to understand why competition develops as a response to environmental enrichment
288 only in females, as both female and male California mice show high levels of territorial
289 aggression towards same-sex conspecifics (Rieger et al., 2019; Trainor et al., 2011).

290 Another important observation in the current studies is that females housed in standard
291 housing conditions showed higher frequency of stereotypic behavior already at P30, in contrast

292 to males who only showed an increase in this behavior by P50. This could be associated with
293 differences in sexual maturity: female California mice molt their juvenile coat earlier than males
294 (Wright et al, unpublished). Future studies assessing the underlying neurobiology of stereotypic
295 backflipping behavior could shed more light on this question.

296

297 *Effects of housing conditions on anxiety-like and exploratory behaviors*

298 In females both large and large+EE affected behavior in a context-dependent manner, in
299 contrast, no effects of housing or stress were observed in males. Under baseline (non-stress)
300 conditions, large and large+EE increased the time females spent in non-social and social
301 exploration without affecting time spent in the open field. This suggests that elevated motivation,
302 rather than reduced anxiety, underlies the effects of housing on these behaviors. Previous
303 studies have found that environmental enrichment has profound effects on the physiology of
304 ventral striatum, a key brain area underlying motivation and reward processing. For example,
305 microdialysis studies in rats showed that environmental enrichment results in elevated levels of
306 extracellular striatal dopamine (Segovia et al., 2010), and studies in mice have shown that
307 enrichment alters the expression of genes key to striatal signaling, including upregulation of
308 Brain-derived neurotrophic factor (BDNF) (Bezard et al., 2003), and the transcription factor
309 delta-Fos B (Solinas et al., 2009).

310 The fact that both large and large+EE had similar effects on exploratory behaviors
311 suggests that increased space and social complexity, in the absence of environmental
312 enrichment, are sufficient to trigger an effect on motivated responses. Studies in female C57
313 mice showed similar results: just the social component of enriched environments could explain
314 an increase in both non-social and social exploration time, which was correlated with an
315 increased number of doublecortin expressing cells in the hippocampus, indicating increased
316 neurogenesis (Moreno-Jiménez et al., 2019).

317 We were particularly interested in learning whether housing conditions would interfere
318 with the sex-specific effects of defeat stress on social interaction behavior that we consistently
319 see in our research program (Duque-Wilckens et al., 2018; Greenberg et al., 2014; Trainor et
320 al., 2011). Remarkably, we found that social defeat reduces time spent in social approach in
321 females of all housing groups without affecting this behavior in males. These results show that
322 female-biased susceptibility to social defeat stress is a very robust phenotype in California mice,
323 which provides even stronger support for using this model as a means of understanding female-

324 biased psychiatric disease. Further, we found that both large and large+EE increase the
325 magnitude of the effects of stress on social approach by reducing the within-group variance in
326 females. This is very relevant as it could translate into fewer animals needed to achieve
327 statistical significance, favoring the second of the three Rs principles underpinning the humane
328 use of animals in scientific research (van Luijk et al., 2013). Overall, the current studies show
329 that larger cages and increased social and environmental complexity effectively delay the
330 appearance of stereotypic behavior. Importantly, these reductions in stereotypic behaviors are
331 associated with reduced variability and increased effects size in behavioral experiments
332 focusing on social anxiety-related behaviors. These results highlight the benefits of paying close
333 attention to housing conditions to improve animal welfare and quality of science.

334

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495 **Figure legends.**

496

497 Fig.1. General experimental timeline (A). Pictures showing three experimental housing
498 conditions: standard, large, and large + Enrichment (EE) (B).

499

500 Fig. 2. Backflipping behavior across development. Experimental timeline (A). Stereotypic
501 behavior increases with age across experimental conditions in males (B) and females (C)
502 (repeated 2-way ANOVAS). In males, large+EE reduces frequency of stereotypic behavior at
503 P50, but not P90 (C-E) (nested 1-way ANOVA). In females, large, but not large+EE, reduces
504 stereotypic behavior at P30 and P50 (H-J) (nested 1-way ANOVA). Percent of animals showing
505 stereotypic behavior within each housing group (F,K) (n=10-19 per group).

506

507 Fig. 3. Behavior in the open field. A. Experimental timeline. Housing conditions do not affect
508 time spent in the open field before or after stress in males (B,C) or females (E,F) (nested 1-way
509 ANOVA). Stress does not affect time spent in the open field in males of any of the housing
510 groups (D). Stress reduces time spent in the open field in females housed in large and
511 large+EE, but not in standard cages (G) (repeated measures ANOVA).

512

513 Fig 4. Behavior during acclimation phase (target absent) A. Experimental timeline. Housing
514 conditions did not affect time spent in proximity to an empty cage in males either before (B) or
515 after stress (C). Unstressed females housed in standard cages spent less time in proximity to an
516 empty cage compared to females housed in large and large+EE (E), but housing had no effect
517 on this measure after stress (F) (nested 1-way ANOVA). In males, stress had no effect on time
518 spent in proximity to an empty cage in any of the housing groups (D). In females housed in
519 standard, but not large or large+EE, stress increased time in cage during acclimation (G,
520 repeated measures ANOVA).

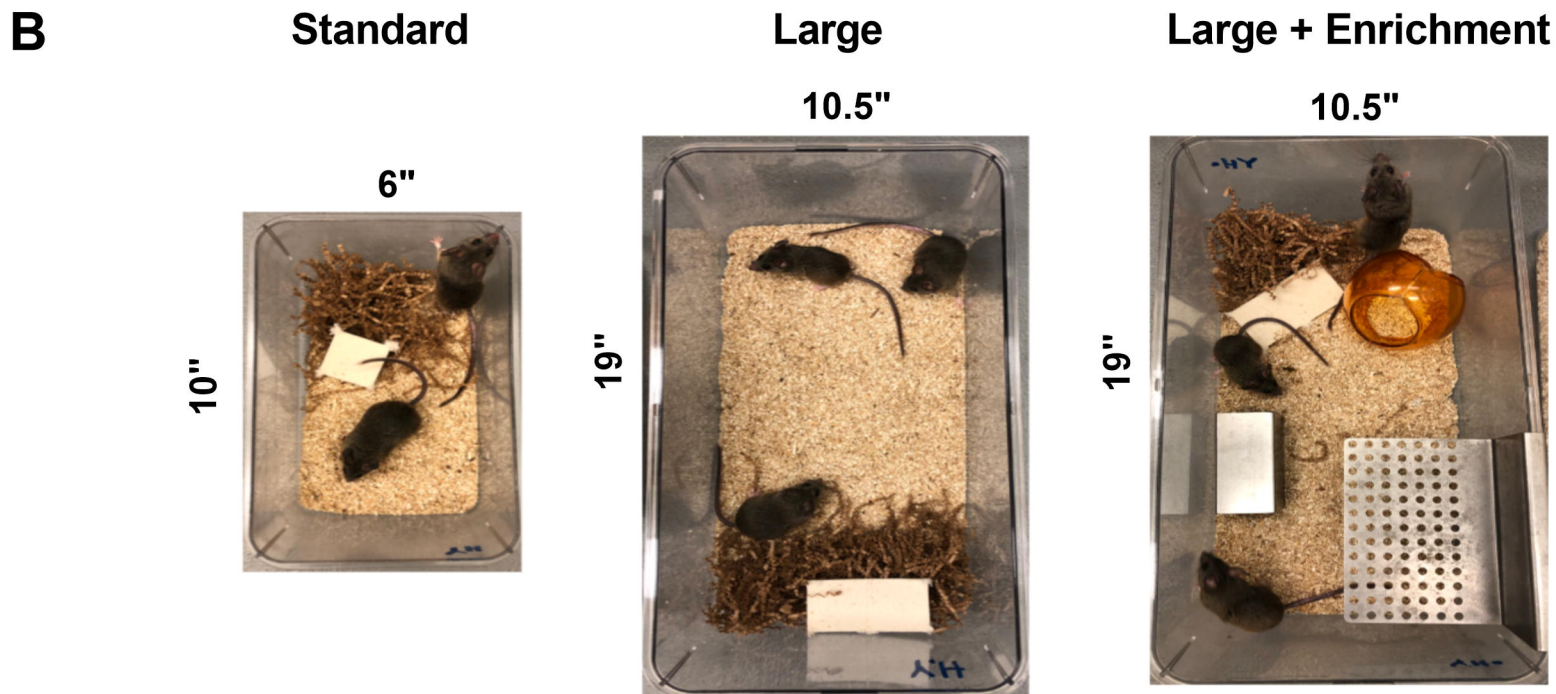
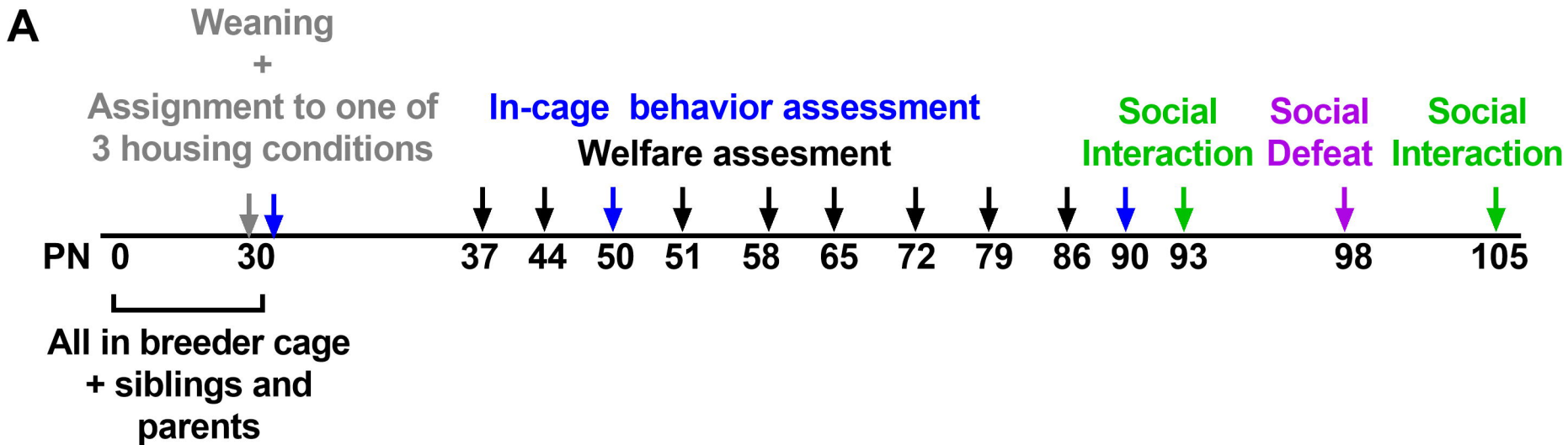
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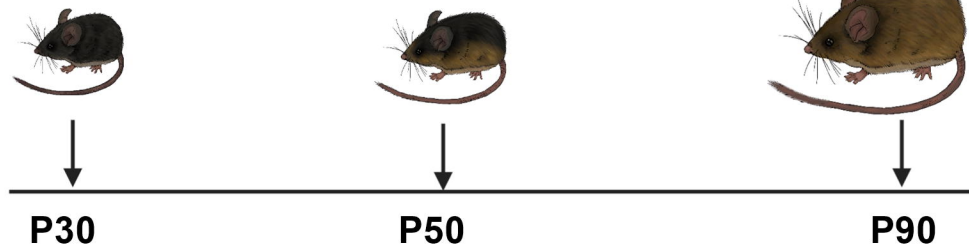
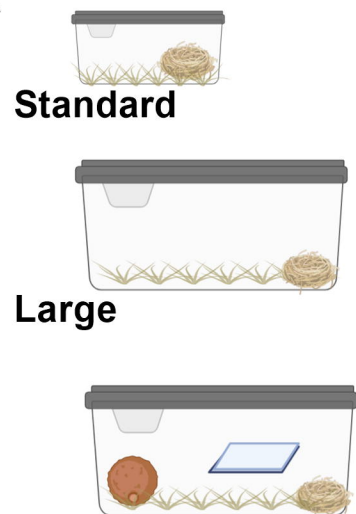
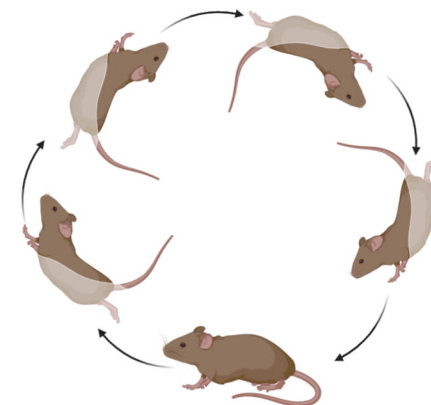
522 Fig. 5. Behavior during interaction phase (target present) A. Experimental timeline. Housing
523 conditions did not affect time spent in proximity to the social stimulus in males either before (B)
524 or after stress (C). Unstressed females housed in standard cages spent less time in proximity to

525 a social stimulus compared to females housed in large and large+EE (E), but housing had no
526 effect on this measure after stress (F) (nested 1-way ANOVA). In males, stress had no effect on
527 time spent in proximity to the social stimulus in any of the housing groups (D). In females of all
528 housing groups, stress increased time in cage during social interaction, but the effect size was
529 smallest in the standard housed females (G, repeated measures ANOVA).

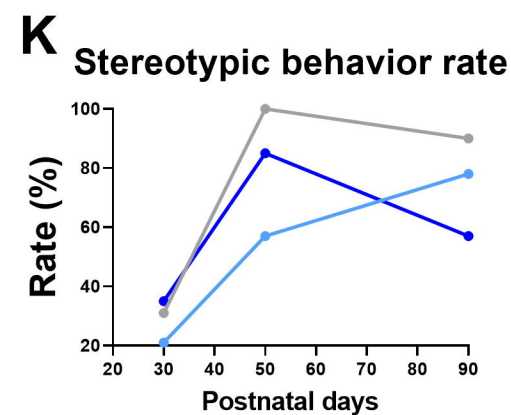
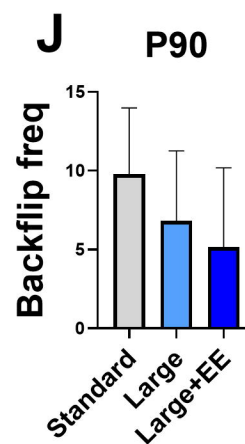
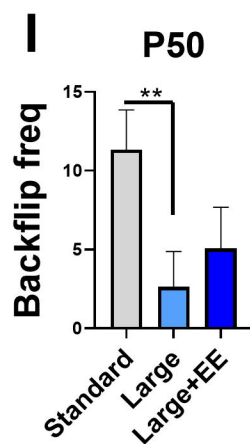
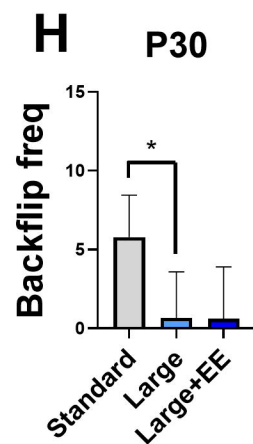
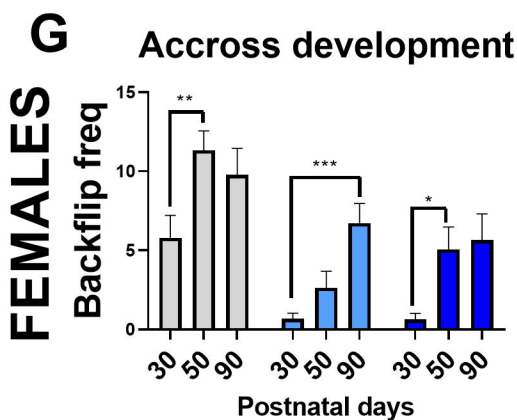
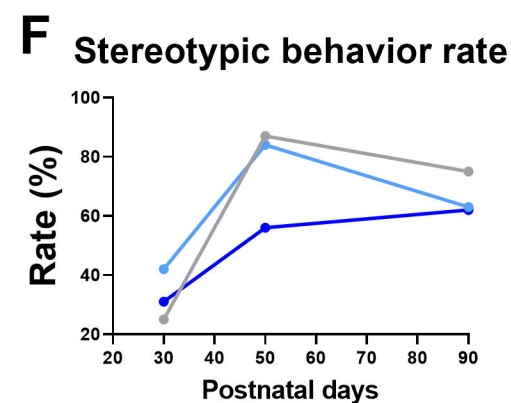
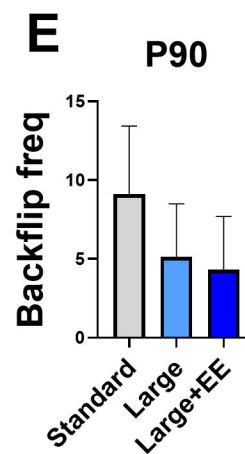
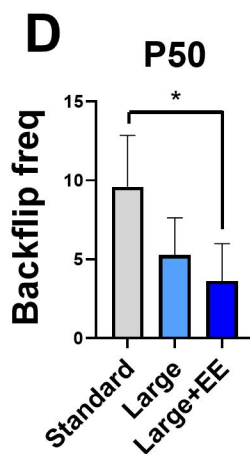
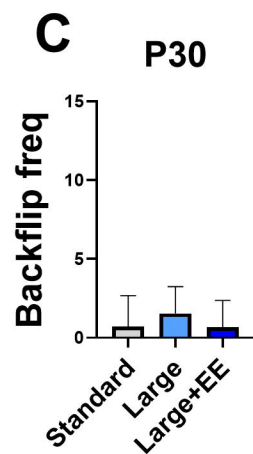
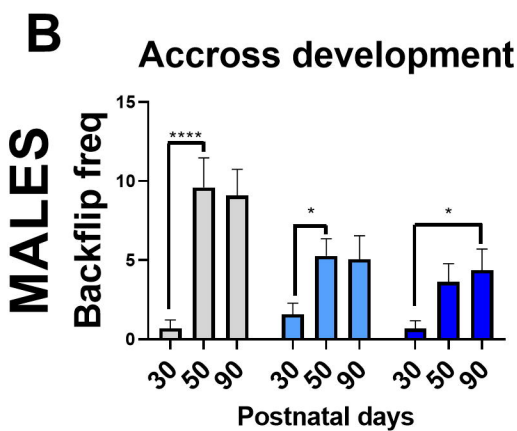
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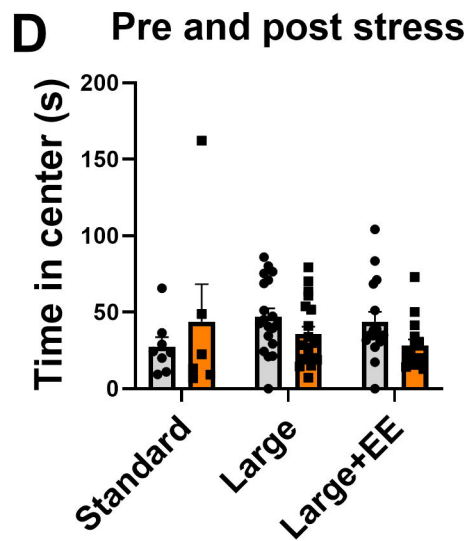
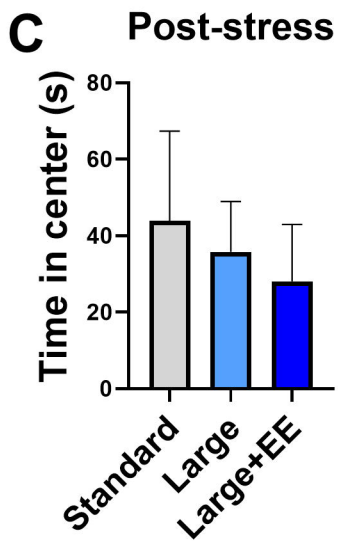
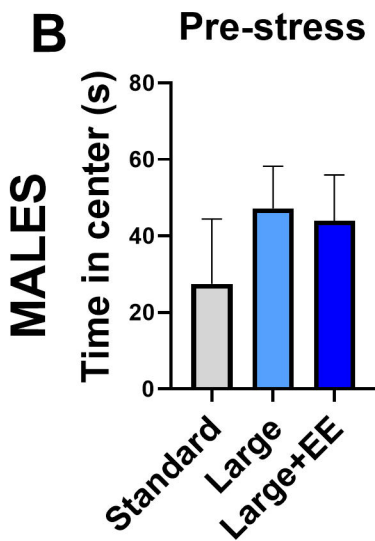
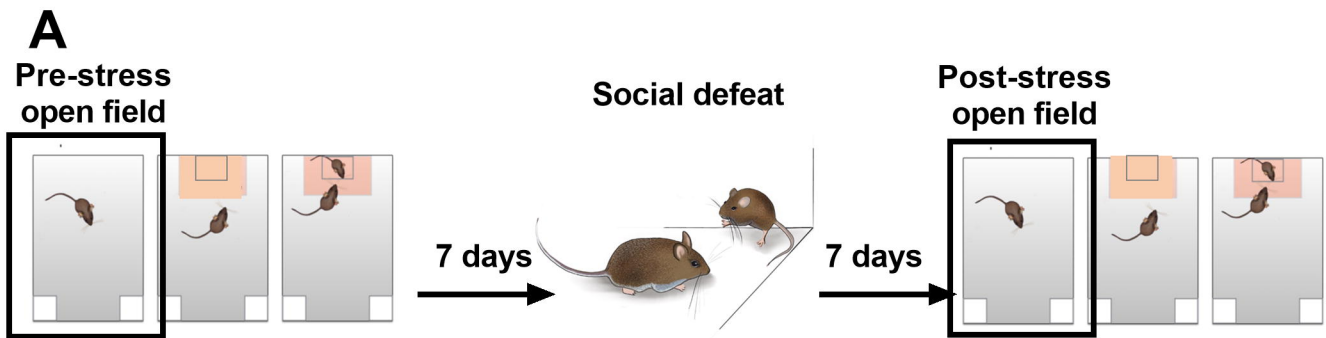
531 Fig. 6. Use of enrichment increased with age in males (A), but decreased in females (E) (1-way
532 ANOVA). There were no differences in body weight between housing groups in males (B) or
533 females (F). There were no differences in body condition between housing groups in males (C).
534 In females large increased body condition score compare to standard at PN79 (2-way repeated
535 measures ANOVA). There were no effects of housing on urination in response to handling in
536 males (D) or females (H).



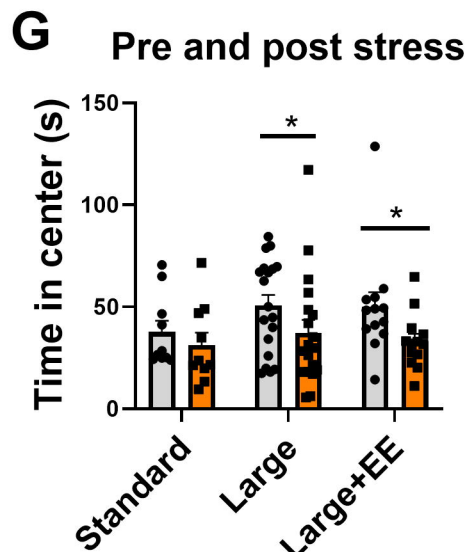
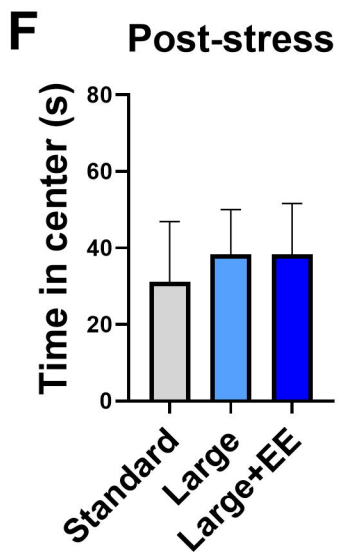
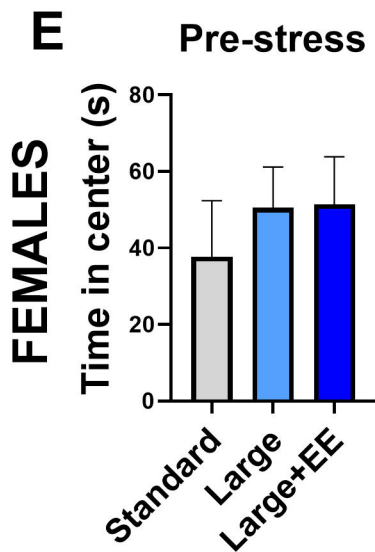
A**In-cage behavioral observations****Backflip behavior****Large + EE**

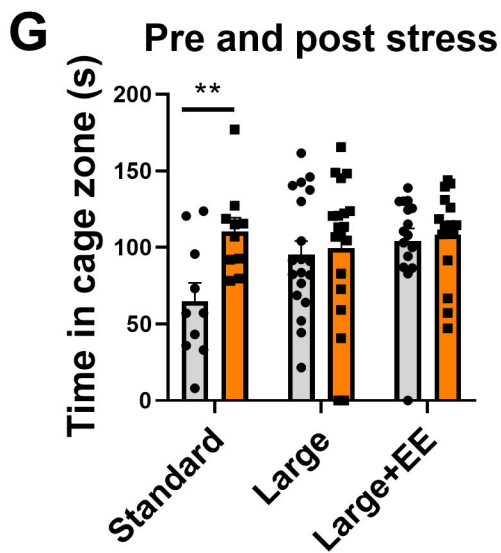
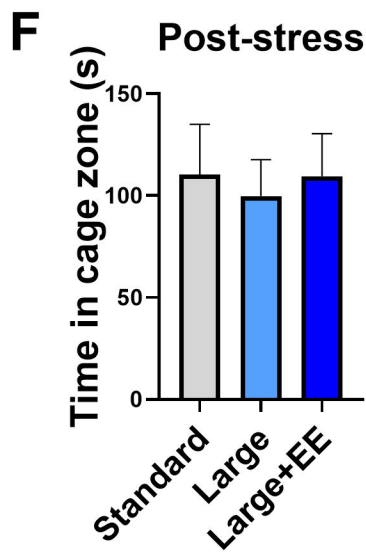
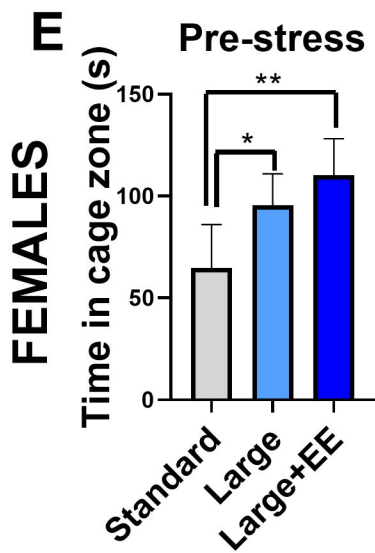
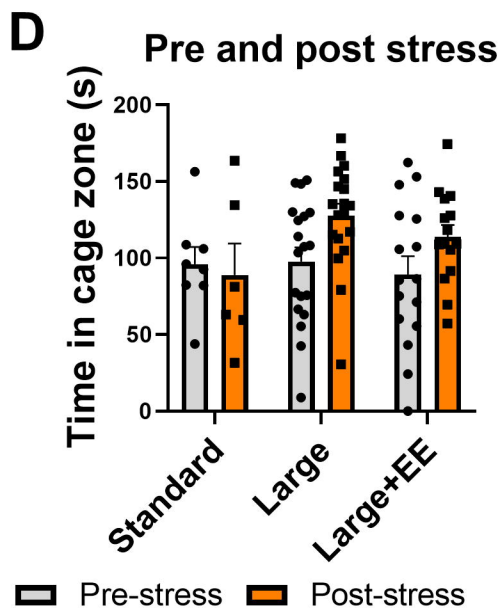
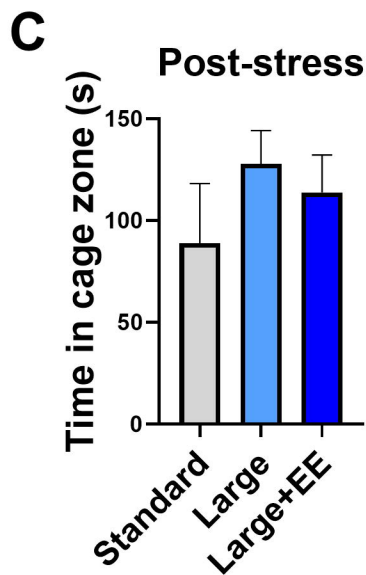
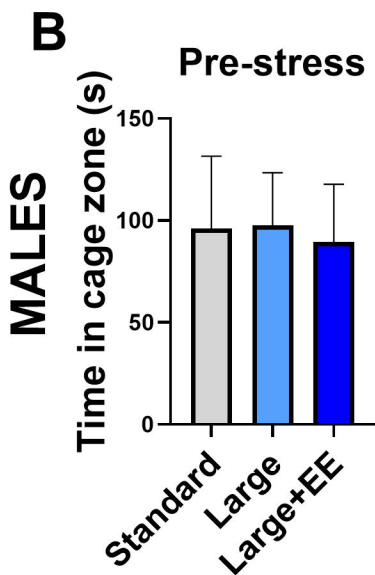
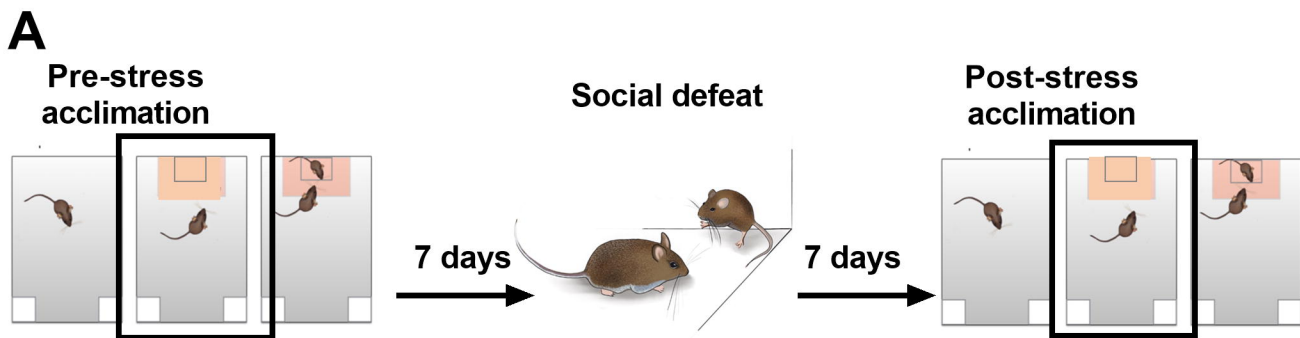
Standard Large Large+EE

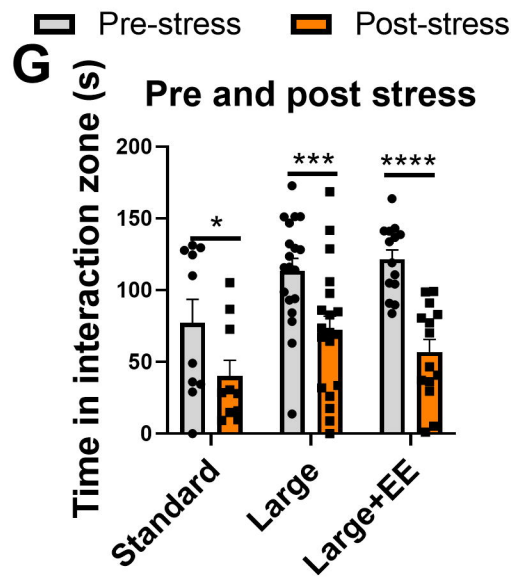
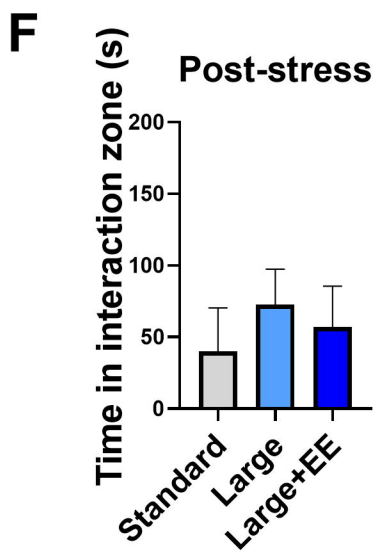
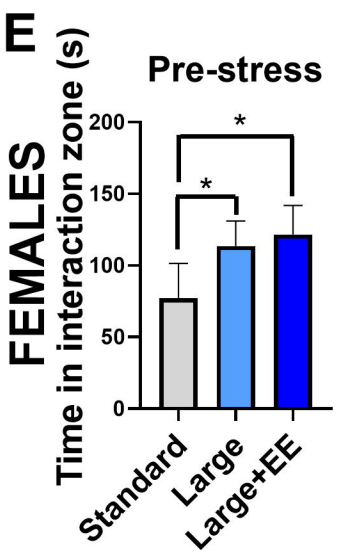
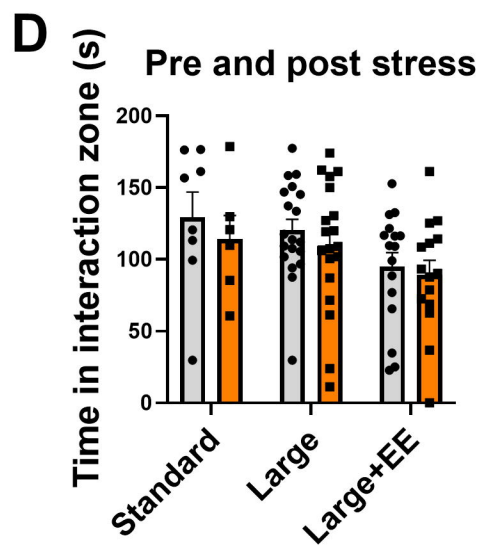
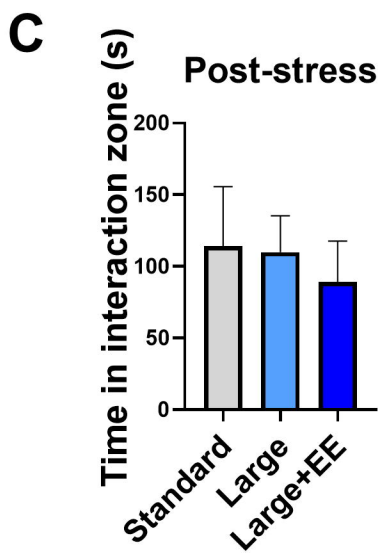
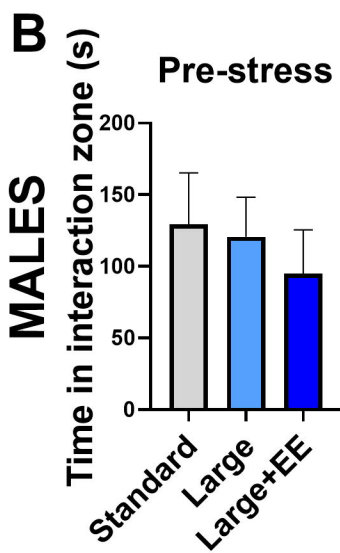


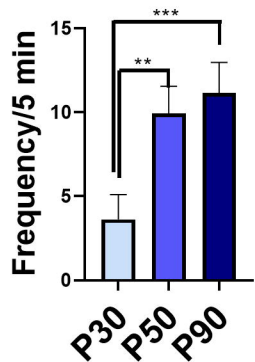
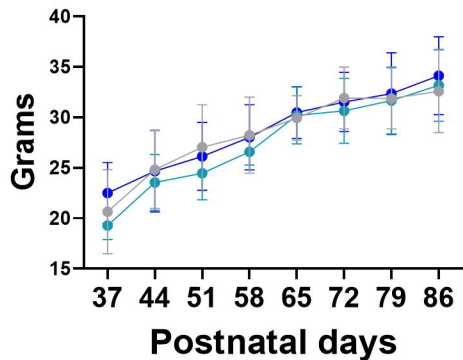


□ Pre-stress □ Post-stress

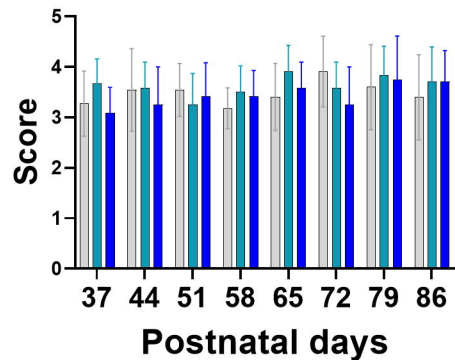




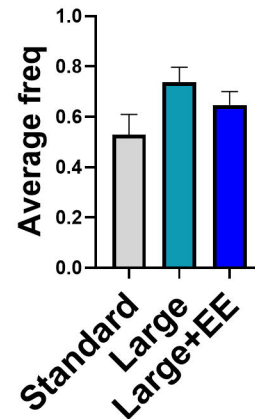
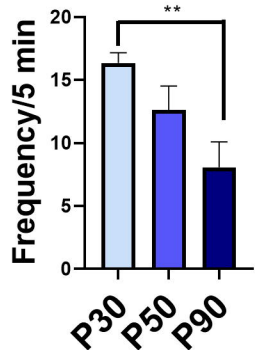
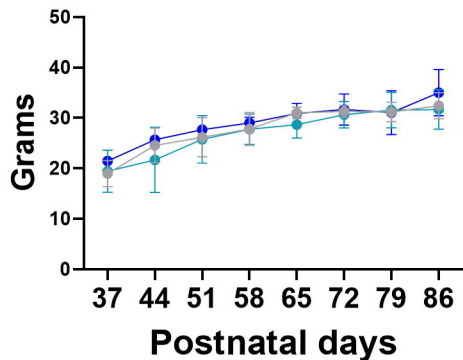
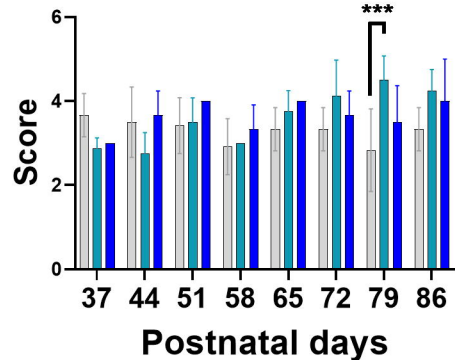


MALES**A Use of Enrichment****B Body weight**

● standard
● large
● large + EE

C Body condition

■ standard
■ large
■ large + EE

D Urination**FEMALES****E Use of Enrichment****F Body weight****G Body condition****H Urination**