Received: 22 February 2022 | Revised: 20 May 2022 | Accepted: 7 June 2022

DOI: 10.1111/jne.13179

ORIGINAL ARTICLE

Journal of Neuroendocrinology WILEY

The emotional impact of disrupted environmental contexts: Enrichment loss and coping profiles influence stress response recovery in Long–Evans rats

Molly Kent[1] | Dmitry Kovalev[2] | Benjamin Hart[2] | Danielle Leserve[2] | Gabriella Handford[1] | Dylan Vavra[2] | Kelly Lambert[2]

[1]Department of Biology, Virginia Military Institute, Lexington, VA, USA

[2]Department of Psychology, University of Richmond, Richmond, VA, USA

Correspondence
Kelly Lambert, B326 Gottwald Science Center, Dept. of Psychology, University of Richmond, Richmond, VA 23173, USA.
Email: klambert@richmond.edu

Abstract

With increasing rates of anxiety and mood disorders across the world, there is an unprecedented need for preclinical animal models to generate translational results for humans experiencing disruptive emotional symptoms. Considering that life events resulting in a perception of loss are correlated with depressive symptoms, the enrichment-loss rodent model offers promise as a translational model for stress-initiated psychiatric disorders. Additionally, predisposed temperament characteristics such as coping styles have been found to influence an individual's stress response. Accordingly, male rats were profiled as either consistent or flexible copers and assigned to one of three environments: standard laboratory housing, enriched environment, or enriched environment exposure followed by downsizing to standard laboratory cages (i.e., enrichment-loss group). Throughout the study, several behaviors were assessed to determine stress, social, and reward-processing responses. To assess recovery of the stress response, fecal samples were collected following the swim stress in 3-h increments to determine the recovery trajectory of corticosterone (CORT) and dehydroepiandrosterone (DHEA) metabolite levels. Upon death, neural markers of neuroplasticity including doublecortin, glial fibrillary acidic factor, and brain-derived neurotrophic factor were assessed via immunohistochemistry. Results indicated the flexible coping animals in the continuous enriched group had higher DHEA/CORT ratios (consistent with adaptive responses in past research); furthermore, the enrichment-loss animals exhibited a blunted CORT response throughout the assessments and enriched flexible copers had faster CORT recovery rates than consistent copers. Standard housed animals exhibited less exploratory behavior in the open field task and continuous enriched, flexible rats consumed more food rewards than the other groups. No differences in neuroplasticity neural markers were observed. In sum, the results of the present study support past research indicating the disruptive consequences of enrichment-loss, providing evidence

that the model represents a valuable approach for the investigation of neurobiological mechanisms contributing to interindividual variability in responses to changing experiential landscapes.

KEYWORDS
coping, corticosterone, dehydroepiandrosterone (DHEA), enrichment loss, stress

1 | INTRODUCTION

With approximately 350 million people diagnosed with depression across the world, depressive disorders have become one of the largest contributors to global disease burden[1–3] and, tragically, a contributing factor for approximately two-thirds of suicidal deaths.[1] Among the life events that are associated with depressive symptoms (and other illnesses) are various forms of psychological loss, including the death of loved ones, divorce, loss of one's home, or diminished autonomy as a result of illness or injury.[2,3] These findings are of special interest amid the recent global pandemic in which personal losses have reached unprecedented rates.[4] Recent surveys suggest that approximately 30% of adults in the USA have reported symptoms of anxiety and depression; furthermore, approximately 10% reported suicidal ideation.[5]

With increasing rates of stress-related disorders, appropriate animal models are necessary to understand critical mechanisms involved in the transition from stress exposure to the emergence of psychiatric disorders or, alternatively, the identification of mechanisms of emotional resilience in these situations. It is important to recognize that resilience is the most typical response to various stressors, whereas stress-related pathology occurs less frequently.[6] Several preclinical animal models of depression exist, including chronic unpredictable stress, social defeat, and early-life stress, which generate relevant neurobiological consequences such as altered responses to stress challenges, diminished sucrose preference, and decreased exploration. However, few innovative treatments have emerged since antidepressant psychotropic interventions increased in popularity through the 1990s.[7]

The recently introduced enrichment-loss rodent model involves removing rats from their familiar enriched/complex environments and rehousing them in standard laboratory cages.[8] In this model, aspects of both physical and social contexts are lost, representing similarities (i.e., face validity) with many forms of human psychological loss. The results suggest that this rodent model of enrichment-loss results in a depression-like phenotype characterized by increased helplessness behavior, increased body weight, and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis.[8,9] This enrichment-loss model corroborates previous findings that abrupt removal from an enriched environment was associated with rats' diminished motivation to explore in a radial arm maze.[10] Recently, enrichment disruption has been explored in mice noting increased aggression and social distancing,[11] as well as impaired cognition in mice exposed to both maternal immune activation and subsequent social isolation following long-term enriched environment exposure.[12] Numerous studies have confirmed that exposure to complex or enriched environments enhances neuroplasticity markers in laboratory animals[13,14] with evidence of individual differences[15,16]; however, the pervasiveness and persistence of these effects, especially when animals are permanently relocated to more impoverished conditions, is a clinically relevant question deserving further investigation.

Neurobiological mechanisms associated with HPA axis dysregulation have been identified as biological markers for depression susceptibility.[17,18] Cortisol, for example, is an adrenal steroid that enhances vigilance, arousal, and targeted attention processes considered to increase an animal's likelihood of surviving a threat, concurrently decreasing energy to long-term functions such as reproduction and growth.[19] Also of interest is the influence of the adrenal steroid, dehydroepiandrosterone (DHEA), which is released synchronously with cortisol and is associated with anti-glucocorticoid effects, resulting in potential neuroprotection during chronic stress effects.[20] Specifically, higher levels of DHEA in rodents, especially in proportion to corticosterone (CORT) levels, are associated with enhanced stress resilience.[21–23] Probable DHEA-regulated stress resilience mechanisms include the interference of 7-hydroxylated DHEA metabolites with hippocampal glucocorticoid receptors[24–26]; additionally, DHEA has been proposed to have an impact on GABA(A) and NMDA receptors.[27,28] During apparently adaptive stress responses, DHEA-regulated mechanisms mitigate excessive stress responses that increase susceptibility to the vulnerable allostatic load state.[29] Accordingly, higher DHEA/cortisol ratios are correlated with larger hippocampal volume in depressed and non-depressed human participants[30]; furthermore, hypercortisolemia and lower DHEA/cortisol ratios are correlated with treatment-resistant depression.[31] Individual differences in HPA axis function[32,33] suggest that an exploration of individual coping strategies may provide important information about resilience in stressful contexts. In general, more active coping styles have been associated with enhanced emotional resilience[34]; however, past research in our laboratory has indicated that animals with flexible coping strategies exhibit more signs of resilience than rats with more consistently active or passive strategies.[35–38]

Of interest in the present study was the impact of enrichment loss and the animal's predisposed coping style on behavioral responses in various assessments of stress and anxiety (open field, swim task), anhedonia (novelty suppressed sweet cereal consumption), and social contact. Additionally, corticosterone and DHEA responses to swim stress over the course of a 6-h recovery period were observed across the varied environment and coping groups.

Finally, because depressogenic symptoms have been associated with hippocampal plasticity measures, a few markers of plasticity including doublecortin (DCX[39]), brain-derived neurotrophic factor (BDNF[40,41]), and glial fibrillary acidic protein (GFAP) were quantified in the dorsal hippocampus.[41,42] It was hypothesized that the flexible coping animals consistently housed in the enriched environment would exhibit the highest degree of emotional resilience with more behavioral exploration, increased interest in social contact, increased rate of food reward consumption, a faster recovery of corticosterone and DHEA to non-stressed levels, and increased markers of hippocampal neuroplasticity.

2 | MATERIALS AND METHODS

2.1 | Animals

All experimental procedures were conducted in accordance with the University of Richmond, Institutional Animal Care and Use Committee. On postnatal day (PND) 21, 30 male Long–Evans (*Rattus norvegicus*) rats were weaned from their on-site bred mothers ($n = 12$) and housed in triads in standard laboratory cages (35.6 × 29.2 × 16.5 cm) with corncob bedding and with food and water provided ad libitum. Once coping profiles were determined, it was confirmed that there were no effects of litter on coping profiles (i.e., no differences among litters) before randomly assigning rats to treatment groups. Additionally, because the number of animals in each coping profile group is variable as a result of the individual characteristics of the animals at the time of assessment, six additional male rats were obtained from Envigo, Inc. on PND 24 to conduct additional profile assessments, so that each group would have the appropriate number of animals. Rats were maintained under a 12:12 h light/dark photocycle (lights on 8:30 a.m.). In all behavioral assessments, a single observer was used when the observational software was not appropriate to use; specific details for each behavioral task are provided below.

2.2 | Coping profile assessment

After 1 week of acclimation to the facilities, all rats were profiled for coping strategies on PND 31 (in accordance with Kent at al.[35]). Briefly, rats were gently restrained for 1 min by being held by the experimenter in a supine position and the number of escape attempts (or repositions) was recorded. On PND 38, rats were again gently restrained for 1 min and the number of escape attempts was recorded. After behavioral assessments, rats were classified as representing one of two coping strategies. Consistent coping rats ($n = 18$) exhibited similar numbers of escape attempts during both profile assessments. A consistent coping rat was characterized by either a consistently high number of repositions (i.e., 8 or more) or a consistently low number of repositions (i.e., 4 or fewer attempts) during both assessments. Rats that varied their escape strategies from high to low or vice-versa, showing variability in their responses were categorized as flexible coping rats (n = 18). In alignment with previous studies in our lab, a difference of at least four escape attempts observed during the two assessments defined the flexible strategy. After coping profiles were determined, all rats were randomly assigned to one of two housing environment groups: standard ($n = 12$, pair-housed with animals matching their coping strategies in standard lab cages), enriched ($n = 24$, housed in groups of 12 in large enclosures: 90 × 60 × 90 cm). Half of the enriched animals were subsequently assigned to an enrichment loss group ($n = 12$). Enrichment loss animals were subsequently pair-housed in standard lab cages based on coping strategy. All environment groups had an equal number of consistent and flexible coping rats.

2.3 | Environmental enrichment

Rats were placed in large enclosures and maintained in enriched environments on PND 38[43] with at least one hiding structure and approximately three objects that could be manipulated (i.e., shells, balls, blocks) and an object that could be climbed on (i.e., ladders, sticks, etc.). The cage floor was covered with a 50:50 mixture of corncob bedding (Envigo) and coconut fiber substrate (Zoo Med) that has a texture similar to dirt aiming to provide a more natural environment and facilitate digging behaviors. Enrichment items were switched every 3 days with bedding changes occurring every week. Enriched rats and standard-housed rats were housed in the same room for the duration of the experiment. On PND 56, the enrichment loss group ($n = 12$) was created by moving 12 rats from the enriched environment to standard laboratory cages, pair-housed with animals matching their coping strategies. The standard housed ($n = 12$), continuous enriched ($n = 12$), and enriched-loss ($n = 12$) rats were maintained in these housing environments for the duration of the experiment (Figure 1).

2.4 | Handling

Starting on PND 26, body weights were recorded on a weekly basis throughout the duration of the study. To obtain accurate weights and prepare the rats for behavioral testing, rats were briefly handled for approximately 1 min each day. Following handling, rats received a food reward (i.e., a piece of Froot Loop® sweet cereal). All rats received the same number of food rewards prior to behavioral testing. The food reward was used as an incentive during behavioral testing.

2.5 | Behavioral assessments

2.5.1 | Open field test

Starting on PND 70, animals were placed in a plexiglass open-field arena (108 × 108 cm). The open-field test consisted of four distinct

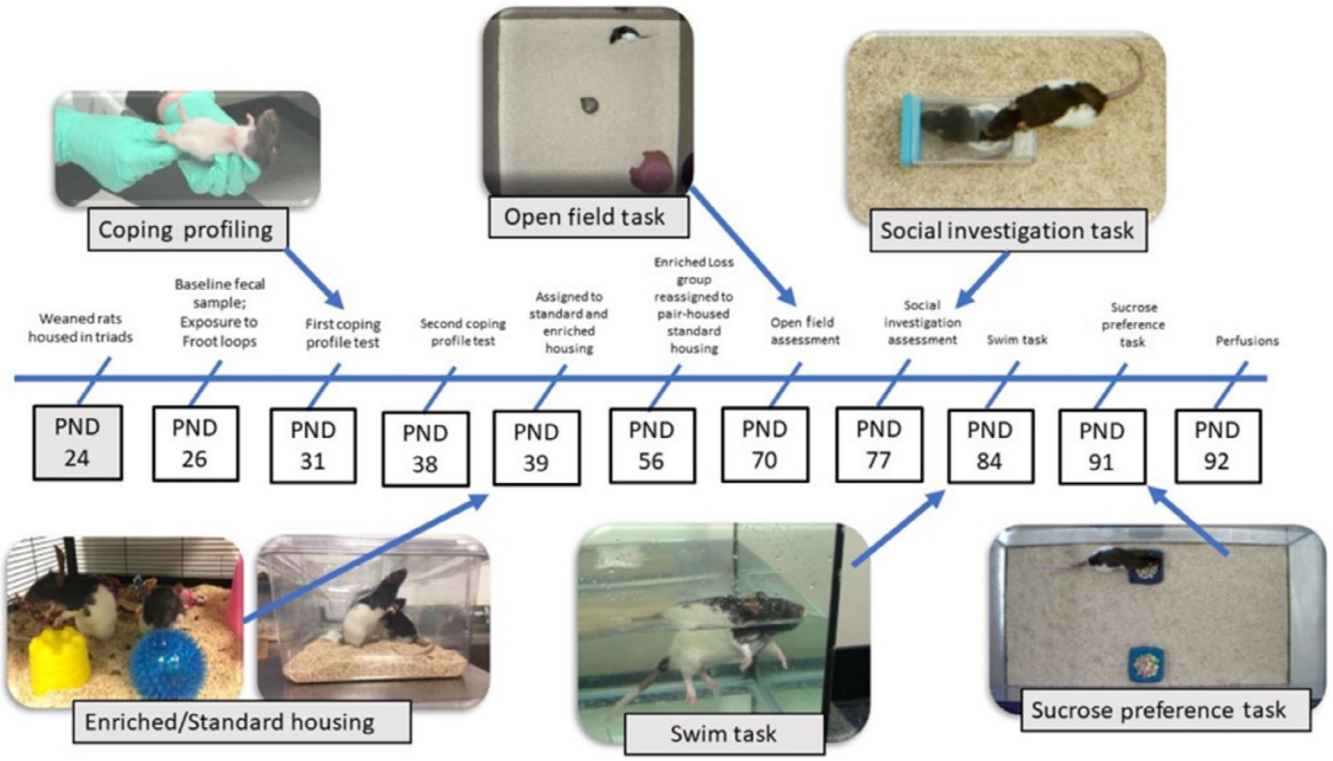

FIGURE 1 Timeline of experiment. As depicted, the timeline starts at postnatal day (PND) 24 and continues with coping profile assessments, environmental assignments, behavioral tasks, and tissue collection.

phases: (1) habituation to testing arena, (2) assessment in the arena with a hiding structure familiar to animals in the enriched environments (small plastic structure shaped like a tree stump), (3) assessment in the arena that included a hiding structure, as well as the addition of a predator scent (teaspoon of soiled cat litter) (Figure 1), and (4) assessment in the arena with a novel hiding structure (upside-down wicker basket) unfamiliar to both groups with a novel predator scent (i.e., from a different, unfamiliar cat). The predator scent was placed in the arena in a small metal bowl and was changed after every third animal to provide a fresh sample. Corncob bedding was redistributed after each trial to remove evidence of distinct paths of the previous animal[36]; additionally, animals were randomly assigned to the testing order to avoid any timing and group bias. The four trials were run consecutively over 4 days, and behaviors were scored both by hand and using Noldus tracking software (Noldus). A random numbers table was used to determine the order each rat assessed in the arena. Trials were 5 min in duration during which frequencies of the following behaviors were scored by blind observers: freezing bouts and rearing responses (internal and external directions). When the hiding structure was introduced to the arena, frequencies of entering, climbing on top of and hiding behind the structure were also recorded. The same behavioral variables were assessed when the predator scent was introduced. Noldus recorded frequency, duration, and latency of movement, zone visits, and thigmotaxis.

2.5.2 | Social investigation assessment

On PND 77, rats were subjected to a social investigation test (SIT) in a glass tank (76 × 32 × 31 cm) with a layer of corncob bedding (3 cm) (Figure 1). The SIT was conducted in two phases. The first phase was habituation to the arena and the plastic holding container. The second phase introduced a novel conspecific inside the plastic holding container (19 × 10 × 10 cm). The duration of all trials was 5 min. During the first phase, rats were habituated to the tank and the plastic holding container. During habituation, rearing (internal and external), and freezing responses were recorded by hand, again by blind observers. Noldus recorded latency to approach, frequency of visits in the central areas, and duration in specific zones of the tank (i.e., proximity zone to holding container and peripheral zone of the arena), as well as mobility and total movement. The same behaviors with the addition of digging bouts were recorded during the second phase of the SIT when a novel conspecific was introduced in a plastic container.

2.5.3 | Swim task

On PND 84, rats were exposed to 3 days of 5-min swim assessments in a large aquarium (76.2 × 31.6 × 55.8 cm) filled with room temperature water. Noldus software was used to record all trials (Figure 1). Days 1 and 3 of the swim task were conducted from 8:30 p.m. to

10:45 p.m. in a dimly lit room, whereas day 2 of the swim task was conducted from 2:00 p.m. to 5:00 p.m. in the same room. The later swim times for the first and third swim trials were used so fecal samples could be collected the subsequent morning to allow for an approximate 12-h delay to appropriately assess metabolites.[23] The following behaviors were scored by a blind observer from recorded videos: frequency of dives (full body swimming underwater) and half dives (head and shoulder area underwater in a swimming movement), frequency of sinks (controlled drop to the bottom of tank, and subsequent return to top of tank), and frequency of head-shake bouts. Noldus observational software was used to record latency, frequency, and duration of floating, as well as latency, frequency, and duration of swimming. Approximately 12 h later, fecal samples were taken after the first and third swim trials so they could be compared with baseline samples collected at the same time of day.

2.5.4 | Sucrose preference task

On PND 91, a modified sucrose preference task (SPT) was performed (Figure 1). This behavioral assessment was a combination of the traditional SPT and the novelty suppressed feeding test. Rats were food-restricted for 3 h prior to testing to enhance food motivation. During habituation, two food bowls were placed inside a glass tank (90.8 × 45.1 × 40 cm). Both food bowls were filled with the same amount of regular food chow to ensure that there was no place preference prior to the test phase. The habituation session lasted 5 min. To test for sucrose preference, rats were again food-restricted and then placed in the testing arena for 5 min the following day. Instead of using sucrose-supplemented water, a sweetened familiar cereal treat was provided for the animals in this task. Total weights of both chow and cereal treats were measured before and after the 5-min trial to mimic the drinking water and sucrose water options in the traditional SPT. Again, observational software (Noldus) was used to record place preference, mobility, latency to move, duration of time in proximity to the food bowls, and duration of time spent in proximity to the walls (thigmotaxic behavior). A blind observer recorded the number of internal and external rear responses (measures of arena exploration and escape responses, respectively), and number of emitted fecal boli.

2.6 | Physiological responses

2.6.1 | Endocrine responses

To assess stress and resilience responses, corticosterone and DHEA were extracted from fecal samples.[23,35] An original baseline fecal sample was obtained at 9:00 a.m., 2 days after the animals were placed in their assigned housing conditions. Fecal samples were collected by briefly placing rats into cages with no bedding and retrieving a fecal bolus once emitted (typically within 5 min). After a sample was collected, the rats were placed back into their home cages. To obtain a hormone sample from the swim test, fecal samples were taken approximately 12 h post stressor to allow for the targeted endocrine metabolites to work through the digestive system for detection in the collected fecal bolus.[23,35] Additionally, to assess recovery from the swim stress, samples were also collected at 12:00 p.m. and 3:00 p.m. so that the animals' stress hormone levels could be assessed for a 6-h period following the time of the stressor. Thus, in addition to the baseline sample, three fecal samples were collected after the first and last swim stress sessions to determine endocrine recovery from stress. Immediately following collection, each sample was stored in a 5-mL centrifuge tube and stored in the freezer at −80°C. Because varying conditions associated with fecal sample collection are known to impact fecal metabolites,[44] all samples were treated consistently (e.g., time of collection, storage of sample, etc.) to increase the reliability of the data.

For endocrine assays, fecal samples were measured to obtain 0.09 g of sample.[23,35] Each sample was then mixed with 100% methanol and vortexed for 30 s. Subsequently, the samples were centrifuged for 5 min at 769 g before extracting the supernatant for corticosterone and DHEA ELISAs (Enzo Life Sciences: ADI-901-097). Samples were run on an automated microplate reader (BioTek, version 2.04.11) and Gen5 software (BioTek, version 2.04.11) to obtain the optical density. Readings were assessed at a wavelength of 405λ. The optical density was then used to extrapolate the hormone concentrations from the standards run on each plate. Numerical values obtained from plates require an R^2 value greater than 98% to be included in the assessment. The CORT assay had a sensitivity of 27 pg mL^{-1} with a range of 32–20,000 pg mL^{-1}. The CORT kit had a cross-reactivity of less than one percent for progesterone (0.046%), testosterone (0.046%), tetrahydrocoticosterone (0.28%), aldosterone (0.18%), and cortisol (0.046%). The intra- and inter-assay coefficients of variations for the CORT assays were 4.43 and 11.15%. The DHEA assay sensitivity was 2.9 pg mL^{-1} and ranged between 12.21 pg mL^{-1} and 50,000 pg mL^{-1}. The DHEA assay had a cross-reactivity of less than 1% for progesterone (0.06%), testosterone (0.1%), aldosterone (0.29%), and cortisol (0.02%). The intra- and inter-assay coefficients of variations were 5.33 and 9.87% for DHEA.

2.6.2 | Histological preparations

Rats were placed in an induction chamber where isoflurane was administered to anesthetize the rat prior to the start of the perfusion. Once the animal was no longer responsive, chilled phosphate-buffered saline (PBS) was perfused through the heart at a rate of 40 ml/min using a MasterFlex perfusion pump to clear blood from the brain (approximately 200 mL of PBS) followed by 200 ml of 4% paraformaldehyde to prepare the tissue for immunohistochemistry. After 24 h in 4% paraformaldehyde at 4°C, brains were transferred sequentially from a 10% sucrose solution to a 30% sucrose solution. Once the brains were cryoprotected with the sucrose solution, 40 μm free-floating sections were sectioned on a cryostat (Thermo

Fisher Scientific) at −25°C. To prevent double-counting individual cells, every fourth consecutive section was taken for a specific immunohistochemical stain, allowing for at least 160 μm between each section analyzed per immunohistochemical stain. Sections were obtained through the dorsal hippocampus (−3.14 to −3.26 from bregma[45]).

2.6.3 | Immunohistology

Following sectioning, brain sections were immunostained for GFAP, BDNF, and DCX. For DCX and GFAP immunohistochemistry, perfused brain sections were first incubated for 10 min in a sodium citrate solution at 100°C water bath for antigen retrieval. Subsequent sections were incubated at room temperature in 0.1% hydrogen peroxide to quench endogenous peroxidase activity. Sections were then blocked for 60 min in 10% normal goat serum (Vector Laboratories) in PBST (0.3% Triton-X, Spectrum Chemical and 0.1% bovine serum albumin), (Jackson Immunoresearch Laboratories). After the blocking step, brains were incubated overnight at 4°C at a concentration of 1:250 for both DCX (rabbit polyclonal, Abcam Inc.: ab18723) and GFAP (mouse polyclonal; Abcam Inc.: ab10062). Following incubation with the primary antibody, sections were exposed to either a goat-anti-rabbit or goat-anti-mouse biotinylated secondary antibody (Vector Laboratories: BA5000, BA9200) at a concentration of 1:200 for 90 min. Following secondary exposure, sections were incubated in Avidin-Biotin Complex (Vector: PK6200) for 90 min. Finally, brain sections were incubated in a pre-3,3′-diaminobenzidine (DAB) solution (0.6% Tris buffer +0.3% NH_3Nis + 0.02% DAB) for 10 min. The DAB peroxidase substrate with 30% H_2O_2 was then added to the free-floating sections for another 10 min. Sections were subsequently floated onto subbed slides prior to being cleared with sequential dilutions of ethanol starting at 70% and ending at 100% before being cleared with Citrasolv and coverslipped with Permount (Electro Microscopy Sciences). The same process was used for the BDNF sections; however, the primary rabbit polyclonal antibody was used at a concentration of 1:500 (Bioss:BS-4989R) and the secondary goat anti-rabbit antibody (Vector) was used at a concentration of 1:200.

2.6.4 | Neuroquantification

BDNF immunoreactive cells in the CA1 and CA3 areas of the hippocampus were quantified using Neurolucida software (Microbrightfield, Inc.) and a Zeiss Axioskop light microscope (Carl Zeiss). The dentate gyrus was imaged for both the GFAP and DCX immunoreactive tissue. CA1 and CA3 were also imaged for GFAP. GFAP and DCX-immunoreactive cells were quantified using light-thresholding software (Bioquant); specifically, a percentage of immunoreactive tissue was calculated based on thresholding the immunoreactive cells in the imaged area (135 × 135 μm at 40× magnification) of the dentate gyrus, CA1 or CA3.

2.7 | Statistical analysis

All data were analyzed using SPSS, version 26 (IBM Corp.) and visualized with Prism, version 8 (GraphPad Software Inc.). A two-way 2 × 3 (coping × housing) analysis of variance (ANOVA) was used to analyze the neuronal data. Additionally, a 2 × 3 ANOVA (coping × housing) was used to analyze the open field, sucrose preference, and social preference behavioral data. A 2 × 3 × 3 mixed ANOVA (coping × housing × time) was used to analyze the swim data collected over the three swim sessions in all groups. For the endocrine assessments, a 2 × 3 × 7 mixed ANOVA (coping × housing × time) was used to analyze the hormone data from seven different time points (i.e., baseline, first swim stress and recovery sampling time points, and third swim stress and recovery time points). For all analyses, p <.05 was considered statistically significant. Individual ANOVAs and Tukey's post-hoc tests were used where appropriate to determine specific group differences. Because the n for each group was relatively low ($n = 6$); the partial eta squared (ηp2) values are reported for all data to provide additional information about the effect size of reported findings. Finally, for the percentage changes from the first to the third hormone assessment for each swim day, the percentage change was recorded as negative or positive, then a constant variable of 100 was added to all scores for further statistical analysis; consequently, scores below the 100% threshold represent negative scores. Please see Appendix for Table including statistical information for nonsignificant findings for all dependent variables.

3 | RESULTS

3.1 | Behavioral assessments

3.1.1 | Open field test

During the habituation stage of the open field test a 2 × 3 ANOVA indicated no significant differences in behavior among the groups. When the hiding structure was introduced into the open field test in the second phase of assessment, a 2 × 3 ANOVA revealed a significant main effect of environment on the number of external rears (i.e., rears directed toward the walls of the arena; $F_{2,30} = 4.835$, $p = .015$, ηp2 = 0.244). Post-hoc analysis indicated the enriched loss group had significantly more external rears compared to the enriched group ($p = 0017$) (Figure 2A). The frequency of climbing on top of the hiding structure also indicated a significant main effect of environment ($F_{2,30} = 11.412$, $p < .005$, ηp2 = 0.432). Further analysis indicated the standard group climbed on top of the hide structure significantly less than both the enriched ($p = .005$) and the enriched loss ($p < .001$) groups (Figure 2B). When the predator odor was first introduced, a 2 × 3 ANOVA indicated a significant interaction between environment and coping strategy on the number of internal rears (directed toward the center of the arena; $F_{2,30} = 4.872$, $p = .015$, ηp2 = 0.245). The flexible coping group exhibited little variability among environments but standard-housed animals in

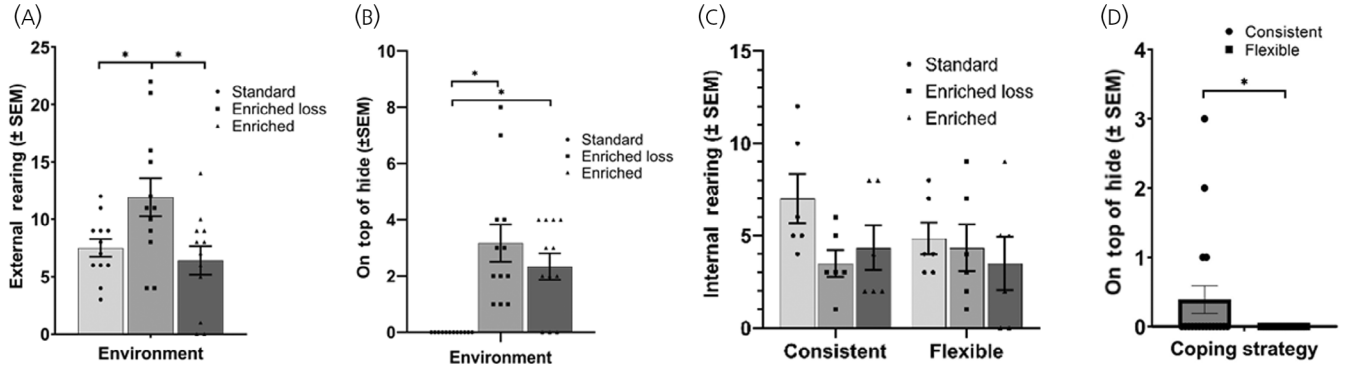

FIGURE 2 Open field tasks. (A) Frequency of external rears after the hiding structure was introduced. (B) Frequency of times the rat climbed on top of the hide structure in the open field arena. (C) Frequency of internal rear responses in phase 3 when predator scent was introduced to consistent and flexible copers in varying environments (data depict the significant coping by environment interaction). (D) Frequency of climbs on hiding structure during the fourth assessment with novel hiding structure and predator scent. Data are expressed as the mean ± SEM. *$p \leq .05$.

the consistent coping group exhibited more rears compared to the enriched-loss group (Figure 2C). During a second predator odor exposure with a novel hiding structure, a 2 × 3 ANOVA indicated a significant main effect of coping on the behavior of climbing on top of the hiding structure ($F_{1,30} = 4.623$, $p = .04$, ηp2 = 0.0.134). Specifically, the consistent coping group climbed onto the hide more than the flexible coping group (Figure 2D). No significant effects were observed in the remaining dependent variables including freezing bouts, zone visits, and thigmotaxis.

3.1.2 | Social functioning task

A 2 × 3 ANOVA indicated no significant differences in behaviors quantified for the social functioning task. The behaviors that were analyzed included internal and external rearing, digging toward and away from the restraint container, and proximity to the container.

3.1.3 | Swim task

A 3 × 2 × 3 repeated-measures ANOVA was used to analyze the differences among groups over the three different swim tasks. An effect of time was observed for all behavioral measures but there was no significant effect of treatment groups. The behaviors analyzed included duration of swimming, latency to commence floating, duration of floating, frequency of swimming bouts, bouts of floating, frequency of dives, and half-dives.

3.1.4 | Sucrose preference task

For the sucrose preference task, a 2 × 3 ANOVA indicated a significant main effect of environment on the amount of cereal consumed ($F_{2,30} = 4.407$, $p = .021$, ηp2 = 0.0.227). Post-hoc analysis indicated a significant difference between the enriched and enriched-loss groups ($p = .028$) and a trend between the enriched and standard groups ($p = .06$). Specifically, the enriched group consumed more cereal treats compared to the other groups, an effect not observed for the chow consumption (Figure 3A). For clarification, no significant differences in body weight were observed among environment groups (Figure 3B); additionally, coping strategy also failed to influence bodyweight throughout the duration of the study.

3.2 | Physiological responses

3.2.1 | Hormone analysis

A three-way mixed ANOVA revealed a three-way interaction of time, environment, and coping strategy on fecal CORT metabolites ($F_{2,174} = 2.343$, $p = .008$, $\eta_p^2 = 0.0.139$) [see Figure 4A for distribution of CORT fecal metabolites for coping × environment at all time points and subsequent graphs (B–E) for specific statistically significant effects]. To determine the sources of this interaction, two-way ANOVAs (environment × coping) were conducted at each time point. As a reminder, three time points were assessed following the first and third consecutive days of swim stressors; as a result of the metabolite delays in the fecal sample, the 9:00 a.m. sample is representative of the stress experience whereas the 12:00 p.m. and 3:00 p.m. samples represent three and 6-h delays following the stressor, respectively.[23] Post-hoc analyses identified a significant main effect of environment at 9:00 a.m. following the day 1 swim stress ($F_{2,29} = 4.28$, $p = .023$, $\eta_p^2 = 0.228$) with the standard housed group exhibiting significantly higher fecal CORT metabolite levels than the enriched loss group ($p = .022$) (Figure 4B). Additionally, at 3:00 p.m. on day 1, a significant effect of coping style was found ($F_{1,29} = 4.67$, $p = .039$, $\eta_p^2 = 0.0139$); specifically, the consistent coping group exhibited higher levels of fecal CORT metabolites compared to the flexible group (Figure 4C). On day 3 of swim stress, post-hoc analyses indicated a significant effect of environment at 12:00 p.m. ($F_{2,29} = 3.59$, $p = .041$, $\eta_p^2 = 0.198$) (Figure 4D). Specifically, the enriched group had significantly higher fecal CORT metabolite

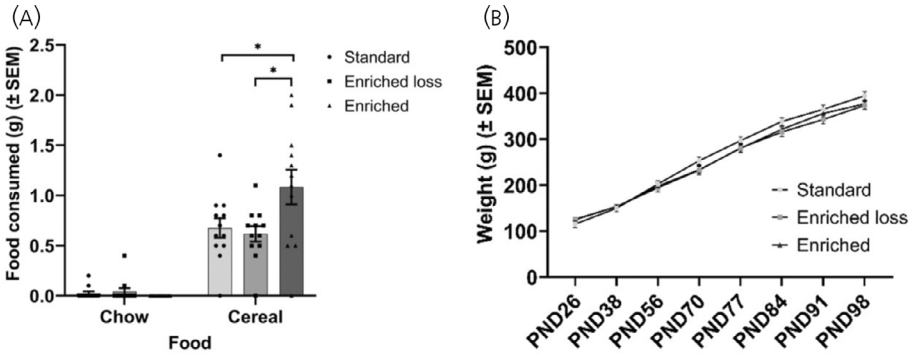

FIGURE 3 Sucrose preference test. (A) Total amount of chow and cereal consumed during preference test. (B) Body weight of animals in each environmental group (grams). Data are expressed as the mean ± SEM; *$p \leq .05$. PND, postnatal day

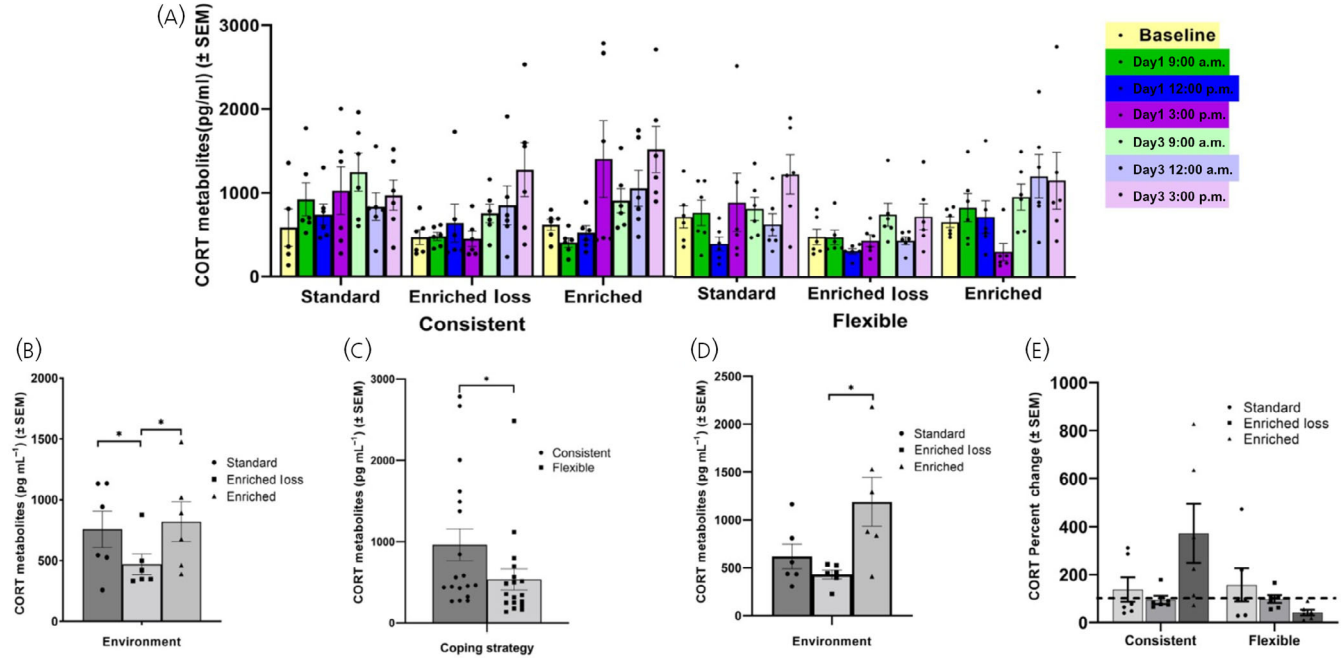

FIGURE 4 Corticosterone (CORT) levels. (A) Distribution of CORT fecal metabolites (pg mL^{-1}) for all coping and environmental groups at each time point. (B) CORT metabolites (pg mL^{-1}) 12 h after the first day of stress swim representing the initial stress experience. (C) CORT metabolites (pg mL^{-1}) 18 h after the first stress swim representing 3 h post initial stress experience. (D) CORT metabolites (pg mL^{-1}) 18 h after the third stress swim representing 6 h post initial stress experience. (E) Percentage change in CORT metabolites from samples representing initial stress exposure and 6-h recovery after the first stress swim; shown as environment × coping groups. Data are expressed as the mean ± SEM percentage change of CORT metabolites; *$p \leq .05$.

levels compared to the enriched- loss group ($p = .045$). To determine recovery efficiency of the CORT response, the percentage change of CORT values from 9:00 a.m. to 3:00 p.m. was determined; accordingly, a significant interaction of coping by environment was observed on day 1 of swim stress ($F_{1,29} = 4.552$, $p = .019$, $\eta_p^2 = 0.76$) (Figure 4E). Specifically, the interaction was influenced by the variability of the enriched animals with the consistent coping enriched animals exhibiting a marked increase in CORT metabolites throughout the recovery period (i.e., over 300% higher than the standard and enriched loss groups), whereas the flexible coping enriched animals exhibited metabolite values that were less than 50% of the comparative housing groups (Figure 4E).

A three-way mixed ANOVA revealed a significant interaction between time and environment on fecal DHEA metabolites ($F_{12,174} = 1.87$, $p = .041$, $\eta_p^2 = 0.0.114$) [see Figure 5A for distribution of DHEA values and subsequent graphs (B–G) for specific statistically significant effects]. Further post-hoc analysis indicated a significant effect at 3:00 p.m. on day 1 of swim stress ($F_{2,32} = 3.65$, $p = .037$, $\eta_p^2 = 0.186$). Specifically, the enriched-loss group had lower levels of DHEA compared to the standard group ($p = .036$) (Figure 5B). Analysis of percentage change of DHEA scores from 9:00 a.m. to 3:00 p.m. following swim stress to monitor the recovery rate 6 h following the stressor indicated a significant main effect of environment on day 1 ($F_{2,29} = 3.913$, $p = .031$, $\eta_p^2 = 0213$) and day 3 ($F_{2,29} = 4.66$, $p = .018$, $\eta_p^2 = 0.0.243$) (Figure 5C,D, respectively). On each day, post hoc analyses indicated that the standard housed animals had higher scores (i.e., maintained higher metabolite levels) than the enriched animals on day 1 ($p = .049$), as well as on day 3 ($p = .018$).

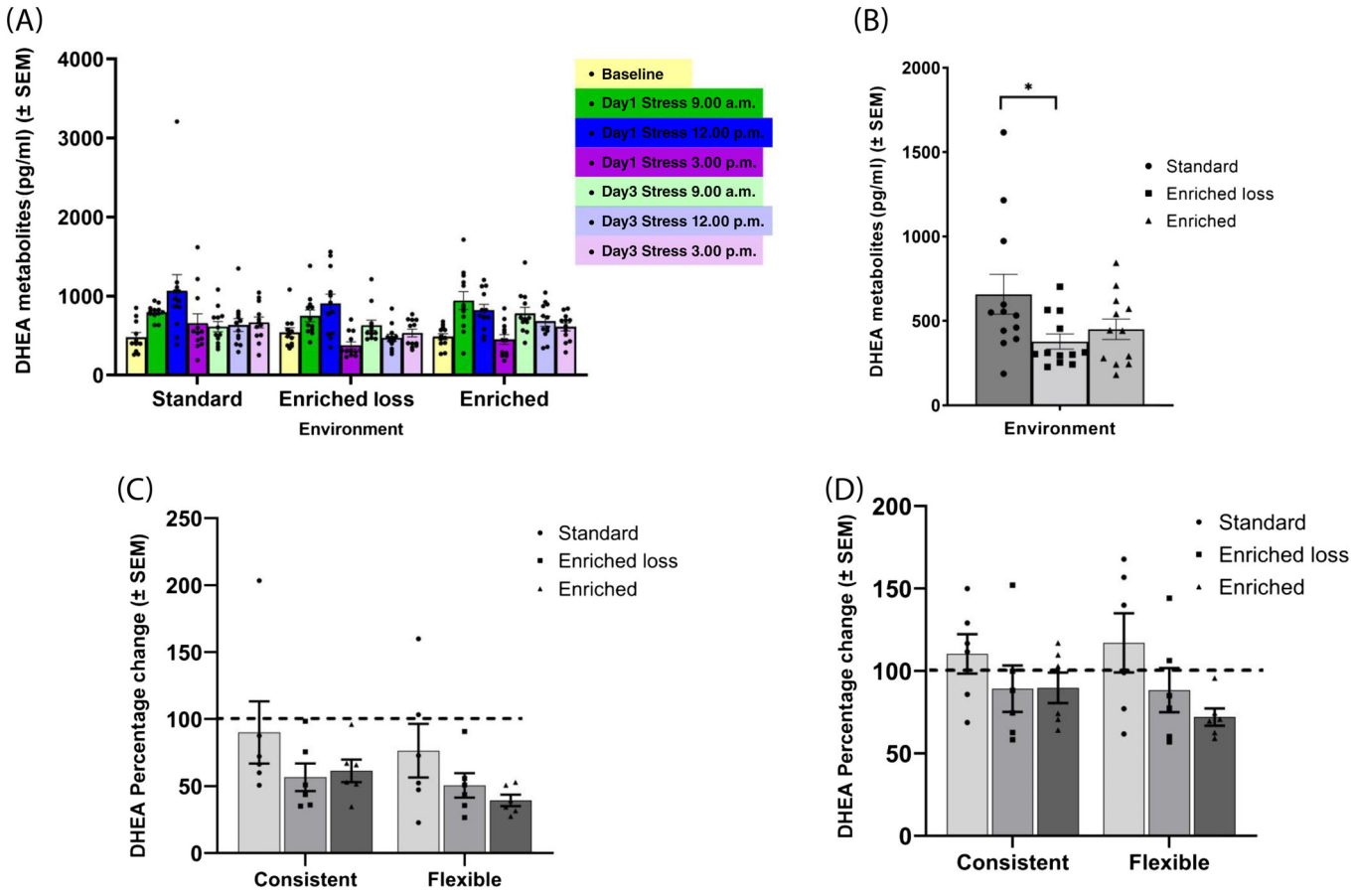

FIGURE 5 Dehydroepiandrosterone (DHEA) levels. (A) Distribution of DHEA fecal metabolites (pg mL^{-1}) samples across all groups and time points; (B) DHEA metabolites (pg mL^{-1}) representing 6 h post the swim stress. (C) Percentage change in DHEA metabolites between 9:00 a.m. and 3:00 p.m. after the first stress swim, representing recovery over the 6 h following swim stress. (D) Percentage change in DHEA metabolites between 9:00 a.m. and 3:00 p.m. once again representing recovery over the 6 h following swim stress. Data are expressed as the mean ± SEM percentage change of DHEA metabolites; in (C) and (D), data are shown as coping groups; $^{*}p \leq .05$.

A three-way mixed ANOVA assessing the ratio of DHEA to CORT fecal metabolites revealed a significant three-way interaction among time, coping, and environment ($F_{12,174} = 2.748$, $p = .002$, $\eta_p^2 = 0.159$) (see Figure 6A for distribution of all DHEA to CORT metabolite ratio values and subsequent graphs for specific statistically significant effects). Further post-hoc between-subjects analyses indicated a significant main effect of environment at 9:00 a.m. on day 1 of swim stress ($F_{2,29} = 3.76$, $p = .035$, $\eta_p^2 = 0.206$). Specifically, post-hoc analysis revealed that the enriched group exhibited a trend toward higher ratios compared to the standard group ($p = .053$) (Figure 6B). At 12:00 p.m. on day 1; however, a significant main effect of environment ($F_{2,29} = 3.667$, $p = .038$, $\eta_p^2 = 0.202$) indicated that the enriched loss group had significantly higher ratios compared to the enriched group ($p = .03$) (Figure 6C). Main effects of coping were also observed. At 12:00 p.m. on day 1, the flexible group had significantly higher ratios compared to the consistent group ($F_{1,29} = 5.74$, $p = .023$, $\eta_p^2 = 0.165$) (Figure 6D). Furthermore, at 3:00 p.m. on day 1, the flexible group continued to have significantly higher levels compared to the consistent group ($F_{2,29} = 6.197$, $p = .019$, $\eta_p^2 = 0.176$). At 12:00 p.m. on day 3, the flexible group continued to exhibit a higher ratio compared to the standard group ($F_{1,29} = 5.977$, $p = .021$, $\eta_p^2 = 0.171$) (Figure 6F). Analysis of the percentage change in DHEA/CORT ratios from 9:00 a.m. to 3:00 p.m. on day 1 indicated a significant interaction of coping strategy and environment ($F_{2,29} = 3.37$, $p = .048$, $\eta_p^2 = .188$) (Figure 6G) characterized by the enriched flexible group exhibiting higher ratios compared to the standard and enriched-loss flexible groups. In the consistent coping animals, however, the standard housed animals had higher ratios compared to the other consistent groups.

3.2.2 | Neuroohistochemical analysis

No significant differences between groups were observed for immunoreactivity of all proteins and brain areas analyzed. Neither the CA1, CA3, nor the dentate gyrus showed differences between groups for GFAP ($p > .05$). The CA1 and CA3 of the hippocampus were analyzed for BDNF immunoreactivity and no significant differences were observed ($p > .05$). The immunoreactivity for doublecortin was analyzed in the dentate gyrus and again no significant differences were observed between groups ($p > .05$).

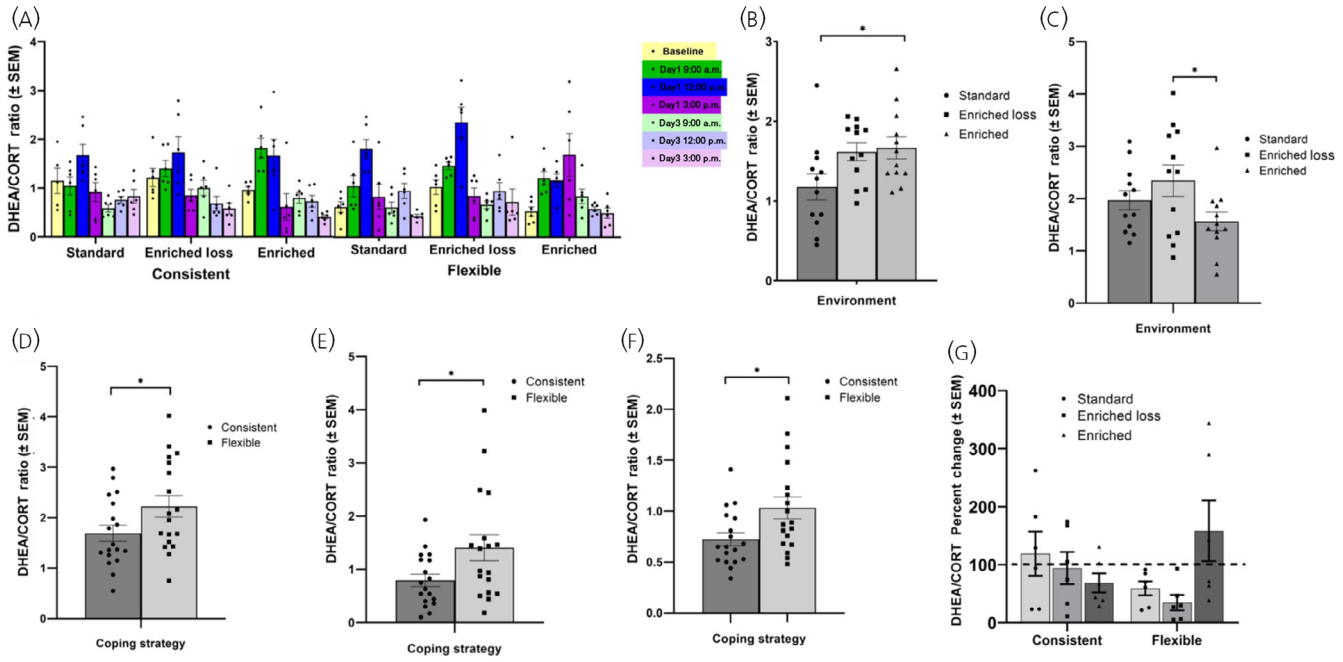

FIGURE 6 Dehydroepiandrosterone (DHEA) to corticosterone (CORT) ratio. (A) Distribution of ratios of DHEA to CORT fecal metabolites across all groups and time points. (B) Ratio of DHEA to CORT metabolites 12 h after the first swim stress representing the initial stress exposure. (C) Ratio of DHEA to CORT metabolites 15 h after the first swim stress representing the 3-h recovery timepoint for animals from the different environments. (D) Ratio of DHEA to CORT metabolites 15 h after the first swim stress for animals in the two coping groups representing the 3 h recovery point. (E) Ratio of DHEA to CORT metabolites 18 h after the first swim stress representing the 6-h recovery timepoint. (F) Ratio of DHEA to CORT metabolites 15 h after the third swim stress. (G) Percentage change in ratios from the initial swim stress to the 6-h recovery timepoint for the first day of swim stress. Data expressed as the mean ± SEM percentage change of the DHEA to CORT ratio; $^*p \leq .05$.

4 | DISCUSSION

In agreement with past research, the results of the present study suggest that the enrichment-loss model is a valuable model for the exploration of depressogenic symptoms, especially for the exploration of predisposed coping strategies and changing environmental habitats on the HPA regulatory system [9]. Briefly, the importance of interactions between coping profiles and environmental conditions was observed following repeated acute swim stress. As discussed below, the recovery DHEA:CORT ratios, an endocrine marker previously associated with emotional resilience in rodents and humans,[35] indicated that the stress endocrine response is complex and context-dependent.

In a previous study of enrichment-loss in male Sprague–Dawley rats, lower corticosterone levels in response to restraint stress were observed in the enrichment-loss animals compared to the continuously enriched animals.[9] Similar endocrine effects were observed in the present study; specifically, the enrichment-loss group exhibited the lowest corticosteroid fecal metabolite levels for each time point, with statistically significant decreases at two time points. By contrast to a previous enrichment-loss study,[8] the animals in the present study were not yet sexually mature, which adds to the robustness of this model; however, it would be interesting to explore the effect of enrichment-loss in more mature adult animals, especially in the context of different coping strategies. Furthermore, because the coping strategies were assessed at a young age post weaning, additional research is necessary to establish the consistency of the coping profiles throughout the lives of the rats.

The lower corticosteroid levels observed in the previous and current enrichment loss studies are noteworthy considering that diminished or dysregulated cortisol responses in humans may lead to insufficient compensatory reactions to stressors.[46,47] Additionally, hypocortisolism has been observed in PTSD,[48] perhaps as a result of altered glucocorticoid receptor sensitivity.[49] Evidence of developmental programming of this response was suggested in an investigation of the offspring of women who were pregnant and working in close proximity to the World Trade Center in New York City on September 11, 2001. Subsequent salivary cortisol assessments of these children revealed lower cortisol levels than observed in children born during a different time period.[50] In the present study, the finding of lower corticosteroid levels in the animals removed from the engaging and familiar enriched environment suggests that the enrichment-loss model represents an impactful stressful experience with translational value for humans. Consequently, it would be interesting to follow these animals throughout their lives to assess various health indices that may be associated with lower corticosteroid levels. Additionally, considering that the duration of enriched environment exposure was relatively short (i.e., 18 days) because of pragmatic limitations, investigations examining longer durations of enrichment would be especially informative.

Whereas previous enrichment-loss studies focused on the corticosterone response, the ratio of DHEA to corticosterone (DHEA:CORT) fecal metabolite levels following repeated acute swim stress sessions

was investigated in the present study. Fecal samples collected on the first and third days of swim stress in 3-h intervals starting at approximately 12 h past the initial stressor[23,36] indicated higher DHEA:CORT ratios in the enriched, flexible rats. Higher DHEA:CORT ratios are associated with emotional resilience considering that lower ratios of DHEA to cortisol levels have been observed to be predictive of treatment-resistant depression and decreased hippocampal volumes.[30,31] Additionally, higher DHEA in proportion to cortisol has been associated with emotional resilience,[35,51] perhaps as a result of the purported antagonistic actions of DHEA to glucocorticoids.[20] Additionally, past research suggests that higher levels of DHEA and DHEA(S) are predictive of emotional resilience.[52,53] For example, higher DHEA levels predicted perseverance and graduation outcomes for an elite airmen training program.[54] Finally, the DHEA:CORT ratio measure utilized in the present study was beneficial in that the relative measure across timepoints is less sensitive to circadian effects than analyses of absolute values of corticosterone and DHEA. Thus, the DHEA:CORT index offers potential as an indicator of an animals' ability to recover to baseline stress hormone levels in an efficient manner that does not result in wear and tear of the affected physiological systems, a response associated with a decreased likelihood of allostatic load and stress-related-illnesses.[55] However, caution should be taken in the translation of DHEA effects from rodents to humans considering that the primary source of DHEA in humans is the adrenal gland whereas the gonads represent the primary source of DHEA synthesis in the rodent.[56]

Focusing on the enrichment-loss animals, it was interesting to see that the DHEA:CORT ratios were different, depending on temperament style, at the 6-h time point on the first day of swim stress. Specifically, the flexible enrichment-loss animals had lower ratios (interpreted as less adaptive) than the consistent coping enrichment-loss group. Although the flexible animals were hypothesized to exhibit more resilience, the negative impact of enrichment loss in the flexible coping animals is reminiscent of a previous study in which flexible-coping rats that were exposed to a contingency-training task (i.e., the effort-based reward task) exhibited higher DHEA:CORT ratios than control rats that received the same number of non-contingent food rewards. However, similar to findings in the present study, when flexible coping rats encountered contexts that were accompanied by a lack of control such as non-contingent training (comparable to enrichment-loss in the present study), they appeared to be more negatively affected by the outcome.[36] One explanation for the vulnerability of the flexible coping animals in these specific contexts is related to the observation that flexible coping animals may be more sensitive and responsive to changing environmental contexts, a response strategy that can be adaptive or maladaptive, depending on the context.[57]

With the interesting endocrine effects observed in the present study, we were surprised at the lack of significant effects of coping profiles and environment on the neuroplasticity-related targets (i.e., BDNF, GFAP, and DCX). Although compelling evidence supports the role of neuroplasticity mechanisms in emotional regulation and dysregulation, the current data failed to indicate the relevance of these mechanisms in the enrichment-loss model. Although an emphasis in the present study was the hippocampus because of its role in neuroplasticity mechanisms,[58] future research should also explore more diverse brain areas implicated in stress responses. This is especially relevant considering the peripheral corticosterone and DHEA effects in the present study, as well as documented interactions between enriched environment exposure and stress responsivity.[59] For example, it would be interesting to explore ratios of glucocorticoid and mineralocorticoid receptors throughout the CA1, CA2, and CA3 areas of the hippocampus considering that mineralocorticoid receptors have been associated with the initial phase of stress appraisal[60] and healthy hippocampal functions.[61]

Enrichment loss has been previously reported to generate additional symptoms typically associated with depression including increased body weight, altered sucrose consumption, and increased floating behavior in the forced swim task, effects that were reversed by imipramine administration.[9] In a subsequent study using females in the enrichment loss paradigm, similar results were reported, with the exception of the observation of decreased sucrose consumption in enrichment loss animals.[9] Biological sex could be a contributing variable to these findings.

Focusing on the open field assessment in the present study, an interaction between coping profile and environmental condition indicated that consistent coping rats housed continuously in the standard environment exhibited less exploration in the presence of predator stimuli than the other groups, an effect not observed in the absence of the predator cues during habituation trials. Furthermore, enrichment-loss animals exhibited more external rearing responses in the habituation period, a response associated with escaping the arena, indicating diminished exploration of the new environment. Remarkably, the standard housed animals failed to jump on top of the hiding structure in subsequent open-field sessions, indicating a potential lack of vertical awareness in rats housed in standard laboratory cages, which is a point of potential concern in the generalization of these animals to their wild-caught conspecifics that exhibit frequent jumping responses[62]

Focusing on the sucrose preference task, sucrose was provided in the form of sweet cereal and presented in an open field arena along with standard chow. Hence, this was a dry food version of the typical sucrose preference test where the sucrose is mixed with water solution. We used this dry version adaptation to avoid consumption errors related to spillage from the sipping tubes of the water bottles. Because the continuously-enriched flexible rats consumed more of the typically preferred froot loop cereal pieces in this novel environment (in the absence of body weight differences), these results suggest that the motivation for reward exceeded anxiety associated with the novel environment, as observed in the closely associated novelty suppressed feeding tasks.[63] Furthermore, considering that motivation for previously-experienced rewards is indicative of the functioning capacity of the reward neurocircuitry known to be vulnerable in depressed individuals,[36,64] it is likely that the reward neurocircuitry of the enriched animals is consistent with adaptive emotional responses. In contradiction to these previous findings, however, no differences in weight gain were observed among the groups.

Interestingly, no effects of coping profile or environment were observed in behaviors assessed in the social and swim tasks. However,

the previously described endocrine results following the swim task suggest that physiological responses were differentially affected by the environment and coping responses, even though those differences were not apparent in the behavioral responses. Additionally, the higher corticosteroid levels and lower DHEA/CORT values on the third day of the swim exposure suggest that the acute nature of the swim stress may have approached the status of a chronic stressor by the time of the final swim. By this time, the animals had likely detected a pattern of stress exposure that likely influenced stress perception and HPA sensitivity, influencing the stress recovery response.

A clear limitation of the present study is the focus on male animals. Although it is desirable to assess both males and females in each investigation, limited resources prohibited the incorporation of additional animals, given the necessity of six different groups to accommodate three environments and two coping conditions. Thus, confirmation in females is necessary before making informed decisions about the generalizability and translational value of the current results. Given past findings related to HPA dysregulation in females exposed to enrichment loss,[9] as well as effects of coping strategies and enriched environments in both males and female rodents,[35] there is considerable evidence indicating that these variables are relevant and require further investigation using sex as a biological variable, especially considering that females are approximately twice as likely to experience the symptoms of depression than males.[65,66]

In conclusion, the findings of the present study reinforce past findings suggesting that animals' responses to the same stressors are differentially influenced by an individual's life history and predisposed coping responses. The enriched, complex environment was associated with adaptive outcomes for the continuously enriched flexible animals, yet this effect was not observed in the enriched animals that were profiled as consistent copers. Interestingly, the CORT response appeared to be more sensitive to coping strategies, whereas the DHEA response was more sensitive to environmental conditions. These results confirm that the stress response is context-specific and that overarching generalizations about high and low levels should be approached with caution.[67] Considering the complexity of psychological loss in humans, multiple variables should be considered in animal models designed to evaluate adaptive stress responses. Multidimensional negative consequences have been observed in families evicted from their homes (another form of enrichment-loss); for example, 50% of displaced mothers report depression symptoms,[68] a result that is consistent with past research indicating the impact of perceived loss and bereavement on stress responses and health outcomes.[69] Accordingly, the enrichment loss model offers promise for future explorations of adaptive responses to ecologically relevant stressors that have translational potential for humans.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Molly Kent: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; supervision; writing – original draft; writing – review and editing. **Dmitry Kovalev:** Conceptualization; data curation; formal analysis; investigation; methodology; writing – review and editing. **Benjamin Hart:** Investigation; methodology. **Danielle Leserve:** Data curation; investigation; methodology. **Gabriella Handford:** Data curation; methodology. **Dylan Vavra:** Conceptualization; data curation; investigation; methodology; project administration. **Kelly Lambert:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jne.13179.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kelly Lambert https://orcid.org/0000-0003-1626-5129

REFERENCES

1. Ledford H. Medical research: if depression were cancer. *Nature*. 2014;515:182-184.
2. Christie-Seely J. Life stress and illness: a systems approach. *Can Fam Physician*. 1983;29:533-540.
3. Sikorski C, Luppa M, Heser K, Ernst A, Lange C. The role of spousal loss in the development of depressive symptoms in the elderly—implications for diagnostic systems. *J Affect Disord*. 2014;161:97-103. Available from: https://www.sciencedirect.com/science/article/pii/S0165032714000810?casa_token=4Nl17nHVt8gAAAAA:YYffyBt-1o6qCvpCVK0Cjz04UxvCMIwVXVx33DNNk7tuYuq2LN0zt11PXy7x8vNSNQBZ9c2DU7Q
4. Zhai Y, Du X. Loss and grief amidst COVID-19: a path to adaptation and resilience. *Brain Behav Immun*. 2020;87:80-81.
5. Czeisler MÉ, Lane RI, Petrosky E, et al. Mental health, substance use, and suicidal ideation during the COVID-19 pandemic - United States, June 24-30, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69:1049-1057.
6. Richter-Levin G, Sandi C. Labels matter: is it stress or is it trauma? *Transl Psychiatry*. 2021;11:1-9.
7. Planchez B, Surget A, Belzung C. Animal models of major depression: drawbacks and challenges. *J Neural Transm*. 2019;126:1383-1408.
8. Smith BL, Lyons CE, Correa FG, et al. Behavioral and physiological consequences of enrichment loss in rats. *Psychoneuroendocrinology*. 2017;77:37-46.
9. Morano R, Hoskins O, Smith BL, Herman JP. Loss of environmental enrichment elicits behavioral and physiological dysregulation in female rats. *Front Behav Neurosci*. 2018;12:287.
10. Franks B, Champagne FA, Higgins ET. How enrichment affects exploration trade-offs in rats: implications for welfare and well-being. *PLoS One*. 2013;8:e83578.
11. Sukegawa M, Yoshihara T, Hou S, Asano M, Hannan AJ, Wang DO. Long-lasting housing environment manipulation and acute loss of environmental enrichment impact BALB/c mice behaviour in multiple functional domains. *Eur J Neurosci*. 2022;55:1118-1140.
12. Zhao X, Tran H, DeRosa H, Roderick RC, Kentner AC. Hidden talents: poly (I:C)-induced maternal immune activation improves mouse visual discrimination performance and reversal learning in a sex-dependent

manner. *Genes Brain Behav*. 2021;20(7):e12755. doi:10.1101/2021.02.15.431275
13. Lambert K, Hyer M, Bardi M, et al. Natural-enriched environments lead to enhanced environmental engagement and altered neurobiological resilience. *Neuroscience*. 2016;330:386-394.
14. Brown M, Tschirhart M, Lambert KG. Paper or plastic? Exploring the effects of natural enrichment on Behavioural and neuroendocrine responses in Long-Evans rats. *J Neuroendocrinol* 2016. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jne.12383?casa_token=HNBEVGI553oAAAAA:kcrKKh-rLr8tR4uNIlD7Iw04i_ir37EFxpNKjy15wGV598-VxIaoHzPF9q7Gmh4Ug8qAuWReA06FqSYE
15. Kempermann G. Environmental enrichment, new neurons and the neurobiology of individuality. *Nat Rev Neurosci*. 2019;20:235-245.
16. Freund J, Brandmaier AM, Lewejohann L, et al. Emergence of individuality in genetically identical mice. *Science*. 2013;340:756-759. doi:10.1126/science.1235294
17. Nandam LS, Brazel M, Zhou M, Jhaveri DJ. Cortisol and major depressive disorder—translating findings from humans to animal models and Back. *Front Psych*. 2020;10:974.
18. Qin D-D, Rizak J, Feng X-L, et al. Prolonged secretion of cortisol as a possible mechanism underlying stress and depressive behaviour. *Sci Rep*. 2016;6:30187.
19. Charney DS. Psychobiological mechanisms of resilience and vulnerability. *Focus*. 2004;2:368-391. doi:10.1176/foc.2.3.368
20. Rosenfeld RS, Hellman L, Roffwarg H, Weitzman ED, Fukushima DK, Gallagher TF. Dehydroisoandrosterone is secreted episodically and synchronously with cortisol by normal man. *J Clin Endocrinol Metab*. 1971;33:87-92.
21. Daskalakis NP, De Kloet ER, Yehuda R, Malaspina D, Kranz TM. Early life stress effects on glucocorticoid—BDNF interplay in the hippocampus. *Front Mol Neurosci*. 2015;8:181.
22. Yehuda R, Brand SR, Golier JA, Yang R-K. Clinical correlates of DHEA associated with post-traumatic stress disorder. *Acta Psychiatr Scand*. 2006;114:187-193.
23. Bardi M, Hampton JE, Lambert KG. Fecal dehydroepiandrosterone (DHEA) immunoreactivity as a noninvasive index of circulating DHEA activity in young male laboratory rats. *Comp Med*. 2010;60:455-460.
24. Makino S, Gold PW, Schulkin J. Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis; comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Res*. 1994;657:141-149.
25. Muller C, Hennebert O, Morfin R. The native anti-glucocorticoid paradigm. *J Steroid Biochem Mol Biol*. 2006;100:95-105. doi:10.1016/j.jsbmb.2006.03.001
26. Chalbot S, Morfin R. Dehydroepiandrosterone metabolites and their interactions in humans. *Drug Metabol Drug Interact*. 2006;22:1-23.
27. Genud R, Merenlender A, Gispan-Herman I, Maayan R, Weizman A, Yadid G. DHEA lessens depressive-like behavior via GABA-ergic modulation of the mesolimbic system. *Neuropsychopharmacology*. 2009;34:577-584.
28. Ratner MH, Kumaresan V, Farb DH. Neurosteroid actions in memory and neurologic/neuropsychiatric disorders. *Front Endocrinol*. 2019;10:169.
29. McEwen BS. Mood disorders and allostatic load. *Biol Psychiatry*. 2003;54:200-207.
30. Jin RO, Mason S, Mellon SH, et al. Cortisol/DHEA ratio and hippocampal volume: a pilot study in major depression and healthy controls. *Psychoneuroendocrinology*. 2016;72:139-146.
31. Markopoulou K, Papadopoulos A, Juruena MF, Poon L, Pariante CM, Cleare AJ. The ratio of cortisol/DHEA in treatment resistant depression. *Psychoneuroendocrinology*. 2009;34:19-26.
32. Kazmi R, Amjad S, Khan D. Individual differences and stress-performance relationship. *J Ayub Med Coll Abbottabad*. 2009;21:172-175.
33. Ebner K, Singewald N. Individual differences in stress susceptibility and stress inhibitory mechanisms. *Curr Opin Behav Sci*. 2017;14:54-64.
34. Dijkstra MTM, Homan AC. Engaging in rather than disengaging from stress: effective coping and perceived control. *Front Psychol*. 2016;7:1415.
35. Kent M, Bardi M, Hazelgrove A, et al. Profiling coping strategies in male and female rats: potential neurobehavioral markers of increased resilience to depressive symptoms. *Horm Behav*. 2017;95:33-43.
36. Lambert KG, Hyer MM, Rzucidlo AA, Bergeron T, Landis T, Bardi M. Contingency-based emotional resilience: effort-based reward training and flexible coping lead to adaptive responses to uncertainty in male rats. *Front Behav Neurosci*. 2014;8:124.
37. Bardi M, Rhone AP, Franssen CL, et al. Behavioral training and predisposed coping strategies interact to influence resilience in male Long-Evans rats: implications for depression. *Stress*. 2012;15:306-317.
38. Hawley DF, Bardi M, Everette AM, et al. Neurobiological constituents of active, passive, and variable coping strategies in rats: integration of regional brain neuropeptide Y levels and cardiovascular responses. *Stress*. 2010;13:172-183.
39. Balthazart J, Ball GF. Doublecortin is a highly valuable endogenous marker of adult neurogenesis in canaries. Commentary on Vellema M et al. (2014): Evaluating the predictive value of doublecortin as a marker for adult neurogenesis in canaries (*Serinus canaria*). J Comparative Neurol 522:1299–1315. *Brain Behav Evol*. 2014;84:1-4.
40. Yang T, Nie Z, Shu H, et al. The role of BDNF on neural plasticity in depression. *Front Cell Neurosci*. 2020;14:82.
41. Viana GSB, Vale EM do, Araujo ARA de, Coelho NC, Andrade SM, Costa RO da, et al. Rapid and long-lasting antidepressant-like effects of ketamine and their relationship with the expression of brain enzymes, BDNF, and astrocytes. *Braz J Med Biol Res* 2020;54:e10107.
42. Eldomiaty MA, Makarenko O, Hassan ZA, Almasry SM, Petrov P, Elnaggar AM. Contribution of glia cells specifically astrocytes in the pathology of depression: immunohistochemical study in different brain areas. *Folia Morphol*. 2020;79:419-428.
43. Neal S, Kent M, Bardi M, Lambert KG. Enriched environment exposure enhances social interactions and oxytocin responsiveness in male Long-Evans rats. *Front Behav Neurosci*. 2018;12:198.
44. Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Ann N Y Acad Sci*. 2005;1046:54-74.
45. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition*. Elsevier; 2006.
46. Dekel S, Ein-Dor T, Rosen JB, Bonanno GA. Differences in cortisol response to trauma activation in individuals with and without comorbid PTSD and depression. *Front Psychol*. 2017;8:797.
47. Kendall-Tackett KA. Physiological correlates of childhood abuse: chronic hyperarousal in PTSD, depression, and irritable bowel syndrome. *Child Abuse Negl*. 2000;24:799-810. doi:10.1016/s0145-2134(00)00136-8
48. Kosten TR, Mason JW, Giller EL, Ostroff RB, Harkness L. Sustained urinary norepinephrine and epinephrine elevation in post-traumatic stress disorder. *Psychoneuroendocrinology*. 1987;12:13-20. doi:10.1016/0306-4530(87)90017-5
49. Yehuda R, Seckl J. Minireview: stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology*. 2011;152:4496-4503.
50. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the world trade center attacks during pregnancy. *J Clin Endocrinol Metab*. 2005;90:4115-4118.
51. Bürgin D, Kind N, Schröder M, et al. The stress of caring-resilience and HPA-Axis activity in hair samples of youth residential caregivers. *Front Psych*. 2020;11:556486.

52. Petros N, Opacka-Juffry J, Huber JH. Psychometric and neurobiological assessment of resilience in a non-clinical sample of adults. *Psychoneuroendocrinology*. 2013;38:2099-2108.
53. Morgan CA III, Rasmusson A, Pietrzak RH, Coric V, Southwick SM. Relationships among plasma dehydroepiandrosterone and dehydroepiandrosterone sulfate, cortisol, symptoms of dissociation, and objective performance in humans exposed to underwater navigation stress. *Biol Psychiatry*. 2009;66:334-340.
54. Jenz ST, Goodyear CD, Graves PT, Goldstein S, Shia MR, Redei EE. Blood and affective markers of stress in elite airmen during a preparatory training course: a pilot study. *Neurobiol Stress*. 2021;14:100323.
55. McEwen BS. Stressed or stressed out: what is the difference? *J Psychiatry Neurosci*. 2005;30:315-318.
56. Gabai G, Mongillo P, Giaretta E, Marinelli L. Do Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) play a role in the stress response in domestic animals? *Front Vet Sci*. 2020;7:588835.
57. Waugh CE, Thompson RJ, Gotlib IH. Flexible emotional responsiveness in trait resilience. *Emotion*. 2011;11:1059-1067.
58. Fuchs E, Flügge G. Adult neuroplasticity: more than 40 years of research. *Neural Plast* 2014;2014: 541870.
59. Smail MA, Smith BL, Nawreen N, Herman JP. Differential impact of stress and environmental enrichment on corticolimbic circuits. *Pharmacol Biochem Behav*. 2020;197:172993.
60. Klok MD, Giltay EJ, Van der Does AJW, et al. A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Transl Psychiatry*. 2011;1:e62.
61. Yau JLW, Seckl JR. Local amplification of glucocorticoids in the aging brain and impaired spatial memory. *Front Aging Neurosci*. 2012;4:24.
62. Price EO, Huck UW. Open-field behavior of wild and domestic Norway rats. *Anim Learn Behav*. 1976;4:125-130.
63. Blasco-Serra A, González-Soler EM, Cervera-Ferri A, Teruel-Martí V, Valverde-Navarro AA. A standardization of the novelty-suppressed feeding test protocol in rats. *Neurosci Lett*. 2017;658:73-78.
64. Franzen J, Brinkmann K. Anhedonic symptoms of depression are linked to reduced motivation to obtain a reward. *Motiv Emot*. 2016;40:300-308.
65. Piccinelli M, Wilkinson G. Gender differences in depression. *Critical Review Br J Psychiatry*. 2000;177:486-492.
66. Bangasser DA, Cuarenta A. Sex differences in anxiety and depression: circuits and mechanisms. *Nat Rev Neurosci*. 2021;22:674-684. doi:10.1038/s41583-021-00513-0
67. Sapolsky RM. Glucocorticoids, the evolution of the stress-response, and the primate predicament. *Neurobiol Stress*. 2021;14:100320.
68. Desmond M, Kimbro RT. Eviction's fallout: housing, hardship, and health. *Soc Forces*. 2015;94:295-324.
69. Kaltman S, Bonanno GA. Trauma and bereavement: examining the impact of sudden and violent deaths. *J Anxiety Disord*. 2003;17:131-147.

How to cite this article: Kent M, Kovalev D, Hart B, et al. The emotional impact of disrupted environmental contexts: Enrichment loss and coping profiles influence stress response recovery in Long–Evans rats. *J Neuroendocrinol*. 2022;34(7): e13179. doi:10.1111/jne.13179

APPENDIX A

Statistical information for dependent variables that were not significantly affected by either environment or coping style.

Behavioral test	Behavior/dependent variable	Independent variable/mean (SEM)
Open field test (habituation)	Internal rear	Environment [Standard = 1.25(0.0.28); Loss = 2.75(0.71); Enrich = 1.67(0.48)] Coping [Consistent = 2.28(0.46); Flexible = 1.5(0.41)]
	External rear	Environment [Standard = 10.9(1.28); Loss = 14.5(1.25); Enrich = 12.4(1.48)] Coping [Consistent = 13.3(1.26); Flexible = 11.9(0.97)]
	Freezing	E Environment [Standard = 1.08(0.45); Loss = 0.08(0.08); Enrich = 1.0(0.33)] Coping [Consistent = 0.83(0.27); Flexible = 0.61(0.29)]
	Crossing center	Environment [Standard = 0.67(0.19); Loss = 1.25(0.48); Enrich = 1.0(0.35)] Coping [Consistent = 0.89(0.34); Flexible = 1.1(0.24)]
Open field test (hide)	Internal rear	Environment [Standard = 3.8(0.89); Loss = 4.4(0.69); Enrich = 3.2(0.59)] Coping [Consistent = 4.1(0.53); Flexible = 3.5(0.66)]
	External rear	Environment [Standard = 7.5(0.77); Loss = 11.9(1.7); Enrich = 6.4(1.2)] Coping [Consistent = 8.9(1.1); Flexible = 8.3(1.2)]
	Freezing	Environment [Standard = 0.08(0.08); Loss = 0.25(0.18); Enrich = 0.58(0.15)] Coping [Consistent = 0.39(0.14); Flexible = 0.22(0.1)]
	Inside hide	Environment [Standard = 4.3(0.57); Loss = 3.6(0.4); Enrich = 3.3(0.37)] Coping [Consistent = 4.0(0.45); Flexible = 3.4(0.28)]
	Behind hide	Environment [Standard = 3.3(0.35); Loss = 3.8(0.27); Enrich = 3.0(0.54)] Coping [Consistent = 3.2(0.31); Flexible = 3.5(0.35)]
	On top of hide	Environment [Standard = 0.0(0); Loss = 3.2(0.66); Enrich = 2.3(0.47)] Coping [Consistent = 1.9(0.51); Flexible = 1.7(0.48)]
Open field test (predator, original hide)	Internal rear	Environment [Standard = 1.8(0.44); Loss = 2.4(0.45); Enrich = 2.6(0.45)] Coping [Consistent = 2.1(0.41); Flexible = 2.5(0.32)]
	External rear	Environment [Standard = 3.8(0.67); Loss = 2.4(0.78); Enrich = 1.6(0.4)] Coping [Consistent = 2.6(0.42); Flexible = 2.7(0.67)]
	Freezing	Environment [Standard = 1.4(0.4); Loss = 0.83(0.17); Enrich = 1.4(0.26)] Coping [Consistent = 1.4(0.26); Flexible = 1.0(0.21)]
	Inside hide	Environment [Standard = 0.92(0.26); Loss = 1.4(0.15); Enrich = 1.3(0.28)] Coping [Consistent = 1.3(0.19); Flexible = 1.1(0.2)]
	Behind hide	Environment [Standard2.1(0.4); Loss = 2.1(0.29); Enrich = 2.0(0.41)] Coping [Consistent = 1.8(0.29); Flexible = 2.3(0.3)]
	On top of hide	Environment [Standard = 0.0(0); Loss = 0.08(0.08); Enrich = 0.42(0.23)] Coping [Consistent = 0.17(0.12); Flexible = 0.17(0.12)]
Open field test (predator, novel hide)	Internal rear	Environment [Standard = 5.9(0.82); Loss = 3.9(0.7); Enrich = 3.9(0.9)] Coping [Consistent = 4.9(0.7); Flexible = 4.3(0.67)]
	External rear	Environment [Standard = 8.6(1.0); Loss = 6.0(1.2); Enrich = 5.3(1.4)] Coping [Consistent = 7.7(1.0); Flexible = 5.6(0.94)]
	Freezing	Environment [Standard = 1.8(1.3); Loss = 0.92(0.26); Enrich = 1.6(0.42)] Coping [Consistent = 1.9(0.86); Flexible = 0.89(0.3)]
	Inside hide	Environment [Standard = 0.75(0.33); Loss = 1.6(0.56); Enrich = 1.3(0.36)] Coping [Consistent = 1.1(0.26); Flexible = 1.4(0.27)]
	Behind hide	Environment [Standard = 4.3(0.61); Loss = 4.0(0.33); Enrich = 3.3(0.57)] Coping [Consistent = 4.2(0.39); Flexible = 3.5(0.44)]
	On top of hide	Environment [Standard = 0.0(0); Loss = 0.08(0.08); Enrich = 0.5(0.29)] Coping [Consistent = 0.39(0.2); Flexible = 0.0(0)]
Social investigation test (habituation)	Internal rear	Environment [Standard = 2.2(0.46); Loss = 2.9(0.61); Enrich = 3.7(0.57)] Coping [Consistent = 3.1(0.44); Flexible = 2.8(0.49)]
	External rear	Environment [Standard = 17.8(1.2); Loss = 18.5(0.9); Enrich = 15.3(1.0)] Coping [Consistent = 18.0(0.8); Flexible = 16.4(0.98)]

(Continues)

Behavioral test	Behavior/dependent variable	Independent variable/mean (SEM)
Social investigation test (conspecific)	Internal rear	Environment [Standard = 2.5(0.45); Loss = 3.5(0.86); Enrich = 4.1(0.8)] Coping [Consistent = 3.6(0.64); Flexible = 3.2(0.56)]
	External rear	Environment [Standard = 17.8(1.6); Loss = 18.6(1.8); Enrich = 15.8(0.9)] Coping [Consistent = 16.7(1.4); Flexible = 18.1(1.1)]
	Digging toward conspecific	E Environment [Standard = 0.17(0.11); Loss = 0.08(0.08); Enrich = 0.08(0.08)] Coping [Consistent = 0.11(0.076); Flexible = 0.11(0.076)]
	Digging away from conspecific	Environment [Standard = 0.17(0.06); Loss = 0.08(0.08); Enrich = 0.0(0)] Coping [Consistent = 0.11(0.11); Flexible = 0.06(0.06)]
Forced swim test (day 1)	Latency to float	Environment [Standard = 105.0(20.3); Loss = 79.9(19.2); Enrich = 119.8(17.7)] Coping [Consistent = 97.3(15.6); Flexible = 105.8(16.1)]
	Duration floating	Environment [Standard = 1.8(0.58); Loss = 2.6(0.92); Enrich = 5.2(2.4)] Coping [Consistent = 2.9(0.74); Flexible = 3.4(1.7)]
	Duration swimming	Environment [Standard = 178.3(0.58); Loss = 177.4(0.92); Enrich = 174.8(2.4)] Coping [Consistent = 177.1(0.74); Flexible = 176.6(1.7)]
	Half dives	Environment [Standard = 3.1(0.65); Loss = 2.3(0.68); Enrich = 1.5(0.49)] Coping [Consistent = 2.6(0.53); Flexible = 2.0(0.5)]
	Frequency of floats	Environment [Standard = 1.6(0.56); Loss = 2.1(0.63); Enrich = 1.8(0.83)] Coping [Consistent = 2.0(0.52); Flexible = 1.7(0.58)]
	Dives	Environment [Standard = 0.67(0.33); Loss = 0.83(0.27); Enrich = 0.58(0.23)] Coping [Consistent = 0.72(0.18); Flexible = 0.67(0.27)]
	Sinks	Environment [Standard = 13.4(1.8); Loss = 8.2(1.1); Enrich = 9.6(1.4)] Coping [Consistent = 10.2(2.1); Flexible = 10.6(1.2)]
	Shakes	Environment [Standard = 21.1(2.9); Loss = 23.1(1.6); Enrich = 27.8(3.5)] Coping [Consistent = 23.1(2.4); Flexible = 24.9(2.3)]
Forced swim test (day 2)	Latency to float	Environment [Standard = 161.9(13.2); Loss = 135.6(23.2); Enrich = 130.6(21.6)] Coping [Consistent = 134.7(16.9); Flexible = 150.7(15.4)]
	Duration floating	Environment [Standard = 0.75(0.39); Loss = 0.5(0.29); Enrich = 1.5(0.62)] Coping [Consistent = 1.0(0.32); Flexible = 0.83(0.43)]
	Duration swimming	Environment [Standard = 179.3(0.39); Loss = 179.5(0.29); Enrich = 178.5(0.62)] Coping [Consistent = 179.0(0.32); Flexible = 179.2(0.43)]
	Half dives	Environment [Standard = 0.47(0.11); Loss = 0.25(0.18); Enrich = 0.18(0.12)] Coping [Consistent = 0.24(0.11); Flexible = 0.17(0.12)]
	Frequency of floats	Environment [Standard = 0.57(0.33); Loss = 0.33(0.14); Enrich = 1.8(0.73)] Coping [Consistent = 0.89(0.33); Flexible = 2.3(1.2)]
	Dives	Environment [Standard = 0.0(0); Loss = 0.08(0.08); Enrich = 0.25(0.13)] Coping [Consistent = 0.17(0.09); Flexible = 0.06(0.06)]
	Sinks	Environment [Standard = 2.3(0.6); Loss = 2.6(0.66); Enrich = 2.0(0.66)] Coping [Consistent = 2.5(0.58); Flexible = 2.1(0.44)]
	Shakes	Environment [Standard = 18.8(1.6); Loss = 18.5(1.6); Enrich = 20.2(2.7)] Coping [Consistent = 19.2(2.0); Flexible = 19.1(1.7)]
Forced swim test (day 3)	Latency to float	Environment [Standard = 84.1(25.5); Loss = 18.7(22.9); Enrich = 117.1(24.4)] Coping [Consistent = 53.5(20.3); Flexible = 83.1(21.0)]
	Duration floating	Environment [Standard = ;2.6(1.0) Loss = 2.9(0.83); Enrich = 3.0(1.5)] Coping [Consistent = 2.7(0.77); Flexible = 3.0(1.1)]
	Duration swimming	Environment [Standard = 177.4(1.0); Loss = 177.1(0.83); Enrich = 177.0(1.5)] Coping [Consistent = 1777.3(0.77); Flexible = 177.0(1.1)]
	Half dives	Environment [Standard = 0.08(0.08); Loss = 0.0(0); Enrich = 0.27(0.14)] Coping [Consistent = 0.12(0.08); Flexible = 0.11(0.08)]
	Frequency of floats	Environment [Standard = 1.8(0.51); Loss = 2.6(0.78); Enrich = 2.0(0.84)] Coping [Consistent = 1.9(0.49); Flexible = 2.4(0.67)]
	Dives	Environment [Standard = 0.08(0.08); Loss = 0.0(0); Enrich = 0.08(0.08)] Coping [Consistent = 0.11(0.08); Flexible = 0.0(0)]
	Sinks	Environment [Standard = 0.58(0.23); Loss = 0.5(0.26); Enrich = 1.9(0.95)] Coping [Consistent = 0.89(0.55); Flexible = 1.1(0.43)]
	Shakes	Environment [Standard = 18.6(2.0); Loss = 17.2(2.1); Enrich = 20.4(2.8)] Coping [Consistent = 19.1(2.0); Flexible = 18.4(1.8)]

Behavioral test	Behavior/dependent variable	Independent variable/mean (SEM)
Sucrose preference test (habituation)	External rear	Environment [Standard = 18.3(2.1); Loss = 22.3(0.97); Enrich = 19.4(1.6)] Coping [Consistent = 19.3(1.3); Flexible = 20.7(1.3)]
	Internal rear	Environment [Standard = 6.7(1.5); Loss = 7.5(1.6); Enrich = 10.0(1.8)] Coping [Consistent = 8.9(1.5); Flexible = 7.2(1.1)]
	Fecal boli	Environment [Standard = 0.75(0.51); Loss = 0.17(0.17); Enrich = 0.75(0.37)] Coping [Consistent = 0.28(0.23); Flexible = 0.83(0.36)]
Sucrose preference test (test)	External rear	Environment [Standard = 12.9(1.3); Loss = 18.2(1.4); Enrich = 9.8(1.3)] Coping [Consistent = 13.4(1.2); Flexible = 12.4(1.3)]
	Internal rear	Environment [Standard = ;4.9(1.0) Loss = 4.5(0.9); Enrich = 4.1(0.81)] Coping [Consistent = 4.5(0.75); Flexible = 4.5(0.74)]
	Fecal boli	Environment [Standard = 0.0(0); Loss = 0.0(0); Enrich = 0.33(0.33)] Coping [Consistent = 0.0(0); Flexible = 0.22(0.22)]
Immunohistochemical stain	**Brain area/dependent variable**	**Independent variable/mean (SEM)**
GFAP	CA1	Environment [Standard = 0.15(0.005); Loss = 0.15(0.005); Enrich = 0.14(0.003)] Coping [Consistent = 0.15(0.004); Flexible = 0.14(0.004)]
	CA3	Environment [Standard = 0.17(0.006); Loss = 0.17(0.01); Enrich = 0.15(0.007)] Coping [Consistent = 0.17(0.01); Flexible = 0.16(0.006)]
	DG	Environment [Standard = 0.21(0.01); Loss = 0.2(0.02); Enrich = 0.23(0.01)] Coping [Consistent = 0.22(0.01); Flexible = 0.21(0.01)]
BDNF	CA1	Environment [Standard = 109.5(4.7); Loss = 97.8(4.3); Enrich = 96.9(4.8)] Coping [Consistent = 98.1(2.9); Flexible = 105.2(4.8)]
	CA3	Environment [Standard = 73.4(2.0); Loss = 71.4(1.9); Enrich = 74.9(2.2)] Coping [Consistent = 73.1(1.9); Flexible = 73.2(1.5)]
DCX	DG	Environment [Standard = 4.9(0.62); Loss = 6.8(0.75); Enrich = 6.1(0.65)] Coping [Consistent = 6.2(0.61); Flexible = 5.6(0.53)]