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Enriched laboratory housing increases sensitivity to social stress in female California mice (*Peromyscus californicus*).

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Abstract

Domesticated mice and rats have shown to be powerful model systems for biomedical research, but there are cases in which the biology of species is a poor match for the hypotheses under study. The California mouse (*Peromyscus californicus*) has unique traits that make it an ideal model for studying biological mechanisms underlying human-relevant behaviors such as intra-female aggression, biparental care, and monogamy. Indeed, peer-reviewed scientific publications using California mouse as a model for behavioral research have more than doubled in the past decade. Critically, behavioral outcomes in captive animals can be profoundly affected by housing conditions, but there is very limited knowledge regarding species-specific housing needs in California mice. Currently, California mouse investigators have to rely on guidelines aimed for more common laboratory species that show vastly different physiology, behavior, and/or ecological niche. This not only could be suboptimal for animals' welfare, but also result in lack of standardization that could potentially compromise experimental reproducibility and replicability across laboratories. With the aim of assessing how different housing systems can affect California mouse behavior both in the home cage as well as the open field and social interaction tests before and after social defeat stress, here we tested three different caging systems: 1. Standard mouse cage, 2. Large cage, and 3. Large cage + environmental enrichment (EE), which focused on increasing vertical complexity based on observations that California mice are semiarboreal in the wild. We found that the effects of housing were largely sex specific: compared to standard cages, in females large + EE reduced home cage stereotypic-like backflipping and rearing behaviors, while large cage increased social interactions. In males, the large+EE cage reduced rearing and digging but did not significantly affect backflipping behavior. Interestingly, while there were no

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significant differences in the open field and social interaction pre-stress behaviors, large and large +EE housing increased the sensitivity of these tests to detect stress induced phenotypes in females. Together, these results suggest that increasing social and environmental complexity affects home cage behaviors in male and female California mice without interfering with, but rather increasing the magnitude of, the effects of defeat stress on the open field and social interaction tests.

Keywords

Welfare; Environmental Enrichment; Stereotypic Behavior; Social Behavior; California mouse; Stress

Introduction

The California mouse (*Peromyscus californicus*) possesses unique behavioral characteristics that make it a powerful model species for testing hypotheses that are difficult or impossible to test with standard rodent lines: California mice are monogamous, biparental, and both males and females show aggression towards intruders of both sexes (Ribble and Salvioni, 1990; Rieger et al., 2019). Male parental behavior and female aggression are present in humans but are very rare in common laboratory rats and mice (Kleiman, 1977; Kleiman and Malcolm, 1981), making the California mouse a great model to study the sex-specific mechanisms underlying social behaviors relevant to human behavior. This is particularly important considering that the new NIH guidelines require consideration of sex as a biological variable in research. Indeed, PubMed Central® database indicates that publications using California mouse in biomedical research have more than doubled in the past decade (fig. 1A).

While the unique behavior of California mice provides great opportunities for research, it can also present challenges for husbandry, as it likely results in unique housing needs (Baumans, 2005). Surprisingly, there is very limited research assessing housing systems for California mice. Currently, investigators have to rely on guidelines aimed for more common laboratory species such as *Mus musculus* or *Rattus norvegicus* (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011), which could be suboptimal for California mice considering that mice and rats show largely different behaviors, physiology, and ecological niches (Gubernick and Alberts, 1987; Krugner-Higby et al., 2006; Panti-May et al., 2016, 2016; Ribble and Salvioni, 1990; Singleton and Hay, 1983). The unavailability of species-specific guidelines could further result in lack of standardization across laboratories, potentially compromising experimental reproducibility and replicability. This is especially relevant for behavioral research considering that environmental conditions can have a major impact on the animal's behavior and physiology both under baseline conditions and in response to stress (Friske and Gammie, 2005; Lehmann and Herkenham, 2011; Olsson and Sherwin, 2006; van Dellen et al., 2000). Specifically, differential environmental housing conditions have been shown to influence susceptibility to develop anxiety and depressive-like behaviors after exposure to social defeat stress (Lehmann and Herkenham, 2011; Schloesser et al., 2010), one of the

main focus of our research program (Duque-Wilckens et al., 2020; Greenberg et al., 2014; Williams et al., 2018).

With the aim of shedding light on the potential impact that different housing conditions can have on California mouse behavior, here we compared the effects of three different caging systems on home cage behaviors and social defeat stress-induced phenotypes in the open field and social interaction tests: standard, larger cages with increased social complexity, and larger cages, increased social complexity, and environmental enrichment. We designed the enrichment program based on observations of California mice in the wild, who build complex nests in a variety of contexts including tree cavities, leaf litters, and creek banks, and spend a significant amount of the day climbing (Dalquest, 1974; Gubernick and Alberts, 1987). Based on previous findings (Lehmann and Herkenham, 2011; Schloesser et al., 2010), we hypothesized that compared to standard housing, environmental enrichment would reduce susceptibility to develop social avoidance phenotypes in California mice.

Materials and Methods

Animals and housing:

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and conformed to NIH guidelines. All mice were bred in our colony (University of California, Davis, Department of Psychology). Original breeders were purchased from the *Peromyscus* Genetic Stock Center, University of South Carolina, which mice are derived from about 60 ancestors collected between 1979 and 1987 in Santa Monica Mts., CA. For breeding pairs setup, 3 month-old females were allowed to choose between two 3 month-old non-sibling males in the mate preference test as previously described (Gleason et al., 2012), after which the pair stayed together for life. A rodent health surveillance was performed monthly by the Comparative Pathology Laboratory of University of California, Davis, during which sample mice were screened for viral, bacterial, and parasitic pathogenic agents. No pathogenic agents were detected in the colony during the duration of this experiment. At weaning (post-natal day 30, P30) animals were ear punched for identification and randomly assigned to one of three conditions (cagemates were a mix of siblings and non-siblings, fig. 1B): **1. Standard:** cage dimensions 15.2cm ×25.4cm ×12cm, 2 same-sex individuals per cage. Total animals in this condition: 10 males, 14 females (Fig.1C) **2. Large:** cage dimensions 48.3cm ×26.7cm × 15.2cm, 3–4 same-sex individuals (Fig. 1D). Total animals in this condition: 19 males, 19 females. Or 3. **Large + EE**: cage dimensions 48.3cm ×26.7cm × 15.2cm, 3–4 same-sex individuals. Total animals in this condition: 19 males, 14 females (fig.1E). Cages from all treatments (Ancare, Bellmore, NY), were made of clear polypropylene and all treatment groups were provided with Sani-chip bedding (P.J. Murphy, Montville, NJ), cotton nestlets (Ancare, Bellmore, NY), and enviro-dri (Shepherd Specialty Papers, Watertown, TN). The large+EE group was additionally provided with a crawl ball™ (Bio-Serv, Flemington, NJ), a stainless-steel loft with holes (Otto environmental, Greenfield, WI), and a 10×15cm stainless-steel tube (Otto environmental, Greenfield, WI). For all animals, municipal tap water and food (Harlan Teklad, Madison, WI) were provided *ad libitum*. All treatment groups were housed in the same room under a 16L:8D light:dark cycle. The room temperature was kept at 20–23°C.

Home cage behavioral observations:

initial data collected during a pilot experiment showed that >95% of the individuals are asleep during the light phase, regardless of sex or treatment (data not shown), which corresponds with previous observations that California mice are nocturnal (Marten, 1973). Therefore, all the home cage behavior data here correspond to observations performed during the dark cycle. Observations were performed at 3 developmental stages (fig.1B): postnatal day 30 (P30), age at which captive California mice are typically weaned (Gubernick and Nordby, 1992; Johnson et al., 2017), postnatal day 50 (P50), which corresponds to adolescence (Gubernick, 1988; Gubernick and Nordby, 1992; Wright et al., 2020), and postnatal day 90 (P90), age at which California mice are fully adults. All observations were done by one experimenter who was blind to the main hypothesis of the study. The experimenter used red light (3 lux) headlamps to help with experimenter vision but minimize potential effects of light exposure on the mice circadian rhythm (Hattar et al., 2003; Provencio and Foster, 1995; Yoshimura and Ebihara, 1996). The observations were done in randomized order. The behavior of each animal within a cage was assessed individually facilitated by individual ear punch patterns. Additional temporary tail marks were made with non-toxic, permanent markers before each data collection. The presence/absence of backflips, autogrooming, prosocial interaction, digging, and rearing were recorded every 15 seconds for 5 minutes (20 recording bouts total per individual per developmental stage). Definitions of each behavioral measures recorded can be found in figure 2A. Although we initially included aggression in our observation sheets, we did not see any intra-cage aggressive encounter.

Individual physical status assessment:

Individual health of all animals was monitored daily by the Department of Psychology, UC Davis animal care staff. No experimental animal showed signs of injuries or disease. Additionally, to assess whether different housing cages could affect body weight, the first cohort of animals (males standard n=11, large n=12, large+EE n=12; females standard n=6, large n=4, large+EE n=3) was weekly weighed since weaning (P30) until the end of the experiment (fig. 1F, G). This analysis was discontinued as the variability was very low between animals and there were no effects of treatment. These analyses also did not have an effect on other reported behaviors in this study.

Open field and social interaction test:

After the last in home-cage observation (PN90), California mice were tested in the open field and social interaction test (Trainor et al., 2011) 7 days prior and again 14 days after exposure to social defeat stress (fig. 3A) during the first three hours of the dark cycle (14:00–17:00). The behavior in the social interaction before and after social defeat stress is a critical component of our research program (Duque-Wilckens et al., 2020; Greenberg et al., 2014; Williams et al., 2018), so we were really interested in assessing whether a change in housing conditions would affect this behavior.

The *open field and social interaction test* consisted of three 3-minute phases during which the behavior of the focal mouse was recorded and automatically scored using Any-maze (Stoelting): open field, acclimation, and social interaction. During the open field phase, the

mouse was placed in the center of an empty testing arena (80 × 63 × 60cm) and was allowed to freely explore. During this phase, automatic scoring included total distance traveled to assess locomotor activity and time spent in the center of the arena (within 8cm of the sides and 14cm of the ends), which was used as a measure of anxiety-like behavior (Seibenhener and Wooten, 2015). During the acclimation phase, an empty wire mesh cage was placed against one of the walls of the arena.

This phase is used to assess interest in non-social novelty. During the interaction phase, the wire mesh cage was replaced by another identical one containing a novel same-sex conspecific (target mouse). During both the acclimation and interaction phase, the time spent within 8cm of the mesh cage (interaction zone) was automatically scored. Immediately after the interaction phase was complete, the animals were returned to their home cage. The estrous status in females was not recorded because conducting vaginal lavage disrupts behavior in California mouse (Silva et al., 2010), but previous studies have shown that social interaction behavior is not affected by the estrous cycle (Trainor et al., 2011) or gonadectomy (Trainor et al., 2013).

For social defeat stress, the experimental mouse was introduced for three consecutive days into the home cage of a same-sex conspecific (previously screened for territorial aggression) for a duration of 7 minutes or until it was attacked 7 times (Trainor et al., 2011) All animals were attacked at least 3 times, and no animal was physically injured. All animals were immediately returned to their home cages after each defeat episode was completed.

Statistical analyses

We conducted all statistical analyses in R (version 4.0.2) and generated all the figures using GraphPad Prism 9. For all analyses we used an alpha level criterion of 0.05 for statistical significance. We tested the normality of our dependent variable using Shapiro-Wilk test. All analyses were performed for each sex separately because all cages contained individuals of only one sex. Further, previous studies in California mice consistently show sex-specific stress behavioral phenotypes across stress-associated behaviors (Greenberg et al., 2015; Laredo et al., 2015).

Home cage observations: as a first evaluation we assessed the total frequency (sum of all events recorded at P30, P50, and P90) of each of the behaviors independent of treatment or age using one-way ANOVA followed by Dunnett's multiple comparisons test.

Next, we assessed each individual behavior including treatment and age as variables. When separated by treatment and age, all behaviors- backflips, autogrooming, social interactions, digging, and rearing - were in violation of normality and therefore we used a Poisson distribution. We used General Linear Models (GLM) with backflips, autogrooming, social interactions, digging and rearing as the dependent variables. We used animal identification code independent of treatment type as a random effect. This approach provides the best linear unbiased predictor for each random subject, which is of interest to this study as it provides the finest look at the treatment effects (Dingemanse et al., 2010; Gelman and Hill, 2006; Martin et al., 2011).Using the package lme4 (Bates et al. 2015), we fitted Generalized Linear Mixed Models (GLMM) with the animal identification code as a random term and

treatment as the fixed term. We constructed GLMMs for each behavior versus treatment with standard cage size as the reference level. To account for possible variation among age groups, we also included age as a covariate with day 30 as the reference level.

Open field and social interaction test: To assess whether home cage affects stress-induced behavioral phenotypes in the open field and social interaction test, 2-way repeated measures ANOVAs were used considering treatment and stress as factors, and individual animal as matched set. Planned comparisons were performed if ANOVAs showed a significant effect, using Sidak to correct for multiple comparisons. Finally, Cohen's D was used to assess the effect size of stress on each treatment in social interaction test in females.

Body weight: To assess whether there was an effect of cage system on body weight throughout development, we used two-way repeated measures ANOVAS using age and treatment as factors.

Results

Home cage behaviors:

Males: For a summary of the results, see table 1.

A one-way ANOVA showed a significant difference between total frequency of behaviors (Fig. 2B, $F_{4,235}=9.1$, $p<0.0001$). A Dunnett's multiple comparisons test revealed that backflipping was significantly more frequent than all other behaviors (backflipping vs. autogrooming $p<0.001$, vs. prosocial $p<0.01$, rearing $p<0.001$, digging $p<0.0001$).

When analyzing each behavior individually, we found no significant effect of housing on backflipping behavior (fig. 2D), autogrooming (fig. 2E), or social interactions (fig. 2F). However, compared to standard housing, large+EE reduced rearing by 47.7% (large+EE GLMM Estimate -0.64 ± 0.27, $p<0.05$, fig. 2G), and digging by 98.9% (large+EE GLMM Estimate -0.70 ± 0.32, $p<0.05$, fig. 2H).

Interestingly, the frequency of backflipping (P50 GLMM Estimate 1.11 ± 0.27, $p=0.0001$, P90 GLMM Estimate 1.2 ± 0.27, $p<0.0001$), autogrooming (P90 GLMM Estimate 0.9 ± 0.3, $p=0.005$) and prosocial behavior (P50 GLMM estimate 1.08 ± 0.29, $p=0.0004$; P90 GLMM estimate 1.15 ± 0.29, $p<0.0005$) increased with age.

Females: For a summary of the results, see table 2. A one-way ANOVA showed a significant difference between total frequency of behaviors (Fig. 2C, $F_{4,235}=16.48$, $p<0.0001$). A Dunnett's multiple comparisons test revealed that backflipping was significantly more frequent than all other behaviors (backflipping vs. autogrooming $p<0.0001$, vs. prosocial $p<0.02$, rearing $p<0.0001$, digging $p<0.0001$).

When analyzing each behavior individually, we found a treatment effect on backflipping, autogrooming, prosocial and rearing behaviors. Females in large and large+EE cages show reduced backflipping behavior by 47.9% and 41.5% compared to standard cages, respectively (large GLMM Estimate -0.66 ± 0.22, $p<0.01$; large+EE GLMM Estimate -0.54 ± 0.23, $p<0.05$, fig. 2I). Compared to standard, females in large cages exhibited 189.5%

increase in autogrooming (large GLMM estimate 1.08±0.29, p<0.01, fig. 2J), and 184.1% increase in prosocial behaviors (large GLMM estimate 1.07±0.34, p<0.01, fig. 2K). Finally, compared to standard, females in large+EE exhibited 52.6% decrease in rearing behavior (large GLMM estimate −0.72±0.22, p<0.01, fig. 2L). There was no effect of treatment on digging (fig. 2M)

Independent of treatment type, there was an age effect on backflipping. Females increased backflipping behavior by 372.4% at day 50 and by 354.5% at day 90 when compared to day 30 (age 50 days GLMM Estimate 1.55±0.27, p<0.01; age 90 days GLMM Estimate 1.51±0.27, p<0.01).

Open field and Social Interaction test

Males: There was a main effect of stress on total distance traveled in the open field (F1,40=9.9, p<0.005, fig. 3B), but no effects of treatment were seen on this behavior. Stress increased total distance traveled only in males housed in large cages (p<0.05). There were no effects of treatment or stress on time spent in the center of the open field (fig. 3C), or time spent in the interaction zone during acclimation (fig. 3D) or social interaction (fig. 3E).

Females: There was a main effect of stress on total distance traveled in the open field (F1,37=7.9, p<0.01, fig. 3F), but no effects of treatment were seen on this behavior. Stress increased total distance traveled only in females housed in standard cages (p<0.01). There was a main effect of stress on time spent in center of the open field (F1,39=12.78, p<0.005, fig. 3G). Sidak's multiple comparisons revealed that stress significantly reduced time in the center only in females housed in large (p<0.05) and large+EE (p<0.05), but no standard cages. There was also an effect of stress during acclimation (F1,42=5.75, p<0.05, fig. 3H), in which females housed in standard (p=0.005), but not large or large+EE, increased the time spent in the interaction zone. Finally, there was an effect of stress (F1,40=52, p<0.00001) and treatment (F2,40=3.8, p=0.03, fig. 3I) on social interaction behavior. Stress reduced the time spent in the interaction zone in all cages, although the effect sizes were different between cages: standard (p<0.05, d=0.83), large (p<0.005, d=1) and large+EE (p<0.0001, d=2.2).

Body weight:

We did not see the effects of housing conditions on body weight in males or females (Fig. 1F,G).

Discussion

In the present study we assessed the effects of three different housing conditions on the home cage behavior and stress-induced phenotypes in the California mouse: 1. Standard mouse cage, 2. Large cage, and 3. Large cage + EE. We found that the effects of housing were largely sex specific: compared to standard cages, in females large and large + EE reduced home cage backflipping behavior, while large cage increased both autogrooming and prosocial interactions. In males, the large+EE cage reduced rearing and digging but did not significantly affect backflipping behavior. Interestingly, while there were no significant

differences in the open field and social interaction pre-stress behaviors, large and large+EE housing increased the sensitivity of these tests to detect stress induced phenotypes in females. Together, these results suggest that increasing social and environmental complexity affects home cage behaviors in male and female California mice without interfering, but rather increasing, the effects of defeat stress on the open field and social interaction tests. To our knowledge, this is the first study assessing the effect of different housing conditions on California mouse behavior.

Home cage backflipping behavior

Out of all the behaviors recorded, we found that backflipping was the most frequent behavior displayed in the home cage by both males and females. Backflip behavior has been previously reported in captive California mice (Greenberg et al., 2015), but, to our knowledge, this has never been observed in the wild. This, together with its rigid and repetitive display and apparent lack of particular function, suggest that this behavior could be classified as abnormal (Garner, 2005; Mason and Latham, 2004a), as it has been described in other captive rodent species (Callard et al., 2000; Hadley et al., 2006; Novak et al., 2016). Abnormal behaviors typically arise as a consequence of inadequate housing conditions, where the animal is exposed to chronic aversive stimuli and/or is chronically prevented from performing species-typical behaviors (Garner, 2005; Gross et al., 2012). The presence of abnormal behavior is problematic because it can be associated with suboptimal welfare(Mason and Latham, 2004b), impaired cognition(Garner and Mason, 2002) and affective state(Novak et al., 2016), and increase variability in behavior and physiology(Garner, 2005). All these can increase variability of experimental outcomes and/or directly interfere with measures used in biomedical research programs. Therefore, efforts to reduce the expression of abnormal behavior in experimental animals should be of high priority.

Environmental enrichment has been proposed as a viable alternative to reduce the display of abnormal behaviors in captive species (Baumans, 2005; Bayne, 2018; Bayne and Würbel, 2014; Bechard et al., 2016; Shyne, 2006). In the present study, the large+EE cages incorporated environmental enrichment focused on providing a variety of nesting opportunities as well as increasing three-dimensional complexity based on the observations that wild California mice build complex nests in a variety of contexts including tree cavities, leaf litters, and creek banks, and spend a significant amount of the day climbing (Dalquest, 1974; Gubernick and Alberts, 1987 and R. Petric personal communication). We found that, compared to standard cages, both large and large+EE significantly reduced-yet did not eliminate- backflipping behavior in females without significantly reducing this behavior in males. Nonetheless, large+EE did reduce digging in males, a behavior that has also been considered abnormal in other captive species when presented in repetitive fashion (Wiedenmayer, 1997). While these results suggest that increasing space and social complexity can partially reduce behaviors that could be considered abnormal in California mice, we do not know if the differences observed are biologically relevant or if they extend beyond the observation intervals. Future studies should focus on expanding the frequency and length of behavioral data collection as well as identifying the underlying physiological mechanisms of repetitive backflipping and digging behaviors in captive California mice.

Effects of housing conditions on behavior in the open field and social interaction tests

We were particularly interested in learning whether housing conditions would interfere with the effects of defeat stress on behavior in the open field and social interaction tests, as our research program largely focuses on uncovering the mechanisms underlying female-biased vulnerability to develop anxiety and depressive-like phenotypes (Duque-Wilckens et al., 2018; Greenberg et al., 2014; Trainor et al., 2011). We found that social defeat reduced social approach in females but not males, which replicates previous findings, and that this effect was independent of housing conditions, suggesting that female-biased susceptibility to social defeat stress is a very robust phenotype in California mice. These results were surprising, as previous studies had found that environmental enrichment exerts a protective effect on defeat-induced depressive-like behaviors (Lehmann and Herkenham, 2011; Schloesser et al., 2010). Nonetheless, these studies used only male C57 mice and a social defeat paradigm consisting of 14 consecutive days of daily exposure to an aggressor. Since our social defeat paradigm -which lasts only 3 days- does not induce depressive-like behaviors in male California mice, it is possible that a ceiling effect could be obscuring a potential protective effect of environmental enrichment in this species. It would be interesting to assess whether in female C57 mice environmental enrichment has a distinct effect on behavioral response to social stress, which could be attained in this species by using protocols such as vicarious social defeat stress (Iñiguez et al., 2018).

Interestingly, while the directionality and sex-specificity of the effect of social defeat on social interaction was not affected by housing conditions, large and large+EE increased the magnitude of the effects of stress in females. This, together with the findings that social defeat reduced time spent in the center of the open field in females housed in large and large +EE, but not standard, suggest that increasing space and/or socioenvironmental complexity increases the sensitivity of the open field and social interaction test to detect stress-induced phenotypes. This is very relevant as it could translate into more accurate identification of specific neurobiological mechanisms underlying stress-induced behaviors and further increase the power of the experiments, reducing the total number of animals needed to reach statistical significance.

Overall, the results of this study show that the effects of housing conditions can be sex-specific, highlighting the importance of including both males and females in every study assessing the effects of environmental enrichment on behavior. Future studies in California mice should include alternative enrichment protocols and a more in-depth evaluation of first, the nature of backflipping behavior, and second, the effects of housing on welfare-related measures.

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Highlights

- Here we assess the effects of three caging systems on male and female California mice behavior
- Larger cages and increased social complexity reduce backflipping behavior in females
- Social defeat reduces social approach in females regardless of cage type
- Larger cages and increased social complexity increase the magnitude of stress effects on behavior

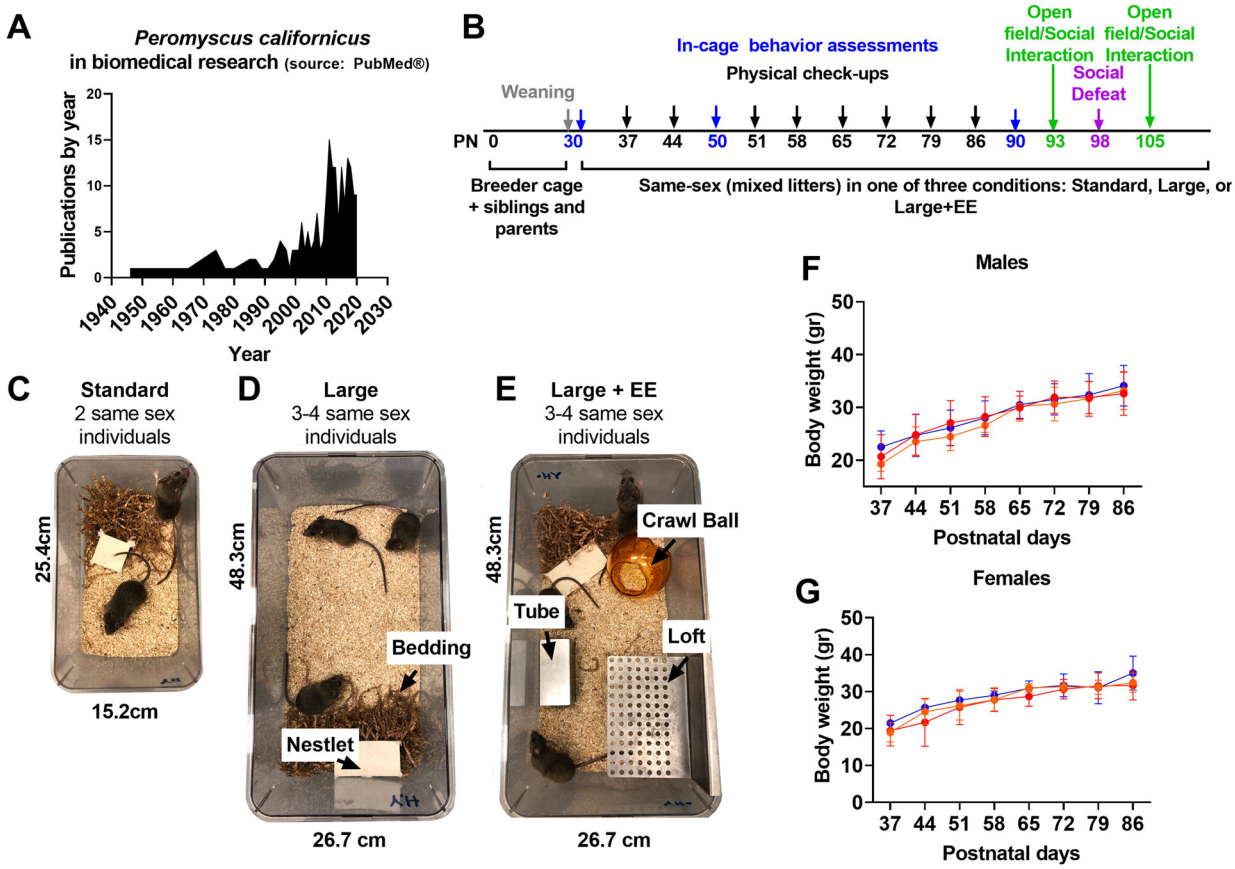

Fig.1.

A: Pubmed® timeline results of publications using *Peromyscus californicus* by year. **B:** Experimental timeline. **C,D,E:** Example pictures of the three experimental housing conditions: standard, large, and large + Enrichment (EE). **F,G:** Mean and standard error of bodyweight measures throughout development were not affected by housing conditions in males (repeated measures two way ANOVA, standard n=11, large n=12, large+EE n=12). or females (standard n=6, large n=4, large+EE n=3).

A

Behavior	Description
Backflipping	Focus animal performs repetitive locomotor pattern which resembles a backwards somersault
Autogrooming	Focus animal licks/pulls at own body/pelage
Prosocial	Focus animal smells, grooms, sniffs, or mounts a cagemate
Rearing	Focus animal puts its weight on its hind legs, raises its forelimbs from the ground and extends its head upwards
Digging	Focus animal moves forepaws on a series of fast alternating movements while scraping bedding

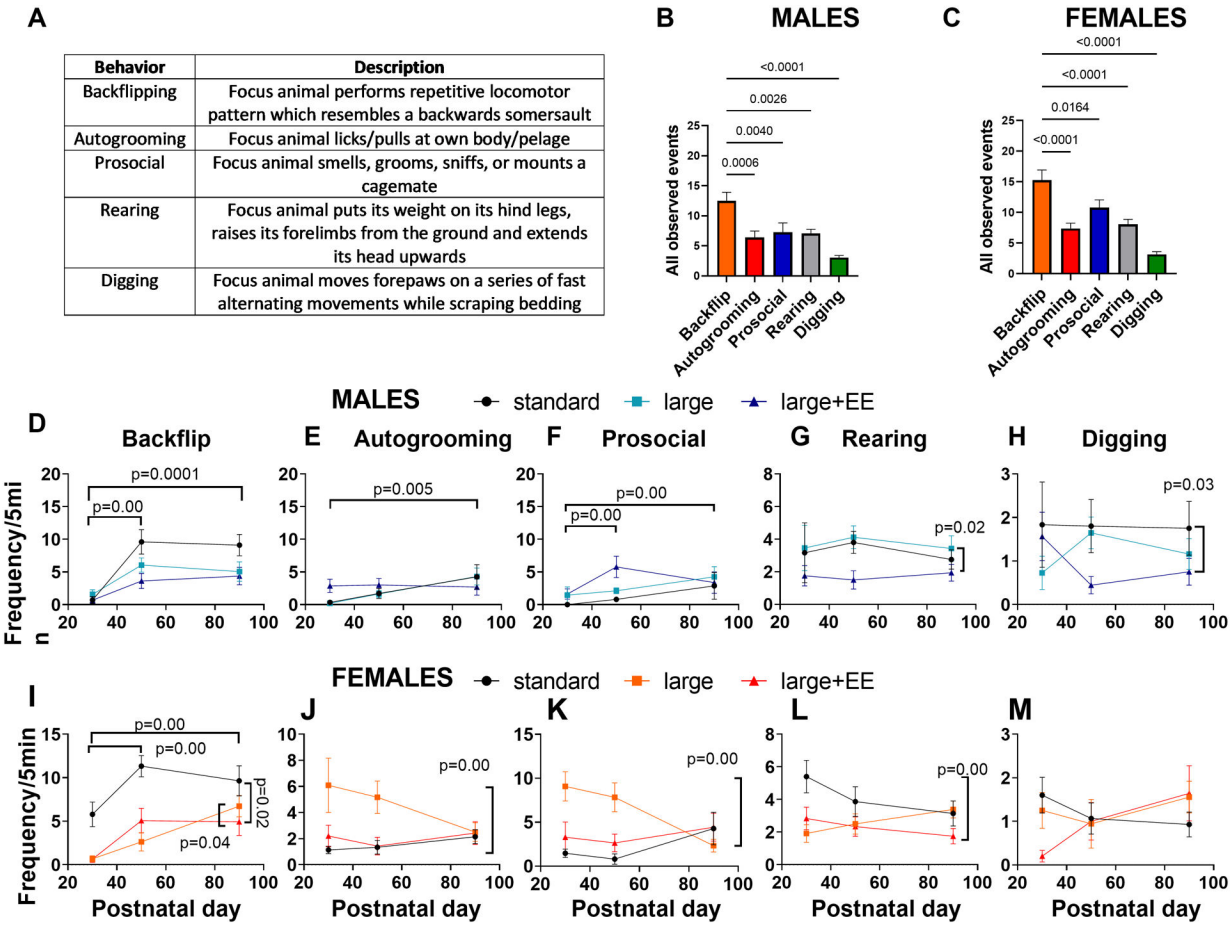

Fig. 2.

A. Table with description of behaviors recorded during home cage observation. **B,C:** Mean and standard error of total frequency (sum of all events recorded at P30, P50, and P50) of each of the behaviors independent of treatment or age using one-way ANOVA followed by Dunnett's multiple comparisons test in males (n=48) and females (n=48). **D–M:** General linear mixed model analyses for each behavior with considering individual animal as a random term, treatment as the fixed term (using standard cage as reference) and age as a covariate (using PN30 as reference) separated by sex. Graphs show mean and standard error.

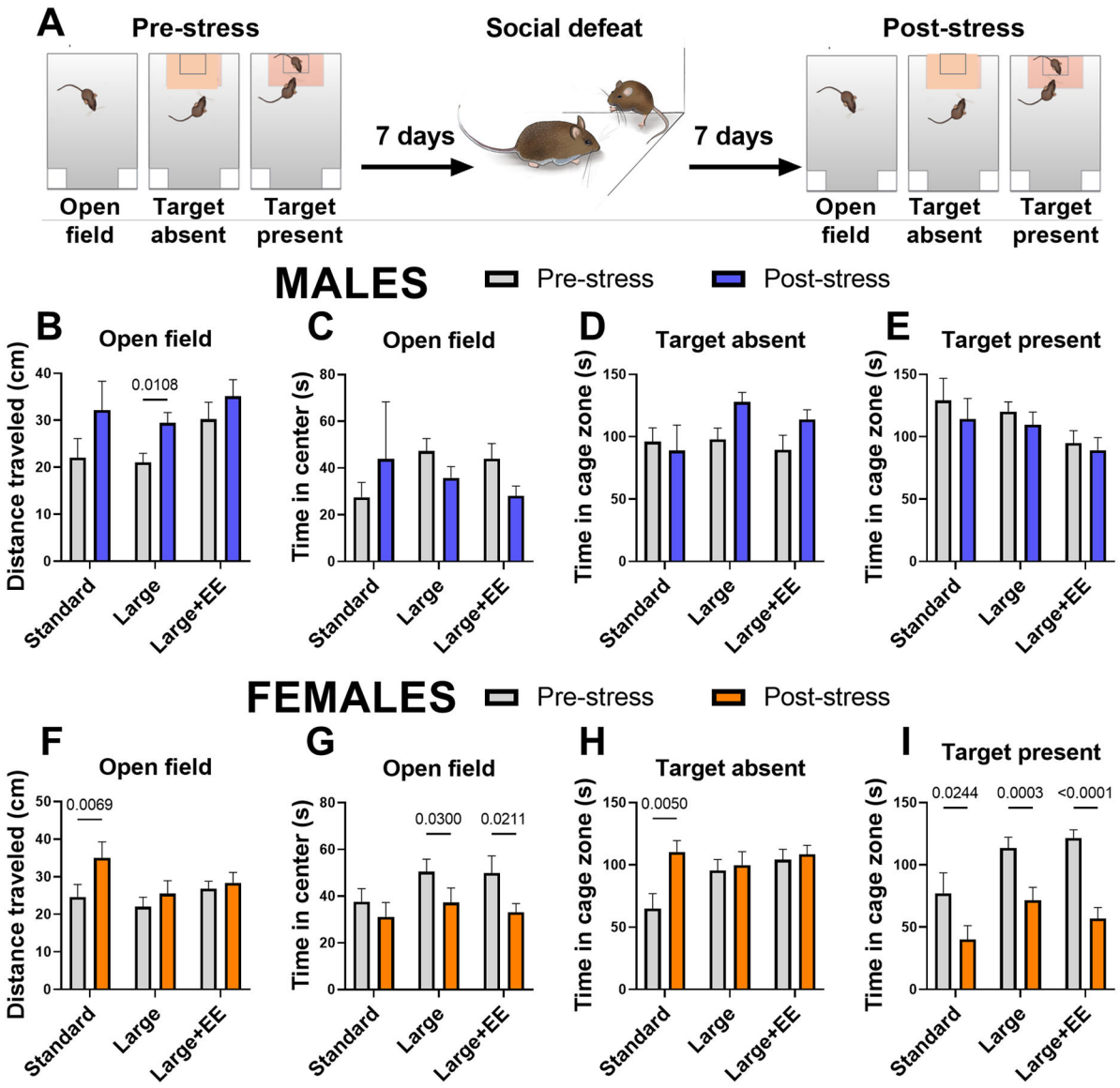

Fig. 3.

A. Drawing representing open field and social interaction tests performed before and after social defeat stress. **B–I**. 2-way repeated measures ANOVAs of behaviors tracked in the open field and social interaction test with Sidak correction for multiple comparisons. Numbers indicate significant (<0.05) p values. All graphs show mean and standard error.

Table 1.

Summary of home cage behavior data in males

Behavior	Treatment	Age	N	Minimum	1st Quantile	Median	Mean	3rd Quantile	Maximum	SD	SE	CI
Backflips	Standard	-	24	0	0.00	4.50	5.75	10.50	14	5.59	1.14	2.36
	Large	-	57	0	0.00	3.00	4.91	10.00	18	5.56	0.74	1.48
	Large+EE	-	48	0	0.00	0.50	3.88	8.00	16	5.26	0.76	1.53
	-	StereoPN30	43	0	0.00	0.00	1.91	2.00	13	3.57	0.54	1.10
	-	StereoPN50	43	0	0.50	5.00	5.81	10.50	18	5.40	0.82	1.66
	-	StereoPN90	43	0	0.00	5.00	6.33	12.50	18	6.07	0.93	1.87
Autogrooming	Standard	-	24	0	0.00	1.00	2.21	2.25	14	3.66	0.75	1.55
	Large	-	47	0	0.00	1.00	2.38	2.00	18	4.15	0.60	1.22
	Large+EE	-	50	0	0.00	1.00	2.86	3.00	19	4.36	0.62	1.24
	-	StereoPN30	33	0	0.00	0.00	1.52	1.00	15	3.11	0.54	1.10
	-	StereoPN50	45	0	0.00	1.00	2.20	3.00	16	3.21	0.48	0.96
	-	StereoPN90	43	0	0.00	1.00	3.70	4.50	19	5.29	0.81	1.63
Dig	Standard	-	24	0	0.00	1.50	1.79	3.00	6	1.91	0.39	0.81
	Large	-	47	0	0.00	1.00	1.23	2.00	4	1.48	0.22	0.43
	Large+EE	-	50	0	0.00	0.00	0.90	1.00	8	1.57	0.22	0.45
	-	StereoPN30	33	0	0.00	0.00	1.33	2.00	8	1.98	0.34	0.70
	-	StereoPN50	45	0	0.00	1.00	1.20	2.00	6	1.50	0.22	0.45
	-	StereoPN90	43	0	0.00	0.00	1.12	2.00	5	1.48	0.23	0.46
Social Interactions	Standard	-	24	0	0.00	0.00	1.29	1.00	17	3.46	0.71	1.46
	Large	-	47	0	0.00	1.00	2.83	3.00	20	4.95	0.72	1.45
	Large+EE	-	50	0	0.00	1.00	3.68	4.00	20	5.98	0.85	1.70
	-	StereoPN30	33	0	0.00	0.00	1.27	1.00	14	3.23	0.56	1.15
	-	StereoPN50	45	0	1.00	1.00	3.29	4.00	19	4.95	0.74	1.49
	-	StereoPN90	43	0	0.00	1.00	3.67	2.50	20	6.39	0.97	1.97
Rear	Standard	-	24	0	1.75	3.00	3.29	4.00	11	2.69	0.55	1.14
	Large	-	47	0	0.00	3.00	3.68	5.50	12	3.49	0.51	1.02
	Large+EE	-	50	0	0.00	0.50	1.72	3.00	8	2.28	0.32	0.65
	-	StereoPN30	33	0	0.00	0.00	25.8	4.00	12	3.67	0.64	1.30
	-	StereoPN50	45	0	1.00	3.00	3.00	5.00	10	2.77	0.41	0.83
	-	StereoPN90	43	0	0.00	2.00	2.74	4.00	12	2.72	0.41	0.84

Table 2.

Summary of home cage behavior data in females

Behavior	Treatment	Age	N	Minimum	1st Quantile	Median	Mean	3rd Quantile	Maximum	SD	SE	CI
Backflips	Standard	-	30	0	3.25	8.00	8.17	13.00	18	5.86	1.07	2.19
	Large	-	57	0	0.00	1.00	4.26	6.00	17	5.56	0.74	1.47
	Large+EE	-	42	0	0.00	2.00	4.79	9.50	17	5.46	0.84	1.70
	-	StereoPN30	43	0	0.00	0.00	1.56	2.50	13	2.81	0.43	0.86
	-	StereoPN50	43	0	1.00	7.00	7.37	13.00	17	6.08	0.93	1.87
	-	StereoPN90	43	0	1.00	7.00	7.09	12.50	18	5.90	0.90	1.81
Autogrooming	Standard	-	44	0	0.00	1.00	1.52	2.00	7	1.78	0.27	0.54
	Large	-	48	0	0.00	2.00	4.40	7.00	19	5.29	0.76	1.54
	Large+EE	-	38	0	0.00	1.00	2.00	3.00	12	2.80	0.45	0.92
	-	StereoPN30	37	0	0.00	1.00	3.03	3.00	19	4.78	0.79	1.59
	-	StereoPN50	47	0	0.00	0.00	2.83	4.00	16	4.11	0.60	1.21
	-	StereoPN90	46	0	0.00	1.50	2.37	3.00	12	2.78	0.41	0.83
Dig	Standard	-	44	0	0.00	1.00	1.20	2.00	4	1.37	0.21	0.42
	Large	-	48	0	0.00	1.00	1.25	2.00	10	1.85	0.27	0.54
	Large+EE	-	38	0	0.00	0.00	1.03	1.00	8	1.81	0.29	0.59
	-	StereoPN30	37	0	0.00	1.00	1.11	2.00	4	1.41	0.23	0.47
	-	StereoPN50	47	0	0.00	0.00	1.00	1.00	10	1.84	0.27	0.54
	-	StereoPN90	46	0	0.00	1.00	1.39	2.00	8	1.72	0.25	0.51
Social Interactions	Standard	-	44	0	0.00	0.50	2.14	2.00	20	4.43	0.67	1.35
	Large	-	48	0	1.00	4.00	6.08	10.00	20	6.14	0.89	1.78
	Large+EE	-	38	0	0.00	1.00	3.47	3.75	18	4.99	0.81	1.64
	-	StereoPN30	37	0	1.00	2.00	4.43	6.00	20	5.50	0.90	1.83
	-	StereoPN50	47	0	0.00	1.00	4.04	8.50	17	5.76	0.84	1.69
	-	StereoPN90	46	0	0.00	1.00	3.57	5.00	20	5.33	0.79	1.58
Rear	Standard	-	44	0	2.00	3.00	4.16	6.00	15	3.50	0.53	1.07
	Large	-	48	0	0.75	3.00	2.69	4.00	10	2.34	0.34	0.68
	Large+EE	-	38	0	0.25	1.00	1.97	3.00	8	2.05	0.33	0.67
	-	StereoPN30	37	0	1.00	3.00	3.62	5.00	15	3.28	0.54	1.09
	-	StereoPN50	47	0	1.00	2.00	2.79	4.00	14	2.88	0.42	0.85
	-	StereoPN90	46	0	0.25	3.00	2.65	4.00	7	2.37	0.35	0.70